

RESEARCH ARTICLE

The metabolic cost of incline locomotion in the Svalbard rock ptarmigan (*Lagopus muta hyperborea*): the effects of incline grade and seasonal fluctuations in body mass

John Lees¹, Lars Folkow², Karl-Arne Stokkan² and Jonathan Codd^{1,*}

¹Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, UK and ²Department of Arctic and Marine Biology, University of Tromsø, Tromsø NO-9037, Norway

*Author for correspondence (jonathan.codd@manchester.ac.uk)

SUMMARY

In a terrestrial environment animals must locomote over varying terrain; despite this, the majority of studies of animal locomotion focus on level locomotion. The influence moving up an inclined surface has on the metabolic cost of locomotion and the efficiency with which animals perform positive work against gravity is still not well understood. Generally speaking, existing data sets lack consistency in the use of grades, further compounded by differences between species in terms of morphology and locomotor gait. Here we investigated the metabolic cost of locomotion using respirometry in the Svalbard ptarmigan (*Lagopus muta hyperborea*). The Svalbard ptarmigan provides a unique opportunity to investigate the cost of incline locomotion as it undergoes a seasonal fluctuation in body mass, which doubles in winter, meaning the requirement for positive mechanical work also fluctuates with season. We demonstrate that at the same degree of incline, the cost of lifting 1 kg by 1 vertical metre remains relatively constant between seasons despite the large differences in body mass from summer to winter. These findings are consistent with the notion that positive mechanical work alone dictates the cost of lifting above a certain body mass. However, our data indicate that this cost may vary according to the degree of incline and gait.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/216/8/1355/DC1>

Key words: Arctic, energetics, ptarmigan, respirometry.

Received 1 August 2012; Accepted 6 December 2012

INTRODUCTION

The metabolic cost of locomotion is a major contributor to the daily energy budget of terrestrial organisms, particularly birds, and is therefore a crucial determinant of fitness (Goldstein, 1988; Goldstein and Nagy, 1985; Tolkamp et al., 2002). Much of the research in this field has focused on level treadmill locomotion, with relatively few studies examining the potential effects of inclines (Bamford and Maloij, 1980; Ellerby et al., 2003; Rubenson et al., 2006; Snyder and Carello, 2008; Warncke et al., 1988). Examining the cost of incline locomotion is important and relevant as organisms seldom experience steady-state conditions, being faced with variable terrain over which energy demands for locomotion are continuously changing.

Some indirect calorimetry studies of terrestrial organisms locomoting on level terrain have presented a general linear increase in energy consumption with speed (U) (Kram and Taylor, 1990; Roberts et al., 1998; Taylor et al., 1982; Taylor et al., 1970). However, this linear trend is not always the case, as non-linearity is commonly found for this relationship both within and between gaits (Baudinette et al., 1992; Dawson and Taylor, 1973; Hoyt and Taylor, 1981; Langman et al., 2012; Luick and White, 1986; Nudds et al., 2011; Rubenson et al., 2004). Linear regressions have, however, facilitated comparisons across species of varying body mass (M_b) and morphology. When moving on an incline, additional work must be done raising the centre of mass (CoM) against gravity, related to the M_b of the organism and its rate of ascent (Biewener,

2003), and this additional work entails a metabolic cost. However, the relative effects of slope angle, U and M_b upon the efficiency of incline locomotion are largely unresolved. Initially it was thought that the metabolic cost of lifting 1 kg of M_b by 1 vertical metre (m_v) should be constant, assuming that the efficiency of muscular work is also constant regardless of M_b (Taylor et al., 1972). Smaller animals, having a relatively higher horizontal minimum cost of transport ($MCoT_h$), would therefore demonstrate increased efficiency of incline locomotion (mechanical work in lifting 1 kg by 1 m_v /metabolic energy in lifting 1 kg by 1 m_v) than larger ones. Data from mice (*Mus musculus*) and chimpanzees (*Pan troglodytes*) running on a 15 deg incline suggested that this constant cost was approximately $15.5 \text{ J kg}^{-1} m_v^{-1}$ (Taylor et al., 1972), which is perhaps surprising given the marked differences in locomotor specialisation between the species chosen for comparison. Subsequent experiments across a broad range of species, however, have shown the cost of lifting to be highly variable between species. Studies across taxa of varying M_b (from 0.8 g to over 400 kg), morphology and locomotor specialisation have yet to reveal a consistent effect of incline upon the metabolic cost of locomotion (Bamford and Maloij, 1980; Cohen et al., 1978; Fancy and White, 1987; Full and Tullis, 1990; Raab et al., 1976; Snyder and Farley, 2011; Wickler et al., 2000; Yousef et al., 1972). For example, the cost of lifting in two species of lizard, *Coleonyx variegatus* and *Eumeces skiltonianus*, of similar M_b on 50 deg inclines differed by nearly $27 \text{ J kg}^{-1} m_v^{-1}$ (Farley and Emshwiller, 1996). Both species, however, have vertical efficiencies

greater than those of cockroaches (*Periplaneta americana*) with lower M_b (Full and Tullis, 1990), but lower values than heavier elk (*Cervus canadensis nelsoni*) (Cohen et al., 1978).

The apparent lack of a correlation between M_b and the overall cost of incline locomotion is compounded by mixed findings regarding the effect of differing inclination angles. For example, dogs (*Canis familiaris*) and guinea fowl (*Numida meleagris*) demonstrate decreased efficiency [calculated using the method of Taylor et al. (Taylor et al., 1972)] with increased inclination angle (Ellerby et al., 2003; Raab et al., 1976), whereas efficiency in quail (*Coturnix coturnix*) on grades of 4, 8 and 12 deg increases with increasing slope (Warncke et al., 1988), in common with findings in humans (Margaria, 1938). However, while kangaroos (*Macropus rufus*) and cockroaches incur an increased metabolic cost of locomotion on inclines, the degree of the incline has relatively little effect (Full and Tullis, 1990; Kram and Dawson, 1998).

The kinematic changes that occur with moving on different grades of incline may provide insight into the associated metabolic cost. The cost of locomotion is dictated by the force that must be generated during the stance phase to support and accelerate the CoM, coupled with the time available for generation of this force (Kram and Taylor, 1990). Given the link between the two, it is surprising that the energetics and kinematics of incline locomotion are rarely assessed in a single study or species. Data from turkeys (*Meleagris gallopavo*), horses (*Equus caballus*), humans and invertebrates on a range of incline grades and speeds reveal constancy in the duration of stance time (t_{stance}) (Full and Tullis, 1990; Gabaldón et al., 2008; Hoyt et al., 2000; Lay et al., 2006). These data suggest that the increased cost of incline locomotion can therefore be attributed solely to the increased positive work performed by stance phase muscles in raising the CoM (Gabaldón et al., 2004; Gottschall and Kram, 2006; Minetti et al., 1993; Roberts et al., 1997; Rubenson et al., 2006). The differences between different species and the lack of an observable pattern in metabolic cost may therefore be attributable to factors influencing the efficiency with which different animals perform positive work against gravity during locomotion. These could be morphological features already thought to influence the efficiency of level locomotion, such as limb number, length and posture, as well as the differing gaits used by organisms (Biewener, 1989; Nudds et al., 2009; Reilly et al., 2007). Additionally, physiological features such as muscular efficiency could influence incline performance, with the muscles of some species being 'tuned' towards certain locomotor strategies (Farley and Emshwiller, 1996). Such factors will vary depending on the level of locomotor specialisation of an organism to moving on level *versus* inclined terrain.

In order to identify what is influencing the cost of incline locomotion, it is necessary to eliminate these confounding factors. One approach has been to use geometrically similar species of differing M_b . Such studies have used two species of quail (*Coturnix chinensis* and *Oreortyx pictus*), rodents (*Mus musculus domesticus* and *Rattus norvegicus*) and crabs (*Ocypode quadrata*) of different M_b and have found the cost of lifting to decrease with M_b up to a critical mass of between 200 g and 1 kg, beyond which it remains constant (Snyder and Carello, 2008; Tullis and Andrus, 2011). Despite some agreement in the findings of these studies, the effects of different grades of incline or locomotor speeds upon the cost of lifting are still unclear. There is also a paucity of data and species diversity among organisms weighing less than 1 kg that have been examined.

Another approach when looking at the cost of incline locomotion is to add artificial loads to the subject and observe the metabolic consequences of increased positive work whilst walking on inclines.

To date, this has only been studied in humans. Borghols et al. (Borghols et al., 1978) found no effect of loads of up to 30 kg (around 40% of M_b) upon the efficiency of incline walking (Borghols et al., 1978). Similarly, Goldman and Iampietro found that at a given speed and grade, adding loads of up to 30 kg had no effect on the mass-specific cost of locomotion (Goldman and Iampietro, 1962). In addition to providing insights into the relative cost of positive work during locomotion on slopes, the influence of mass gain upon the metabolic cost of incline locomotion may be relevant to species that undergo large seasonal variations in M_b . In particular, many avian species seasonally acquire large fat reserves to fuel migration and see them through periods of food scarcity. During such periods, energy conservation may be critical to survival; however, no data currently exists regarding the effect of the impacts of mass changes upon the cost of incline locomotion in birds.

The Svalbard rock ptarmigan (*Lagopus muta hyperborea* Sundevall 1845) is an ideal species in which to study the metabolic cost associated with incline locomotion. This terrestrial phasianid inhabits steep, rocky hills on the archipelago of Svalbard and is well adapted to life in the high Arctic (Lees et al., 2010; Lees et al., 2012a; Nudds et al., 2011; Stokkan, 1992). In particular, the acquisition of fat reserves in winter means that these birds experience a seasonal doubling of M_b , from approximately 500 g between March and August to approximately 900 g in mid-November. At this time, fat content comprises as much as 32% of M_b (Grammeltvedt and Steen, 1978; Mortensen et al., 1983; Stokkan et al., 1986). It has previously been found that Svalbard ptarmigan have adaptations for locomotor efficiency in both summer (Nudds et al., 2011) and winter (Lees et al., 2010), and when young (Lees et al., 2012b); however, it is unknown how the cost of incline locomotion is affected by the dramatic seasonal changes in M_b . Because of their difference in mass, the work that the birds must do against gravity will differ whilst the birds are geometrically identical. Measuring the efficiency with which they perform this work and the associated kinematic parameters will therefore provide novel insights into the influence of M_b upon the cost of incline locomotion. In addition, the use of multiple grades of incline will clarify the relationship between inclination angle and metabolic cost.

In light of previous findings in humans, we hypothesise that in the absence of any kinematic differences, the mass-specific cost of lifting will be dictated solely by the ability to do positive work and will remain constant during both seasons despite the variation in M_b .

MATERIALS AND METHODS

Animals

Adult male Svalbard rock ptarmigan were housed at the Department of Arctic and Marine Biology, University of Tromsø, Norway [these birds were previously used in research conducted by our group (Lees et al., 2010; Lees et al., 2012a; Lees et al., 2012b; Nudds et al., 2011)]. Birds were housed in wooden cages with wire mesh floors and had *ad libitum* access to food and water. Temperature and light conditions ensured that birds underwent their natural seasonal physiological changes. Summer trials all took place in July, with level walking trials during 2009 ($N=6$, mean \pm s.e.m. $M_b=485.8\pm 21.3$ g) and incline walking trials during 2010 ($N=5$, $M_b=528.1\pm 11.6$ g). Winter trials all took place during November, with level trials during 2009 ($N=7$, $M_b=733\pm 14.7$ g) and incline trials during 2010 ($N=7$, $M_b=917.1\pm 31.0$ g). Prior to the collection of incline data, level data were first obtained and compared with previous results in order to confirm accuracy and comparability of data to previous work and between seasons. All experimental procedures were covered by a UK Home Office project licence (40/3001) held by J.C. and performed

under ethical approval of the National Animal Research Authority of Norway (permit number 1333/2008).

Energetics and kinematics

The rates of O₂ consumption (\dot{V}_{O_2} ; ml min⁻¹ kg⁻¹) and CO₂ production (\dot{V}_{CO_2} ; ml min⁻¹ kg⁻¹) were measured using an open-flow respirometry system. The system consisted of a Perspex box (30×26×61.7 cm) mounted onto a treadmill (Bremshy Trail Sport, Almere, The Netherlands). Air was pulled through the box at ~52 l min⁻¹ during all trials. The flow was then sub-sampled into a carboy at 6 l min⁻¹ and further sub-sampled at 0.115 l min⁻¹ for gas analysis. Water vapour pressure (P_{WV}) and relative humidity measurements were recorded using an RH300 (Sable Systems International, Las Vegas, NV, USA). Water was then scrubbed using calcium chloride (2–6 mm granular; Merck, Darmstadt, Germany). The sample was then drawn through a Foxbox-C (Sable Systems International). CO₂ was recorded before being scrubbed using SodaLime with indicator (2–5 mm granular; Sigma-Aldrich, Steinheim, Germany), and finally O₂ was measured. Prior to calculation of rates of gas exchange, a corrected flow (F_c) was calculated from the primary flow rate (F), taking into account measured barometric pressure (P_B) and P_{WV} values, using Eqn 1 [eqn 8.6 in Lighton (Lighton, 2008)]:

$$F_c = \frac{F(P_B - P_{WV})}{P_B} \quad (1)$$

Rates of gas exchange for O₂ and CO₂ were calculated using Eqns 2 and 3, respectively (Lighton, 2008)

$$\dot{V}_{O_2} = \frac{F_c(\Delta O_2)}{(1 - 0.2095)} \quad (2)$$

$$\dot{V}_{CO_2} = \frac{[F_c(\Delta CO_2) - 0.0004(\dot{V}_{O_2})]}{(1 - 0.0004)} \quad (3)$$

where ΔO_2 and ΔCO_2 are the differences between excurrent and background O₂ and CO₂ concentrations, respectively. The respiratory quotients (RQ) of exercising birds were then calculated as $\dot{V}_{CO_2}:\dot{V}_{O_2}$ and used to calculate the rate of energy metabolism (W) (Brody, 1945). These values were then used to calculate mass-specific metabolic rate (P_{met} ; W kg⁻¹) during locomotion at different speeds (representing the cost of locomotion). The accuracy of the respirometry setup ($\pm 2\%$ across all treadmill speeds) was validated using a N₂ dilution test (Fedak et al., 1981), as previously described (Nudds et al., 2011; Tickle et al., 2010).

Three walking speeds were examined in the present study, as previous research over a wider range of speeds had indicated that there was a linear increase in P_{met} across the walking gait (Lees et al., 2010; Lees et al., 2012a; Lees et al., 2012b; Nudds et al., 2011). Therefore, we used representative speeds over this range of 0.22, 0.5 and 0.75 ms⁻¹ on the level and at inclines of 4.2 and 7.4 deg. These inclines represented the 6 and 12% incline settings on our treadmill (which when measured with a clinometer were 7.3 and 12.9%). Trials consisted of first placing birds into the respirometry chamber and monitoring the trace until it remained stable for at least 2 min. Birds were then run at a randomized speed until the oxygen trace plateaued (changed by no more than $\pm 0.001\%$) for no less than 2 min, indicating a stable value from which to determine \dot{V}_{O_2} . Birds were rested for 5–10 min between trials until a steady resting trace was obtained. Room temperature during all trials ranged between 18 and 20.5°C during summer trials and between 9 and 14.5°C during winter trials, which is within the seasonally appropriate thermoneutral range of the birds (Lees et al., 2010).

In order to determine values of kinematic parameters, high-speed video was taken of the walking birds using a Sony Handycam HDR-XR520 (Sony, Tokyo, Japan) at a frame rate of 100 Hz. Birds were filmed from a lateral view and footfall events were quantified using tracker.exe software versions 2.6 and 3.1 (Open Source Physics, www.opensourcephysics.org) for level and incline trials, respectively. The left foot was tracked over five to 10 strides during which birds were maintaining a stable treadmill position, and duty factor (DF), f_{stride} , stride length (l_{stride}), stance time (t_{stance}) and swing time (t_{swing}) were calculated.

In order to calculate the efficiency of incline locomotion, it was first necessary to calculate the metabolic cost of lifting as:

$$\frac{MCoT_{in} - MCoT_h}{\sin \theta} \quad (4)$$

where θ is the inclination angle (deg) and $MCoT_{in}$ and $MCoT_h$ are the minimum cost of transport during incline and horizontal locomotion, respectively (J kg⁻¹ m⁻¹). $MCoT$ values were taken as the lowest values in a plot of total cost of transport (J kg⁻¹ m⁻¹, calculated by dividing P_{met} by U) versus U (Tullis and Andrus, 2011). Efficiency could then be calculated as the mechanical work in lifting 1 kg by 1 m, divided by the metabolic cost of lifting.

Statistical analyses

Within seasons, we tested for differences in the slopes and intercepts of the regressions relating energetic and kinematic variables and U on the different inclines using analysis of covariance (ANCOVA). When slopes were found to be similar, ANCOVAs were repeated, omitting the interaction term (incline× U), and a Tukey’s *post hoc* test was used to establish individual differences in the intercepts of the common slopes. In order to test for differences in the $MCoT$, ANOVAs were performed on values at 0.75 ms⁻¹ (consistently representing the speed at which the lowest $MCoT$ values were obtained) during horizontal and incline treatments. All means are displayed as means \pm s.e.m. ANCOVAs were carried out using the statistics toolbox in MATLAB (R2007b, The MathWorks, Natick, MA, USA) and ANOVAs were conducted in SPSS (version 19.0.0, IBM, Armonk, NY, USA).

RESULTS

Energetics

Summer birds

P_{met} increased linearly with speed U (ms⁻¹) during walking on the level and on inclines of 4.2 and 7.4 deg (Fig. 1A). ANCOVA showed no difference in the slopes of these lines (representing the $MCoT$), indicating a uniform incremental energetic response to changes in U on differing gradients (incline× U , $F_{2,3}=2.66$, $r^2=0.05$, $P=0.22$). Subsequent analysis of the intercepts of the lines relating P_{met} and U based upon their common slope showed a significant effect of incline (ANCOVA; incline, $F_{2,5}=13.72$, $r^2=0.43$, $P=0.0093$; U , $F_{1,5}=31.42$, $r^2=0.49$, $P=0.0025$). A *post hoc* test showed that P_{met} was on average 5.3 W kg⁻¹ higher during 4.2 deg than on the level (95% CI=[1.95, 8.6]) and 3.4 W kg⁻¹ higher at 7.4 deg (95% CI=[0.11, 6.76]). Intercepts of 4.2 and 7.4 deg were not significantly different from each other (mean difference=1.84 W kg⁻¹, 95% CI=[-1.49, 5.17]). Together, these data indicate that although P_{met} was elevated during graded walking, this elevated value was independent of the degree of incline.

Winter birds

As in summer birds, P_{met} increased linearly with U during level, 4.2 and 7.4 deg treatments in winter birds (Fig. 1B). Again there

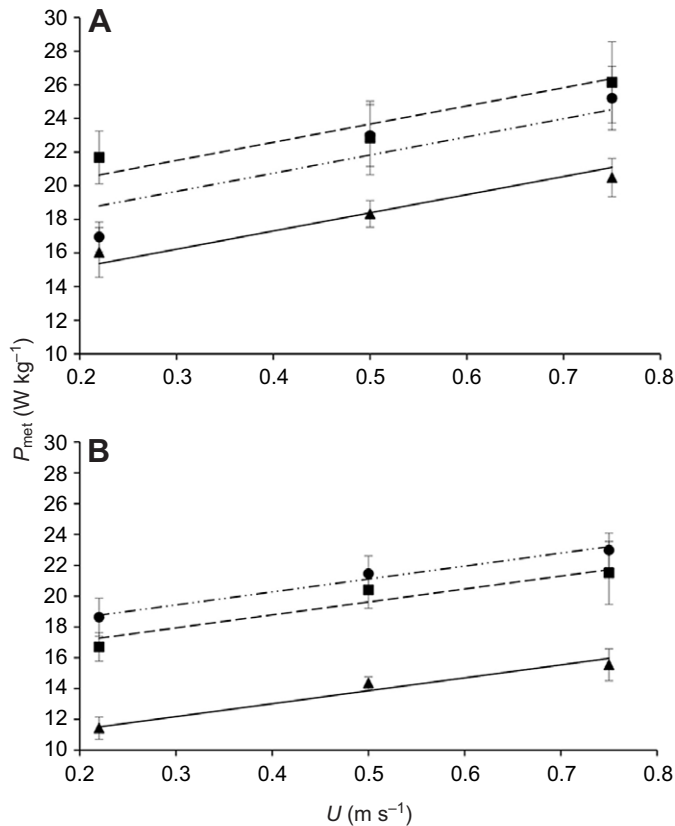


Fig. 1. Mass-specific metabolic power consumption (P_{met}) in Svalbard ptarmigan plotted against walking speed (U) on the level (closed triangles, solid lines) and at inclines of 4.2 deg (closed squares, dashed lines) and 7.4 deg (closed circles, dotted and dashed lines). During both summer (A) and winter (B), P_{met} was elevated when walking on an incline; however, the effects of 4.2 and 7.4 deg inclines were not significantly different.

was no significant difference in the slope of this relationship with differing inclines (ANCOVA; $\text{incline} \times U$, $F_{2,3}=0.13$, $r^2=0.0011$, $P=0.88$). The intercepts of the parallel lines obtained from the ANCOVA were different (incline, $F_{2,5}=137.32$, $r^2=0.74$, $P<0.001$; U , $F_{1,5}=92.93$, $r^2=0.25$, $P<0.001$). A *post hoc* test showed P_{met} to be $5.8 W kg^{-1}$ (95% CI=[4.26, 7.26]) and $7.3 W kg^{-1}$ (95% CI=[5.75, 8.75]) higher at 4.2 and 7.4 deg, respectively, than on the level. The effects of the two inclines were not significantly different (mean difference= $1.5 W kg^{-1}$, 95% CI=[-2.99, 0.014]).

Seasonal comparison

The mean relative increase in P_{met} across all speeds at an incline of 4.2 deg was $5.28 \pm 0.38 W kg^{-1}$ during summer and $5.76 \pm 0.25 W kg^{-1}$ during winter (see supplementary material Table S1). This increase was not significantly different between seasons (independent samples *t*-test; $t=-1.06$, d.f.=4, $P=0.347$). At an incline of 7.4 deg, this relative increase in P_{met} was $3.44 \pm 1.26 W kg^{-1}$ during summer and $7.25 \pm 0.1 W kg^{-1}$ during winter. These values were not different between seasons (independent samples *t*-test; $t=-3.03$, d.f.=2.03, $P=0.093$).

The cost of lifting

It has previously been found that winter birds exhibit a reduced metabolic cost of locomotion (Lees et al., 2010). As a result, absolute values of P_{met} cannot be used for comparison between the seasons. However, this problem can be overcome by comparing birds based

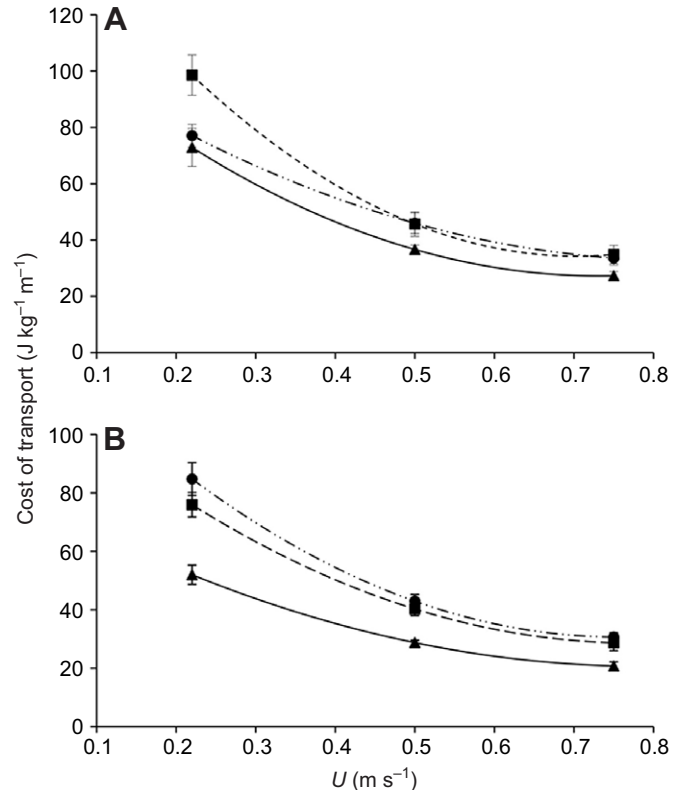


Fig. 2. Total cost of transport in Svalbard ptarmigan plotted against walking speed (U) on the level (closed triangles, solid lines) and at inclines of 4.2 deg (closed squares, dashed lines) and 7.4 deg (closed circles, dotted and dashed lines) during the summer (A) and winter (B). The minimum cost of transport, used to calculate the cost of lifting, occurred at the highest speed ($0.75 m s^{-1}$) in all cases.

on their cost of lifting, as this parameter represents the cost of walking on an incline relative to the cost of level locomotion. Traditionally, differences in the slopes of the regression lines relating metabolic cost and U (representing the MCoT) are used to calculate the cost of lifting (Eqn 4). As a result of the lack of a difference in these slopes upon the different treatments, MCoT values were instead obtained by plotting the total cost of transport (P_{met}/U) against U under the various treatments (Fig. 2). During summer, the average total MCoT_{in} was not significantly different from MCoT_h on inclines of 4.2 or 7.4 deg (ANOVA; incline , $F_{2,15}=2.95$, $P=0.09$; Fig. 2A). Using these values to calculate the cost of lifting (Tullis and Andrus, 2011) gave a cost of 103.1 and $48.9 J kg^{-1} m_v^{-1}$ on inclines of 4.2 and 7.4 deg, respectively, representing efficiencies of 9.5 and 20.1%. During winter, MCoT_{in} was significantly affected by incline (ANOVA; incline , $F_{2,15}=8.06$, $P<0.01$ Fig. 2B); a *post hoc* test showed MCoT to be $8 J kg^{-1} m^{-1}$ (95% CI=[0.63, 15.26]) and $10 J kg^{-1} m^{-1}$ (95% CI=[2.6, 17.23]) higher on inclines of 4.2 and 7.4 deg, respectively. The cost of lifting was calculated as 108.5 and $77 J kg^{-1} m_v^{-1}$ at 4.2 and 7.4 deg, representing efficiencies of 9 and 12.7%, respectively.

Kinematics

Summer birds

DF decreased linearly with U under all treatments, and the slope of this relationship was not significantly affected by treatment (ANCOVA; $\text{incline} \times U$, $F=0.75$, $r^2<0.01$, $P=0.55$; Fig. 3A). In a

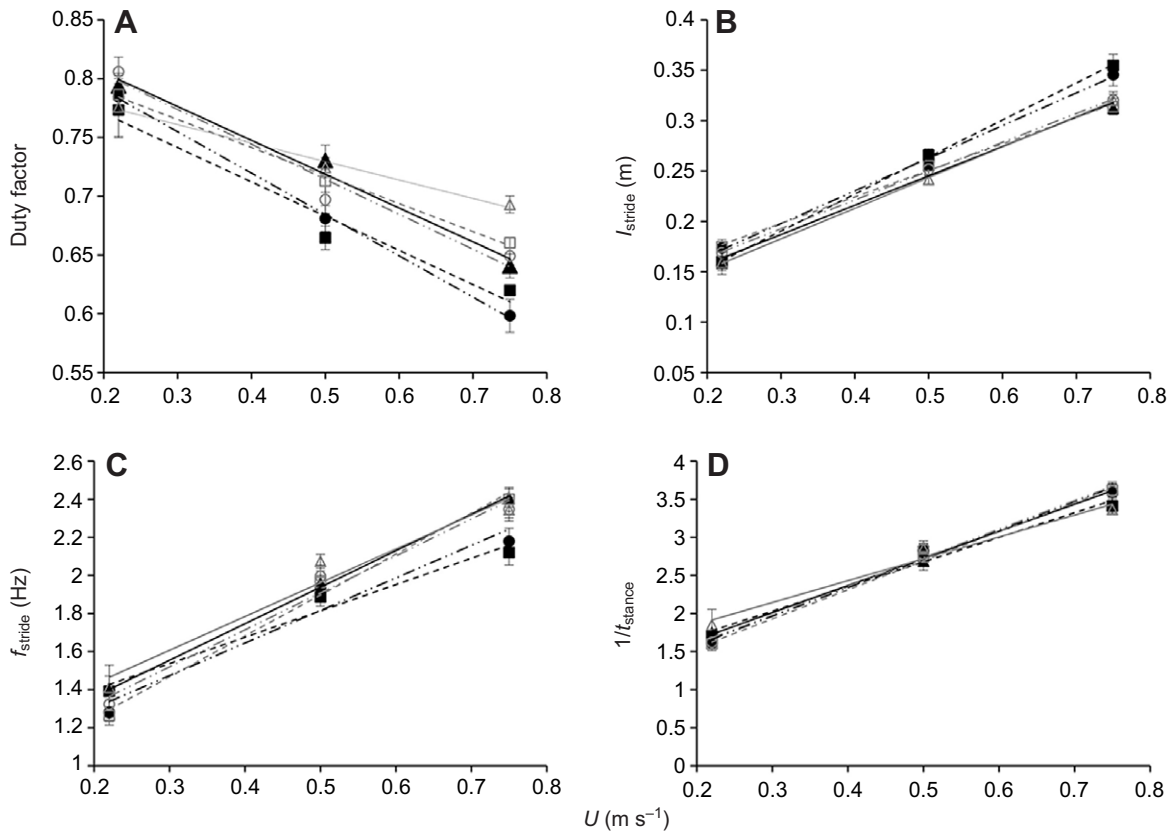


Fig. 3. Gait kinematic parameters – duty factor (A), stride length (l_{stride} ; B), stride frequency (f_{stride} ; C) and the inverse of contact time ($1/t_{\text{stance}}$; D) – in Svalbard ptarmigan plotted against walking speed (U) on the level (triangles, solid lines) and at inclines of 4.2 deg (squares, dashed lines) and 7.4 deg (circles, dotted and dashed lines) during the winter (grey, open symbols) and summer (black, closed symbols). There was no significant affect of incline upon kinematic parameters with the exception of a small elevation in the duration of the swing phase on both inclines during the summer.

simplified ANCOVA comparing intercepts based upon their common slope (-0.3), DF was not significantly affected by incline (incline, $F_{2,5}=5.48$, $r^2=0.054$, $P=0.055$; U , $F_{1,5}=184.88$, $r^2=0.92$, $P<0.001$).

l_{stride} increased linearly with U with a common slope (0.33) under all treatments (ANCOVA; incline $\times U$, $F_{2,3}=3.026$, $r^2<0.01$, $P=0.19$; Fig. 3B). Parallel lines based upon this common slope did not differ in their intercepts (ANCOVA; incline, $F_{2,5}=2.51$, $r^2=0.013$, $P=0.18$; U , $F_{1,5}=369.74$, $r^2=0.97$, $P<0.001$).

f_{stride} increased linearly with U under all treatments (level and incline) and the slope of this relationship was independent of incline (ANCOVA; incline $\times U$, $F_{2,3}=1.006$, $r^2=0.016$, $P=0.46$; Fig. 3C). f_{stride} was not significantly elevated during any of the treatments when comparing intercepts based upon these common slopes (1.67) (ANCOVA; incline, $F_{2,5}=1.4$, $r^2=0.023$, $P=0.33$; U , $F_{1,5}=114.74$, $r^2=0.94$, $P<0.001$).

t_{swing} remained constant across U and this relationship was not significantly affected by incline (ANCOVA; incline $\times U$, $F_{2,3}=0.54$, $r^2=0.021$, $P=0.63$). The intercepts of the lines based upon their common slope (0.019) were significantly different (ANCOVA; incline, $F_{2,5}=25.48$, $r^2=0.84$, $P<0.01$; U , $F_{1,5}=4.74$, $r^2=0.077$, $P=0.081$). Tukey's *post hoc* tests revealed that t_{swing} was elevated at inclines of both 4.2 deg (mean difference= 0.031 , 95% CI=[0.016 , 0.046]) and 7.4 deg (mean difference= 0.026 , 95% CI=[0.011 , 0.041]).

t_{stance} decreased curvilinearly with U and was unaffected by treatment. The inverse of this contact time ($1/t_{\text{stance}}$) increased linearly with U and the slope of this relationship was not significantly

affected by incline (ANCOVA; incline $\times U$, $F_{2,3}=0.6$, $r^2<0.01$, $P=0.6$; Fig. 3D). Comparing the intercepts of the common slopes (3.51) showed no significant effect of incline (ANCOVA; incline, $F_{2,5}=0.11$, $r^2<0.001$, $P=0.9$; U , $F_{1,5}=378.73$, $r^2=0.99$, $P<0.001$).

Together, these data indicate that during the summer, with the exception of small elevations in the duration of their swing phase, there was no significant effect of incline upon the relationships between the kinematic parameters measured and U .

Winter birds

DF decreased linearly with U in winter birds with a common slope (-0.23) among gradients (ANCOVA; incline $\times U$, $F_{2,3}=4.03$, $r^2=0.057$, $P=0.14$; Fig. 3A). Comparing the intercepts of these common slopes revealed no effect of incline upon DF (ANCOVA; incline, $F_{2,5}=0.42$, $r^2=0.013$, $P=0.68$; U , $F_{1,5}=58.63$, $r^2=0.91$, $P<0.001$).

l_{stride} increased linearly with U both on the level and on inclines with a common slope (0.28) (ANCOVA; incline $\times U$, $F_{2,3}=4.23$, $r^2<0.01$, $P=0.13$; Fig. 3B). Comparing the intercepts of these common slopes revealed no effect of treatment (ANCOVA; incline, $F_{2,5}=1.58$, $r^2<0.01$, $P=0.29$; U , $F_{1,5}=1062.4$, $r^2=0.99$, $P<0.001$).

f_{stride} increased linearly with U with the same slope regardless of incline treatment (ANCOVA; incline $\times U$, $F_{2,3}=0.402$, $r^2<0.01$, $P=0.7$; Fig. 3C). A simplified ANCOVA comparing the intercepts based on this common slope (1.96) found no effect of incline upon f_{stride} (incline, $F_{2,5}=0.42$, $r^2<0.01$, $P=0.68$; U , $F_{1,5}=171.25$, $r^2=0.97$, $P<0.001$).

t_{swing} was unaffected by U or treatment (ANCOVA; $\text{incline} \times U$, $F_{2,3}=5.31$, $r^2=0.33$, $P=0.103$). Similarly, the intercepts of the common slopes (-0.017) were not significantly different (ANCOVA; incline , $F_{2,5}=2.37$, $r^2=0.4$, $P=0.19$; U , $F_{1,5}=2.09$, $r^2=0.18$, $P=0.208$).

t_{stance} decreased curvilinearly with U and this relationship was similar between incline treatments. The slope of the relationship between $1/t_{\text{stance}}$ and U was therefore similar regardless of incline (ANCOVA; $\text{incline} \times U$, $F_{2,3}=2.204$, $r^2=0.015$, $P=0.26$; Fig. 3D), as were the intercepts of the common slopes (ANCOVA; incline , $F_{2,5}=0.056$, $r^2<0.001$, $P=0.95$; U , $F_{1,3}=194.07$, $r^2=0.97$, $P<0.001$).

Together, these data indicate that during winter there was no significant effect of incline upon the relationships between U and the kinematic parameters measured.

DISCUSSION

The present data are the first to reveal the cost associated with incline locomotion in a single species exhibiting large natural seasonal M_b fluctuations. In agreement with our initial hypothesis, the relative increase in P_{met} as a result of incline locomotion was similar during both seasons, despite winter birds exhibiting a 300 g (60%) increase in M_b . The cost of lifting data yielded slightly different results. At a 4.2 deg incline, winter birds demonstrate a $5.4 \text{ J kg}^{-1} \text{ m}_v^{-1}$ higher cost of lifting than during the summer and a $28.1 \text{ J kg}^{-1} \text{ m}_v^{-1}$ increase at an incline of 7.4 deg (Fig. 4). Such values are large compared with those in humans on similar gradients, which show no difference in the cost of lifting with artificial loads of up to 57% of M_b (Borghols et al., 1978; Haisman, 1988). It must be noted, however, that comparisons with human data are difficult as a result of non-linearity in the relationship between energy consumption and U . The observed differences in the cost of lifting are in fact small in comparison to those found in geometrically similar species of different mass. For example, button and mountain quail differing in M_b by 168 g exhibited a $45 \text{ J kg}^{-1} \text{ m}_v^{-1}$ difference in the cost of lifting on a 10 deg incline, with the smaller species entailing the larger cost (Snyder and Carello, 2008). Similarly, our largest difference of $28.1 \text{ J kg}^{-1} \text{ m}_v^{-1}$ is within the variation found within single species as a result of changing grade. An avian example is that of quail, which undergo a $42 \text{ J kg}^{-1} \text{ m}_v^{-1}$ reduction in the cost of lifting when moving from a 4 deg to an 8 deg incline. Such findings are in keeping with current theories that the cost of lifting (within the same degree of incline and at the M_b range used) is in fact constant, regardless of M_b , and therefore solely as a result of the positive work that must be performed in raising the CoM against gravity (Snyder and Carello, 2008; Taylor et al., 1972; Tullis and Andrus, 2011). Any differences observed in this cost are therefore a result of differences in the efficiency with which this work is performed. In ptarmigan, it appears that despite a large gain in M_b , winter birds are able to perform this work as efficiently as during summer. However, even our lowest value for the cost of lifting of $48.9 \text{ J kg}^{-1} \text{ m}_v^{-1}$ is higher than previously predicted values of $15.5 \text{ J kg}^{-1} \text{ m}_v^{-1}$ (Taylor et al., 1972) and $27 \text{ J kg}^{-1} \text{ m}_v^{-1}$ (Cohen et al., 1978; Snyder and Carello, 2008), and is apparently high in comparison with much of the existing data set shown in Fig. 4. It seems unlikely that Svalbard ptarmigan are inefficient at incline locomotion compared with the species used to obtain these values given the predominantly sloping terrains that these birds inhabit. An alternative explanation may lie in methodological differences in calculating the cost of lifting. The only other study to use measured MCoT values to calculate the cost of lifting also found a higher value ($59.4 \text{ J kg}^{-1} \text{ m}_v^{-1}$) than those previously reported (Tullis and Andrus, 2011). The authors also found that reprocessing the data from the literature and determining

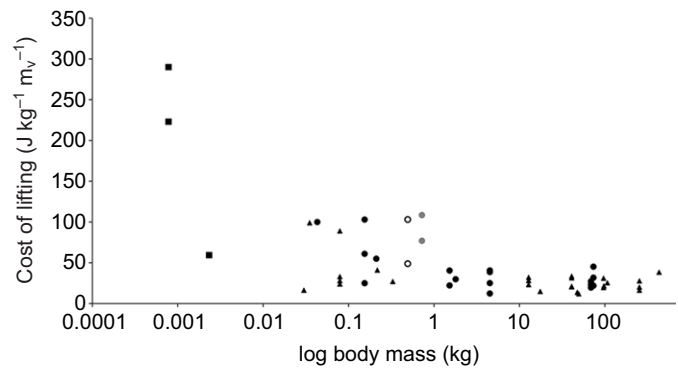


Fig. 4. The cost of lifting ($\text{J kg}^{-1} \text{ m}_v^{-1}$) plotted against log body mass (kg) for a variety of bipedal (circles), quadrupedal (triangles) and polypedal (squares) species and incline grades, ranging from 1.15 to 90 deg. The present data for Svalbard ptarmigan are shown during summer (open circles) and winter (grey circles). Multiple data points at a single body mass represent the use of multiple grades in a single study. Data were taken from the literature for the following species: burro, *Equus asinus* (Yousef et al., 1972); caribou, *Rangifer tarandus granti* (Fancy and White, 1987); chimpanzee, *Pan troglodytes* (Taylor et al., 1972); cockroach, *Periplaneta americana* (Full and Tullis, 1990); crab, *Ocyropsis quadrata* (Tullis and Andrus, 2011); dog, *Canis familiaris* (Raab et al., 1976); elk, *Cervus canadensis nelsoni* (Cohen et al., 1978); goose, *Branta leucopsis* (Nudds and Codd, 2012); guinea fowl, *Numida meleagris* (Ellerby et al., 2003); horse, *Equus caballus* (Wickler et al., 2000); human, *Homo sapiens* (Margaria et al., 1963; Snyder and Farley, 2011; Yousef et al., 1972); kangaroo, *Macropus rufus* (Kram and Dawson, 1998); mouse, *Mus musculus* (Taylor et al., 1972; Snyder and Carello, 2008); button quail, *Coturnix chinensis* (Snyder and Carello, 2008); mountain quail, *Oreortyx pictus* (Snyder and Carello, 2008); quail, *Coturnix coturnix* (Warncke et al., 1988); rat, *Rattus norvegicus* (Snyder and Carello, 2008; Armstrong et al., 1983); reindeer, *Rangifer tarandus groenlandicus* (White and Yousef, 1978); sheep, *Orvis aries* (Clapperton, 1964); squirrel, *Tamiasciurus hudsonicus* (Wunder and Morrison, 1974); and stork, *Leptoptilos crumeniferous* (Bamford and Maloio, 1980).

the cost of lifting from actual MCoT values gave more similar results to those found presently. Indeed, our values of between 48.9 and $108.5 \text{ J kg}^{-1} \text{ m}_v^{-1}$ are in keeping with their calculated average of $103 \text{ J kg}^{-1} \text{ m}_v^{-1}$ for vertebrates.

During the winter, Svalbard ptarmigan demonstrate a reduced cost of locomotion (Lees et al., 2010). One of the suggested mechanisms for this was improved elastic storage in the tendons. However, the present data suggest that elastic storage and recovery are unlikely to be a mechanism by which winter birds economically carry their increased mass. Indeed, if improved elastic savings contribute to this reduced cost during winter, we may have expected to observe improved incline efficiency in winter birds (assuming elastic mechanisms operate on moderate inclines) (Snyder et al., 2012). The current findings also preclude the possibility of improved efficiency of muscular work as an explanation of the increased winter economy of locomotion, as this appears constant during both seasons. However, the findings do suggest that, at least at low grades of incline, it may be more economical for ptarmigan to walk up relatively steeper slopes to achieve a given vertical distance. Whether this influences their behaviour in the wild is yet to be ascertained.

When attempting to examine the scaling relationship between the cost of incline locomotion and M_b , data are often taken from a broad range of incline grades (2 to >90 deg) (Clapperton, 1964; Full and Tullis, 1990). Using data from different grades assumes a consistent relationship between the level of incline and the associated

metabolic cost across species of differing size, geometry and gait. In reality, however, this is not the case, as illustrated in Fig. 4. In the present study, despite exhibiting a similar efficiency at the same incline treatments, birds demonstrated improved efficiency with increasing incline in both seasons. Improved efficiency at higher inclines has also been demonstrated in quail, which show a near doubling of efficiency with a doubling of slope (Warncke et al., 1988). Amongst non-avian bipeds, humans have also demonstrated improved efficiency of walking up to an incline of approximately 11 deg (the point at which positive work alone contributes to metabolic cost) (Margaria, 1938; Margaria, 1976). In contrast, guinea fowl have reduced efficiency with increasing incline (Ellerby et al., 2003), as do storks on inclines of 5 to 9 deg, but not 11 deg (Bamford and Maloij, 1980). The nature of this relationship among quadrupeds is also unclear. Efficiency has been shown to increase (Raab et al., 1976; Wunder and Morrison, 1974), decrease (White and Yousef, 1978) and remain unchanged (Cohen et al., 1978; Kram and Dawson, 1998) with increases in grade. Many other studies of incline locomotion only use single grades, further hampering any comparisons that can be made. Furthermore, those that do test multiple inclines are often not comparable due to the differences in the grades used between studies. This is possibly due to limitations imposed by restricted incline grades on treadmills or inaccuracies in the reading of slope, as was found in the present study. These factors preclude the establishment of a general relationship, as the efficiency of locomotion appears to vary with grade and species. This may be particularly problematic if, as has been found in humans, efficiency is most variable at the lowest incline grades (less than 11 deg) that are almost exclusively used for comparisons (Margaria, 1938) or if extreme grades are used for comparison (Full and Tullis, 1990). Clearly, expansion of the data set is needed to resolve the nature of this relationship and care must be taken when formulating scaling relationships using data from differing incline treatments.

The mechanisms underlying the improved efficiency of walking at higher inclines in the ptarmigan and some other species are unclear. In the present study the answer appears to lie in the fact that P_{met} undergoes a step increase upon incline walking that doesn't change between 4.2 and 7.4 deg. The result is a reduced cost of lifting with incline grade and therefore increased efficiency with slope. The cost of terrestrial locomotion is linked to the time available to generate force during stance in addition to factors influencing the efficiency of force generation, such as the dynamics of muscular contraction and the mechanical advantage of the muscles as a result of posture (Kram and Taylor, 1990). Presently, in agreement with previous data, no differences in t_{stance} were found, suggesting that these alternative factors may explain the observed pattern of cost on different incline grades. During level locomotion in birds, force is primarily generated by economical, isometric contractions of the stance-phase muscles in order to support and accelerate the body during stance (Daley and Biewener, 2003; Gabaldón et al., 2004; Roberts et al., 1997). During incline locomotion, however, there is an increase in positive work to lift the CoM (Gabaldón et al., 2004; Roberts et al., 1997). This increase is met through modulations in the contractile behaviour of the proximal and distal stance muscles, primarily towards shortening contractions, which are metabolically more costly. Significant differences in the underlying contractile behaviour of leg muscles has been demonstrated in turkeys, which, despite exhibiting no change in t_{stance} on inclines of 6 and 12 deg, show significant differences in the underlying contractile behaviour of their leg muscles, linked to the requirement of net positive work and

increased muscle strain (Gabaldón et al., 2004; Gabaldón et al., 2008; Roberts et al., 2007; Roberts et al., 1997). Such inefficient shortening contractions required three times the volume of muscle to generate the same amount of force as on the level (Roberts et al., 1997). Similar findings have been observed in guinea fowl, which exhibit significant increases in blood flow (and therefore energy consumption) to stance-phase muscles during incline running (Rubenson et al., 2006). In the present study, it is possible that the changes in muscle contractility and recruitment that occur upon the transition to walking on an incline of 4.2 deg entail a significant metabolic cost. This cost may, however, be large compared with the additional cost of positive work when moving from an incline of 4.2 deg to one of 7.4 deg. Such an observation may explain the present finding that P_{met} is not significantly different when comparing the two incline treatments tested. In addition to the elevated incline cost imposed by changes in muscle contractile behaviour, there may be a reduction in the force-generating capacity of the locomotor muscles as a result of altered limb posture (Higham and Biewener, 2008). Such changes have been observed in a number of quadrupedal species (Carlson-Kuhta et al., 1998; Lammers et al., 2006; Lee, 2011; Vilensky et al., 1994). Although posture was not quantified in the present study, the intercepts of the lines relating P_{met} to U have been postulated to represent a 'postural cost' separate from the cost of moving and that of resting metabolism. If this is the case, then the finding that our P_{met} regressions share a common, elevated intercept on both inclines in both seasons may suggest an elevated postural cost, which is unaffected by the level of incline. This, in turn, may also contribute to the apparent step change in the cost of incline locomotion, which is independent of grade at the inclines tested. Indeed, more crouched postures have been shown to be associated with incline locomotion in both quadrupeds (Carlson-Kuhta et al., 1998) and bipeds (Daley and Biewener, 2003; Lay et al., 2006; Leroux et al., 2002), and may be associated with an increased metabolic cost as a result of less efficient force generation (McMahon et al., 1987).

The common incremental response of P_{met} to changing U regardless of treatment highlights a complication regarding the methodology used in the calculation of the cost of lifting. This will affect calculations of the efficiency of incline locomotion, commonly used in comparisons between species. Many previous studies have calculated the cost of lifting as the difference between the horizontal and incline regressions of \dot{V}_{O_2} and U (Armstrong et al., 1983; Bamford and Maloij, 1980; Cohen et al., 1978; Raab et al., 1976; Snyder and Carello, 2008; Wunder and Morrison, 1974). This method, however, relies on a difference between the regression slopes. In the present study, in common with findings in mice, crabs, quail and more recently geese, the MCoT did not differ upon incline locomotion in either season (Nudds and Codd, 2012; Taylor et al., 1972; Tullis and Andrus, 2011; Warncke et al., 1988). Applying the traditional approach to such data would therefore reveal no additional cost of lifting despite absolute energy expenditure values clearly being higher during incline locomotion. A more appropriate approach therefore seems to be to test for differences between actual MCoT values derived from the respirometry data. In doing so with the ptarmigan data, we find differences in the efficiency of incline locomotion that are not revealed by the slope of P_{met} versus U regression data. The reasons for the observed differences in the relationship between P_{met} and U on different inclines compared with those in previous studies, however, are unclear.

One explanation could be in the differing speed ranges reported by authors. An increase in the MCoT with incline is expected only if efficiency (mechanical work/energy expended) is constant with

changes in U (Nudds and Codd, 2012). However, this condition may not be a reality and could be affected by the changing mechanics and energetics associated with increasing U . In the present study, only walking speeds were used. Many studies looking at the relationship between metabolic cost and U on differing inclines have, however, included data spanning walking, grounded running and running speeds, although there is little reason to assume that this relationship is unaffected by gait. Non-linearity in the relationship between metabolic cost and U has been demonstrated in a number of species including birds and humans and is associated with changes in the biomechanics of locomotion during different gaits (Baudinette et al., 1992; Hoyt and Taylor, 1981; Margaria et al., 1963; Nudds et al., 2011; Rubenson et al., 2004). Walking, for example, is associated with pendular biomechanics, in which the kinetic energy of forward motion (E_{kh}) and the sum of the potential and vertical kinetic energies ($E_p + E_{kv}$) of the CoM are out of phase (Cavagna et al., 1977; Cavagna et al., 1976). Conversely, during running the mechanical energies of the CoM are in phase and spring-like mechanics dominate (Biewener, 2006; Farley et al., 1993). In addition, different gaits may be marked by abrupt changes in kinematic parameters such as f_{stride} and contact time, known to affect the cost of locomotion (Gatesy and Biewener, 1991; Heglund and Taylor, 1988). The variation observed in different species as a result of different locomotor specializations in terms of footfall, posture, joint kinematics and even functional separation between the fore- and hind-limbs (Carlson-Kuhta et al., 1998; Full and Tullis, 1990; Gabaldón et al., 2008; Hoyt et al., 2000; Lammers et al., 2006; Lee, 2011; Vilensky et al., 1994) could have a significant influence upon the energetic cost of incline locomotion both within and between gaits. This has been demonstrated in sheep, for example, in which the cost of lifting is independent of incline grade but is higher during low-speed gaits (Clapperton, 1964). Pooling of the data across gaits would have resulted in an inaccurate value for the cost of lifting which masked a more complex underlying pattern. Similar findings have been demonstrated in humans, although the curvilinear nature of metabolic cost during walking makes comparison with avian data problematic (Margaria et al., 1963). In the only avian studies to use solely walking gaits, quail on inclines of 4, 8 and 12 deg shared a common slope and geese on inclines of 7 deg showed no difference in MCoT compared with that on level ground (Nudds and Codd, 2012; Warncke et al., 1988). The results of both studies are in agreement with our findings, highlighting the importance of gait when measuring the energetic cost of locomotion.

In summary, Svalbard ptarmigan demonstrate a similar efficiency of lifting during the summer and winter while walking at inclines of 4.2 and 7.4 deg. This is despite large differences in M_b , which suggests that the cost of lifting is largely dictated by the increased positive work required to lift the CoM during graded walking. However, the efficiency with which individuals perform this work is not proportional to the degree of incline and efficiency increases with grade during both seasons. Clearly, investigations into the cost of incline locomotion should combine energetic and kinematic measurements and consider the potential impacts of gait and degree of incline upon the metabolic cost of locomotion.

LIST OF SYMBOLS AND ABBREVIATIONS

CoM	centre of mass
DF	duty factor
E_{kh}	kinetic energy of forward motion
E_{kv}	vertical kinetic energy
E_p	potential energy of the centre of mass
F	flow rate
F_c	corrected flow rate

f_{stride}	stride frequency
l_{stride}	stride length
M_b	body mass
MCoT _h	minimum cost of horizontal transport
MCoT _{in}	minimum cost of transport on an incline
m_v	vertical metre
P_B	barometric pressure
P_{met}	metabolic power consumption
P_{WV}	water vapour pressure
RQ	respiratory quotient
t_{stance}	stance duration
t_{swing}	swing duration
\dot{V}_{CO_2}	carbon dioxide production
\dot{V}_{O_2}	oxygen consumption
θ	inclination angle

ACKNOWLEDGEMENTS

We would like to thank the technicians (Magnus Folkow, Hans Lian, Hans-Arne Solvang and John Ness) at the Department of Arctic and Marine Biology, Tromsø, for assistance with experiments and animal husbandry throughout this project. We would also like to thank Anne-Marit Vik for training the birds prior to experimentation.

AUTHOR CONTRIBUTIONS

J.L. conducted the study and interpreted the findings, and drafted and revised the article. K.A.S. and L.F. were involved in execution of the study, and drafting and revising the article. J.C. was involved in conception, design and execution of the study, interpretation of the findings, and revising the article.

COMPETING INTERESTS

No competing interests declared.

FUNDING

The Biotechnology and Biological Sciences Research Council (grant G01138/1) funded this research, and J.J.L. was supported by a Natural Environment Research Council doctoral training award PhD stipend.

REFERENCES

- Armstrong, R. B., Laughlin, M. H., Rome, L. and Taylor, C. R. (1983). Metabolism of rats running up and down an incline. *J. Appl. Physiol.* **55**, 518-521.
- Bamford, O. S. and Maloiy, G. M. (1980). Energy metabolism and heart rate during treadmill exercise in the Marabou stork. *J. Appl. Physiol.* **49**, 491-496.
- Baudinette, R. V., Snyder, G. K. and Frappell, P. B. (1992). Energetic cost of locomotion in the tammar wallaby. *Am. J. Physiol.* **262**, R771-R778.
- Biewener, A. A. (1989). Scaling body support in mammals: limb posture and muscle mechanics. *Science* **245**, 45-48.
- Biewener, A. A. (2003). *Animal Locomotion*. New York: Oxford University Press.
- Biewener, A. A. (2006). Patterns of mechanical energy change in tetrapod gait: pendula, springs and work. *J. Exp. Zool. A* **305**, 899-911.
- Borghols, E. A., Dresen, M. H. and Hollander, A. P. (1978). Influence of heavy weight carrying on the cardiorespiratory system during exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* **38**, 161-169.
- Brody, S. (1945). *Bioenergetics and Growth*. New York: Reinhold.
- Carlson-Kuhta, P., Trank, T. V. and Smith, J. L. (1998). Forms of forward quadrupedal locomotion. II. A comparison of posture, hindlimb kinematics, and motor patterns for upslope and level walking. *J. Neurophysiol.* **79**, 1687-1701.
- Cavagna, G. A., Thys, H. and Zamboni, A. (1976). The sources of external work in level walking and running. *J. Physiol.* **262**, 639-657.
- Cavagna, G. A., Heglund, N. C. and Taylor, C. R. (1977). Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. *Am. J. Physiol.* **233**, R243-R261.
- Clapperton, J. L. (1964). The energy metabolism of sheep walking on the level and on gradients. *Br. J. Nutr.* **18**, 47-54.
- Cohen, Y., Robbins, C. T. and Davitt, B. B. (1978). Oxygen utilization by elk calves during horizontal and vertical locomotion compared to other species. *Comp. Biochem. Physiol.* **61A**, 43-48.
- Daley, M. A. and Biewener, A. A. (2003). Muscle force-length dynamics during level versus incline locomotion: a comparison of *in vivo* performance of two guinea fowl ankle extensors. *J. Exp. Biol.* **206**, 2941-2958.
- Dawson, T. J. and Taylor, C. R. (1973). Energetic cost of locomotion in kangaroos. *Nature* **246**, 313-314.
- Ellerby, D. J., Cleary, M., Marsh, R. L. and Buchanan, C. I. (2003). Measurement of maximum oxygen consumption in Guinea fowl *Numida meleagris* indicates that birds and mammals display a similar diversity of aerobic scopes during running. *Physiol. Biochem. Zool.* **76**, 695-703.
- Fancy, S. G. and White, R. G. (1987). Energy expenditures for locomotion by barren-ground caribou. *Can. J. Zool.* **65**, 122-128.
- Farley, C. T. and Emshwiller, M. (1996). Efficiency of uphill locomotion in nocturnal and diurnal lizards. *J. Exp. Biol.* **199**, 587-592.

- Farley, C. T., Glasheen, J. and McMahon, T. A. (1993). Running springs: speed and animal size. *J. Exp. Biol.* **185**, 71-86.
- Fedak, M. A., Rome, L. and Seeherman, H. J. (1981). One-step N₂-dilution technique for calibrating open-circuit V_{O₂} measuring systems. *J. Appl. Physiol.* **51**, 772-776.
- Full, R. J. and Tullis, A. (1990). Energetics of ascent: insects on inclines. *J. Exp. Biol.* **149**, 307-317.
- Gabaldón, A. M., Nelson, F. E. and Roberts, T. J. (2004). Mechanical function of two ankle extensors in wild turkeys: shifts from energy production to energy absorption during incline versus decline running. *J. Exp. Biol.* **207**, 2277-2288.
- Gabaldón, A. M., Nelson, F. E. and Roberts, T. J. (2008). Relative shortening velocity in locomotor muscles: turkey ankle extensors operate at low V/V(max). *Am. J. Physiol.* **294**, R200-R210.
- Gatesy, S. M. and Biewener, A. A. (1991). Bipedal locomotion: effects of speed, size and limb posture in birds and humans. *J. Zool.* **224**, 127-147.
- Goldman, R. F. and Iampietro, P. F. (1962). Energy cost of load carriage. *J. Appl. Physiol.* **17**, 675-676.
- Goldstein, D. L. (1988). Estimates of daily energy expenditure in birds: the time-energy budget as an integrator of laboratory and field studies. *Am. Zool.* **28**, 829-844.
- Goldstein, D. L. and Nagy, K. A. (1985). Resource utilization by desert quail: time and energy, food and water. *Ecology* **66**, 378-387.
- Gottschall, J. S. and Kram, R. (2006). Mechanical energy fluctuations during hill walking: the effects of slope on inverted pendulum exchange. *J. Exp. Biol.* **209**, 4895-4900.
- Grammelvedt, R. and Steen, J. B. (1978). Fat deposition in Spitzbergen ptarmigan (*Lagopus mutus hyperboreus*). *Arctic* **31**, 496-498.
- Haisman, M. F. (1988). Determinants of load carrying ability. *Appl. Ergon.* **19**, 111-121.
- Heglund, N. C. and Taylor, C. R. (1988). Speed, stride frequency and energy cost per stride: how do they change with body size and gait? *J. Exp. Biol.* **138**, 301-318.
- Higham, T. E. and Biewener, A. A. (2008). Integration within and between muscles during terrestrial locomotion: effects of incline and speed. *J. Exp. Biol.* **211**, 2303-2316.
- Hoyt, D. F. and Taylor, R. (1981). Gait and the energetics of locomotion in horses. *Nature* **292**, 239-240.
- Hoyt, D. F., Wickler, S. J. and Cogger, E. A. (2000). Time of contact and step length: the effect of limb length, running speed, load carrying and incline. *J. Exp. Biol.* **203**, 221-227.
- Kram, R. and Dawson, T. J. (1998). Energetics and biomechanics of locomotion by red kangaroos (*Macropus rufus*). *Comp. Biochem. Physiol.* **120B**, 41-49.
- Kram, R. and Taylor, C. R. (1990). Energetics of running: a new perspective. *Nature* **346**, 265-267.
- Lammers, A. R., Earls, K. D. and Biknevicius, A. R. (2006). Locomotor kinetics and kinematics on inclines and declines in the gray short-tailed opossum *Monodelphis domestica*. *J. Exp. Biol.* **209**, 4154-4166.
- Langman, V. A., Rowe, M. F., Roberts, T. J., Langman, N. V. and Taylor, C. R. (2012). Minimum cost of transport in Asian elephants: do we really need a bigger elephant? *J. Exp. Biol.* **215**, 1509-1514.
- Lay, A. N., Hass, C. J. and Gregor, R. J. (2006). The effects of sloped surfaces on locomotion: a kinematic and kinetic analysis. *J. Biomech.* **39**, 1621-1628.
- Lee, D. V. (2011). Effects of grade and mass distribution on the mechanics of trotting in dogs. *J. Exp. Biol.* **214**, 402-411.
- Lees, J. J., Nudds, R. L., Stokkan, K.-A., Folkow, L. P. and Codd, J. R. (2010). Reduced metabolic cost of locomotion in Svalbard rock ptarmigan (*Lagopus muta hyperborea*) during winter. *PLoS ONE* **5**, e15490.
- Lees, J. J., Nudds, R. L., Folkow, L. P., Stokkan, K.-A. and Codd, J. R. (2012a). Understanding sex differences in the cost of terrestrial locomotion. *Proc. Biol. Sci.* **279**, 826-832.
- Lees, J. J., Stokkan, K.-A., Folkow, L. P. and Codd, J. R. (2012b). Locomotor development in the Svalbard rock ptarmigan (*Lagopus muta hyperborea*). *Polar Biol.* **35**, 867-874.
- Leroux, A., Fung, J. and Barbeau, H. (2002). Postural adaptation to walking on inclined surfaces: I. Normal strategies. *Gait Posture* **15**, 64-74.
- Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual For Scientists*. New York: Oxford University Press.
- Luick, B. R. and White, R. G. (1986). Oxygen Consumption for locomotion by caribou calves. *J. Wildl. Manage.* **50**, 148-152.
- Margaria, R. (1938). Sulla fisiologia e specialmente sul consumo energetico della marcia e della corsa a varie velocità ed inclinazioni del terreno. *Atti. Acc. Naz. Lincei* **7**, 299-368.
- Margaria, R. (1976). *Biomechanics and Energetics of Muscular Exercise*. Oxford: Clarendon Press.
- Margaria, R., Cerretelli, P., Aghemo, P. and Sassi, G. (1963). Energy cost of running. *J. Appl. Physiol.* **18**, 367-370.
- McMahon, T. A., Valiant, G. and Frederick, E. C. (1987). Groucho running. *J. Appl. Physiol.* **62**, 2326-2337.
- Minetti, A. E., Ardigò, L. P. and Saibene, F. (1993). Mechanical determinants of gradient walking energetics in man. *J. Physiol.* **472**, 725-735.
- Mortensen, A., Unander, S., Kolstad, M. and Blix, A. S. (1983). Seasonal changes in body composition and crop content of Spitzbergen ptarmigan *Lagopus mutus hyperboreus*. *Ornis Scand.* **14**, 144-148.
- Nudds, R. L. and Codd, J. R. (2012). The metabolic cost of walking on gradients with a waddling gait. *J. Exp. Biol.* **215**, 2579-2585.
- Nudds, R. L., Codd, J. R. and Sellers, W. I. (2009). Evidence for a mass dependent step-change in the scaling of efficiency in terrestrial locomotion. *PLoS ONE* **4**, e6927.
- Nudds, R. L., Folkow, L. P., Lees, J. J., Tickle, P. G., Stokkan, K.-A. and Codd, J. R. (2011). Evidence for energy savings from aerial running in the Svalbard rock ptarmigan (*Lagopus muta hyperborea*). *Proc. Biol. Sci.* **278**, 2654-2661.
- Raab, J. L., Eng, P. and Waschler, R. A. (1976). Metabolic cost of grade running in dogs. *J. Appl. Physiol.* **41**, 532-535.
- Reilly, S. M., McElroy, E. J. and Biknevicius, A. R. (2007). Posture, gait and the ecological relevance of locomotor costs and energy-saving mechanisms in tetrapods. *Zoology* **110**, 271-289.
- Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113-1115.
- Roberts, T. J., Kram, R., Weyand, P. G. and Taylor, C. R. (1998). Energetics of bipedal running. I. Metabolic cost of generating force. *J. Exp. Biol.* **201**, 2745-2751.
- Roberts, T. J., Higginson, B. K., Nelson, F. E. and Gabaldón, A. M. (2007). Muscle strain is modulated more with running slope than speed in wild turkey knee and hip extensors. *J. Exp. Biol.* **210**, 2510-2517.
- Rubenson, J., Heliams, D. B., Lloyd, D. G. and Fournier, P. A. (2004). Gait selection in the ostrich: mechanical and metabolic characteristics of walking and running with and without an aerial phase. *Proc. Biol. Sci.* **271**, 1091-1099.
- Rubenson, J., Henry, H. T., Dimoulas, P. M. and Marsh, R. L. (2006). The cost of running uphill: linking organismal and muscle energy use in guinea fowl (*Numida meleagris*). *J. Exp. Biol.* **209**, 2395-2408.
- Snyder, G. K. and Carello, C. A. (2008). Body mass and the energy efficiency of locomotion: lessons from incline running. *Comp. Biochem. Physiol.* **150A**, 144-150.
- Snyder, K. L. and Farley, C. T. (2011). Energetically optimal stride frequency in running: the effects of incline and decline. *J. Exp. Biol.* **214**, 2089-2095.
- Snyder, K. L., Kram, R. and Gottschall, J. S. (2012). The role of elastic energy storage and recovery in downhill and uphill running. *J. Exp. Biol.* **215**, 2283-2287.
- Stokkan, K.-A. (1992). Energetics and adaptations to cold in ptarmigan in winter. *Ornis Scand.* **23**, 366-370.
- Stokkan, K.-A., Mortensen, A. and Blix, A. S. (1986). Food intake, feeding rhythm, and body mass regulation in Svalbard rock ptarmigan. *Am. J. Physiol.* **251**, R264-R267.
- Taylor, C. R., Schmidt-Nielsen, K. and Raab, J. L. (1970). Scaling of energetic cost of running to body size in mammals. *Am. J. Physiol.* **219**, 1104-1107.
- Taylor, C. R., Caldwell, S. L. and Rowntree, V. J. (1972). Running up and down hills: some consequences of size. *Science* **178**, 1096-1097.
- Taylor, C. R., Heglund, N. C. and Maloiy, G. M. O. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 1-21.
- Tickle, P. G., Richardson, M. F. and Codd, J. R. (2010). Load carrying during locomotion in the barnacle goose (*Branta leucopsis*): the effect of load placement and size. *Comp. Biochem. Physiol.* **156A**, 309-317.
- Tolkamp, B. J., Emmans, G. C., Yearsley, J. and Kyriazakis, I. (2002). Optimization of short-term animal behaviour and the currency of time. *Anim. Behav.* **64**, 945-953.
- Tullis, A. and Andrus, S. C. (2011). The cost of incline locomotion in ghost crabs (*Ocyropsis quadrata*) of different sizes. *J. Comp. Physiol. B* **181**, 873-881.
- Vilensky, J. A., Moore, A. M. and Libii, J. N. (1994). Squirrel monkey locomotion on an inclined treadmill: implications for the evolution of gaits. *J. Hum. Evol.* **26**, 375-386.
- Warncke, G., Bandholtz, J. and Schultze-Motel, P. (1988). Metabolic cost and body temperatures during grade running in quail (*Coturnix coturnix*). *Comp. Biochem. Physiol.* **89A**, 93-96.
- White, R. G. and Yousef, M. K. (1978). Energy expenditure in reindeer walking on roads and on tundra. *Can. J. Zool.* **56**, 215-223.
- Wickler, S. J., Hoyt, D. F., Cogger, E. A. and Hirschbein, M. H. (2000). Preferred speed and cost of transport: the effect of incline. *J. Exp. Biol.* **203**, 2195-2200.
- Wunder, B. A. and Morrison, P. R. (1974). Red squirrel metabolism during incline running. *Comp. Biochem. Physiol.* **48A**, 153-161.
- Yousef, M. K., Dill, D. B. and Freeland, D. V. (1972). Energetic cost of grade walking in man and burro, *Equus asinus*: desert and mountain. *J. Appl. Physiol.* **33**, 337-340.

Table S1. Relative increase in P_{met} (W kg^{-1}), the cost of transport ($\text{J kg}^{-1} \text{m}^{-1}$), the cost of lifting ($\text{J kg}^{-1} \text{m}_v^{-1}$) and % efficiency during incline locomotion at 0.22, 0.5 and 0.75 m s^{-1} and at inclines of 4.2 and 7.4 deg

Season	Body mass (g)	<i>N</i>	Incline (deg)	Speed (m s^{-1})	P_{met} increase over level (W kg^{-1})	Cost of transport ($\text{J kg}^{-1} \text{m}^{-1}$)	Cost of lifting ($\text{J kg}^{-1} \text{m}_v^{-1}$)	% Efficiency
Summer	485.8±21.3	6	0	0.22		72.9±6.7		
				0.5		36.7±1.6		
				0.75		27.3±1.5		
Summer	528.1±11.6	5	4.2	0.22	5.7*	98.5±7.2		
				0.5	4.5*	45.7±4.4		
				0.75	5.7*	34.9±3.2	103.1	9.5
Summer	528.1±11.6	5	7.4	0.22	0.9*	77.1±4		
				0.5	4.7*	46±3.7		
				0.75	4.7*	33.6±2.5	48.9	20.1
Winter	733±14.7	7	0	0.22		52±3.3		
				0.5		28.7±0.8		
				0.75		20.7±1.4		
Winter	917.1±31	7	4.2	0.22	5.3*	75.9±4.2		
				0.5	6.1*	40.2±2.3		
				0.75	6*	28.7±2.7	108.5*	9
Winter	917.1±31	7	7.4	0.22	7.2*	84.7±5.6		
				0.5	7.1*	42.9±2.3		
				0.75	7.4*	30.6±1.5	77*	12.7

Values marked with an * indicate those that are significantly different from values obtained for level locomotion.