UNIVERSITY OF TROMSØ UiT

FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS
DEPARTMENT OF ARCTIC AND MARINE BIOLOGY

Terrestrial locomotion in the Svalbard ptarmigan (*Lagopus muta hyperborea*)

How does treadmill running compare with running overground?

Photo: Bob van Oort

Anne Marit Vik

BIO-3910 Master`s thesis in Biology

May 2013

Table of Contents

Abstract

The Svalbard rock ptarmigan (*Lagopus muta hyperborea*) is a resident bird on the Svalbard achipelago, living in an environment where it experiences extreme climatic and photoperiodic conditions. The bird's most striking adaption to this environment is, prior to the onset of winter, its deposition of fat, comprising up to 30% of body mass. Moving around with this excess mass may prove difficult as it is likely to infer an extra energetic cost, especially at a time of food deprivation and low temperatures, where saving of energy is at prime concern. In order to study the impact of locomotion on the birds' energy budget, treadmill studies have previously been carried out looking into both the bird's energetics and biomechanical parameters. To validate these treadmill studies, I have conducted overground running studies to compare the running patterns of the two experimental environments. Do the birds display the same biomechanical patterns on the treadmill and in the natural environment? Additionally, blood gas and lactate analysis have been carried out while birds ran on the treadmill in order to assess their aerobic capacity, to validate the use of indirect calorimetry in the previous energetic studies.

I: Introduction

Treadmill experiments have long been used to study animal locomotion under controlled conditions. Having an animal run indoors in a closely controlled environment provides stable conditions and allows the person carrying out the experiment to carefully regulate experimental conditions, such as ambient temperature, running speed and duration, according to needs and the hypothesis to be tested, and to observe the animals closely. This is, however, an artificial environment in which to study animal locomotion, and there is evidently a need to validate treadmill-based data in a more realistic setting. Various factors like substrate material and composition, incline of the ground, temperature and air friction are all likely to differ in nature as compared to in the laboratory. By conducting and comparing treadmill data with outdoor studies, and in the long term field studies, we would be able to assess the relevance of treadmill experiments for field conditions. This master thesis deals with locomotory biomechanics in the Svalbard ptarmigan during overground running, and with bloodgas chemistry during treadmill running, and partially supplements previously conducted studies on treadmill running in this bird, as performed by a team lead by Professor Jonathan M. Codd, University of Manchester, UK [1, 2, 3, 4, 5].

Study species: the Svalbard ptarmigan

The Svalbard ptarmigan *(Lagopus muta hyperborea)*, a sub-species of the rock ptarmigan *(Lagopus muta)*, is a ground-dwelling bird species resident on, and exclusive to, the Svalbard archipelago and Franz Josef Land [6]. Living under the harsh conditions in Svalbard, it experiences long, dark winters with grim climate conditions and food shortage. Alternating sub-zero temperatures and rain, causing freezing of the range, may periodically make food even more scarce. Thus, the Svalbard ptarmigan has acquired a set of morphological and physiological traits that enable the species to endure these very challenging conditions to which it is exposed, the most remarkable being its profound seasonal fluctuations in body

mass. The birds are between 35-40 cm long, and weigh between 500-550g in summer, and 900-1200g in winter [7]. Birds also undergo changes in plumage (fig. 1), with summer birds displaying a golden brown molt much like that of the willow ptarmigan (*Lagopus lagopus*) (females) or white molt, and winter birds (both female and male) having a white plumage with black outer tail feathers.

Figure 1: Svalbard ptarmigan. Hen in summer plumage (left) and cock in winter plumage (right). (Photos: Eirik Reierth (left) and Bob van Oort (right).

Males additionally display red coloured supraorbital combs above the eyes during the breeding season [8]. Both seasons plumages provide the birds with excellent camouflage, with the white snow during winter and rocky terrain during summer. Molting is casually related to changes in daylength [9, 10]. Mating takes place in late May, and egglaying takes place in early to mid-June. These eggs are usually hatched in late June to early July. All this takes place in a period when ambient temperature is favourable, and plants are most nutritious, ensuring increased survival for the chicks [8]. From late August to mid November, body mass increases by up to 50-100% [9, 7], with fat comprising up to 30% of total body mass [7]. The fat reserves are used during the long winter, and are almost all gone by late February, by which time birds are very lean and continue to be so throughout the spring and summer months. Interestingly, body mass increase coincides with a decrease in feed intake during the autumn. Feeding resumes in April, to peak in June [9]. Metabolic rate is reduced during the winter months [11]. Since the birds still keep feeding to some extent during winter, the fat reserves are thus more of a safety margin during periods of acute food shortage than a pure reservoir [12]. The mass and feeding

changes coincide with conspicious day length changes on Svalbard (fig.2), and appears to be brought about by a photoperiodically controlled endocrine pathway, via melatonin and metabolic hormones like GH or thyroid hormone [13, 14, 15].
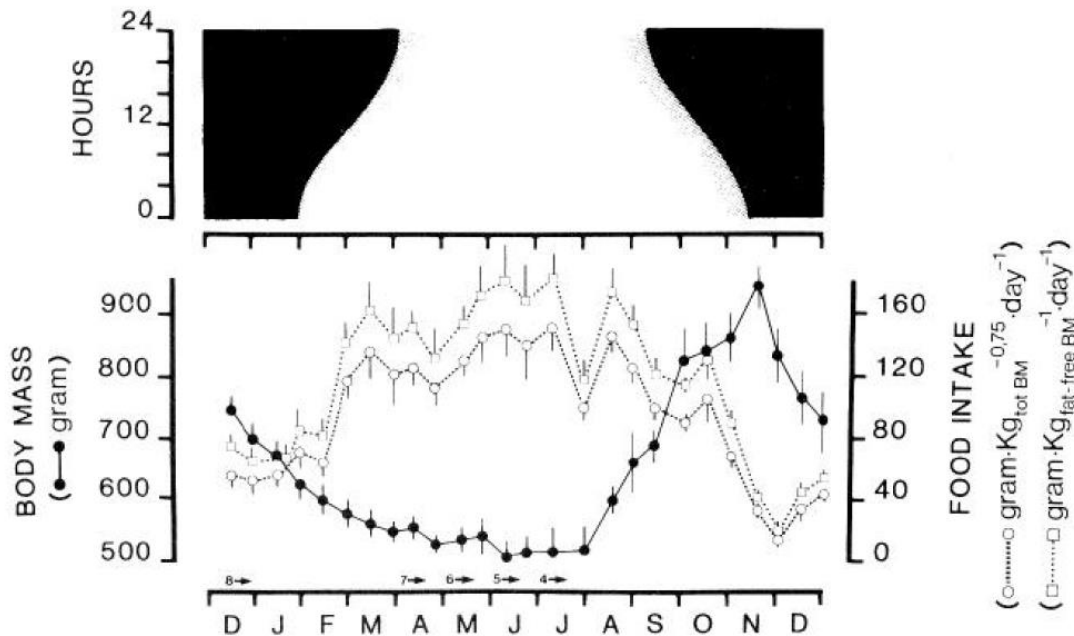

Figure 2: Seasonal changes in body mass (BM) (closed circles) and food intake expressed as gram · Kg total BM$^{-0.75}$· day^{-1} (*open circles*) and as gram · Kg fat free BM^{-1}· day^{-1} (*open squares*) (*bottom*) in captive Svalbard rock ptarmigan exposed to natural temperature and light (*top*) conditions for 13 mo at Svalbard (79°N). During this period, birds were given standardized high-quality feed and snow or water ad libitum. Period when sun is above horizon is shown in *white*, night in *black*, and civil twilight by hatched area. Number of birds is indicated on *abscissa*, and *vertical bars* indicate SE (Stokkan et al., 1986).

Having all this excess body mass in autumn/winter may theoretically impose extra energetic costs for the birds when it comes to moving around. Even though the birds are fairly inactive during winter [9, 16], lying still in snow burrows which also protects them from wind and predators and give extra insulation against the cold, they must occasionally move around to search for what little food is available, often in places where patches of grass have been exposed by the digging activities of the Svalbard reindeer *(Rangifer tarandus platyrhynchus)* [6]. This poses yet another problem when it comes to the birds' energy requirement, which is crucial to maintain at a minimum during this time of food scarcity, so there is a dilemma between finding fuel and not using too much energy. Excess mass would seem to imply that it is energetically more costly for the animal to move around, especially in

4

the snowy terrain, and also put excess strain on the respiratory system, considering respiration is mostly brought about through sternal movements [17]. Since the fat is predominantly deposited around the sternum and trunk walls, structures that play a vital part in respiration, a heavier load has to be lifted during respiration inferring an increased energetic cost in the form of heavier muscle work [18], and loading experiments in other species have already shown that excess weight on the sternum may have substantial impact on energy use during respiration [17]. The use of more energy could be fatal if the search for food is inadequate or if it fails altogether. Still, these birds survive the winter and manage to overcome these problems, posing a question related to whether they may display different locomotory mechanics at different times of the year, depending on body mass.

Recent treadmill experiments suggest that this is indeed the case, as the birds, somewhat paradoxically, have been shown to use less energy when moving during winter than during summer, despite them being up to 47% heavier [1].

Locomotory biomechanics

For all animals, locomotion is a normal activity of daily life, and essential to the survival of the species. Being able to move allow animals to hunt, graze, migrate and escape from potential predators. Like previously stated, locomotion is an energetically costly process that needs to be balanced by energy intake in order for the body to be in energy homeostasis, and this is particularly challenging in species experiencing extreme climates. The mechanics of locomotion are therefore closely related to the energetic costs of an organism, and it is thought that animals are gait-selective for various speeds in order to minimize energy expenditure during locomotion [19]. Most of the existing studies on terrestrial locomotion and on the cost of transport (COT), have been done on humans (walk and run), horses (walk, trot, gallop) and kangaroos (hopping) (fig. 3), but in later years more focus has been directed towards birds. COT is mainly determined by the force that must be generated during stance phase to support and accelerate the animals centre of mass (COM), coupled with the time available for generation of this force [20]. Variations of

this parameter with speed U are steeped in the optimality approach, and this may give indication to evolutionary significant speeds (fig. 3). Being a bipedal species, birds typically walk at lower speeds, and adopt an aerial running phase, in which both feet are off the ground at once, at top running speeds. In addition, there is an intermediate gait known as grounded running, or "groucho running", used at intermediate speeds [21, 22, 23]. The birds are then running, but without an aerial phase.
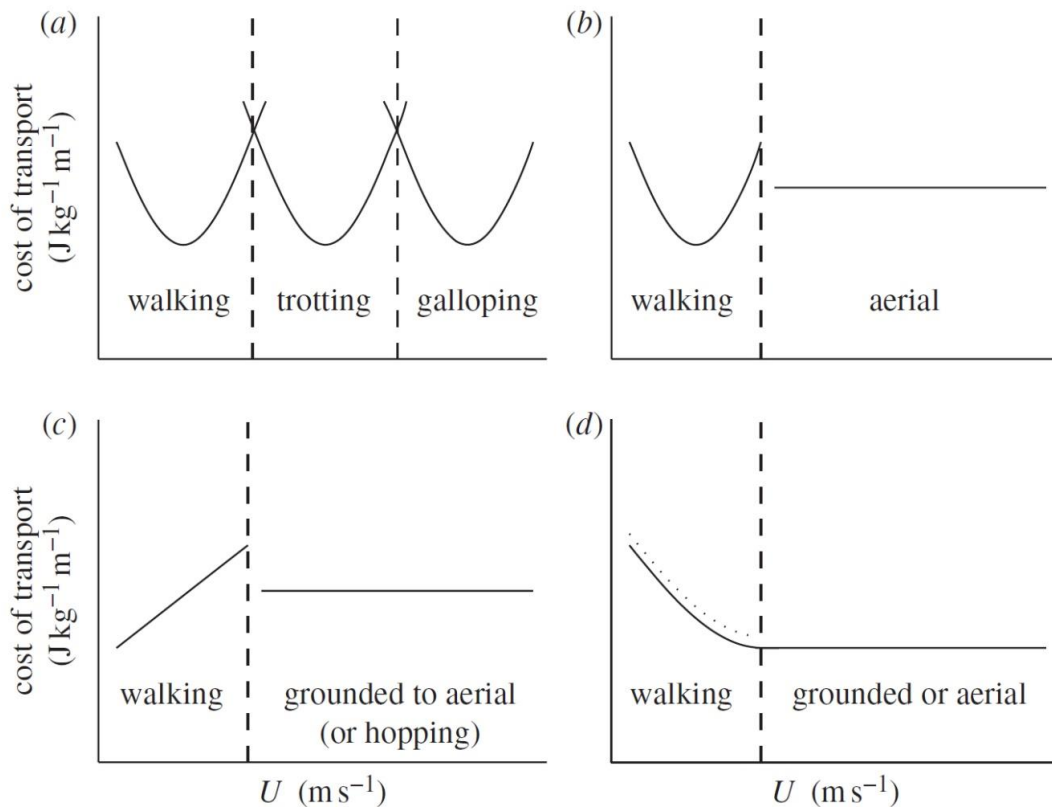

Figure 3: Generalized relationships between the COT (J kg^{-1}m^{-1}) and forward speed (U) across different gaits for: (a) horse; (b) human, donkey and camel; (c) ostrich and kangaroo; and (d) barnacle goose (dotted line), and platypus and small mammals (solid line). In (c) grounded and aerial running refers to the ostrich, and hopping refers to the kangaroo. In (d) the type of running (grounded or aerial) is not known. (Nudds et al., 2011).

The body can be viewed in light of models of physics. When an animal moves at its lowest speeds, essentially walking, the body behaves like an inverted pendulum, where the gravitational kinetic and potential energies of the body's COM relative to the limbs fluctuate close to 180° out of phase, causing a transfer of energy between kinetic energy, E_k and potential energy, E_p, resulting in saving of metabolic energy

[19]. At higher speeds, the phase relationships goes awry, more active muscle power is invested, and E_k and E_p are no longer exchanged to the same extent (fig. 4). Instead, during running, energy is saved from the transfer between the COMs E_k and the elastic energy stored in elastic muscles and tendons [24], thus leading to the body acting like a spring.
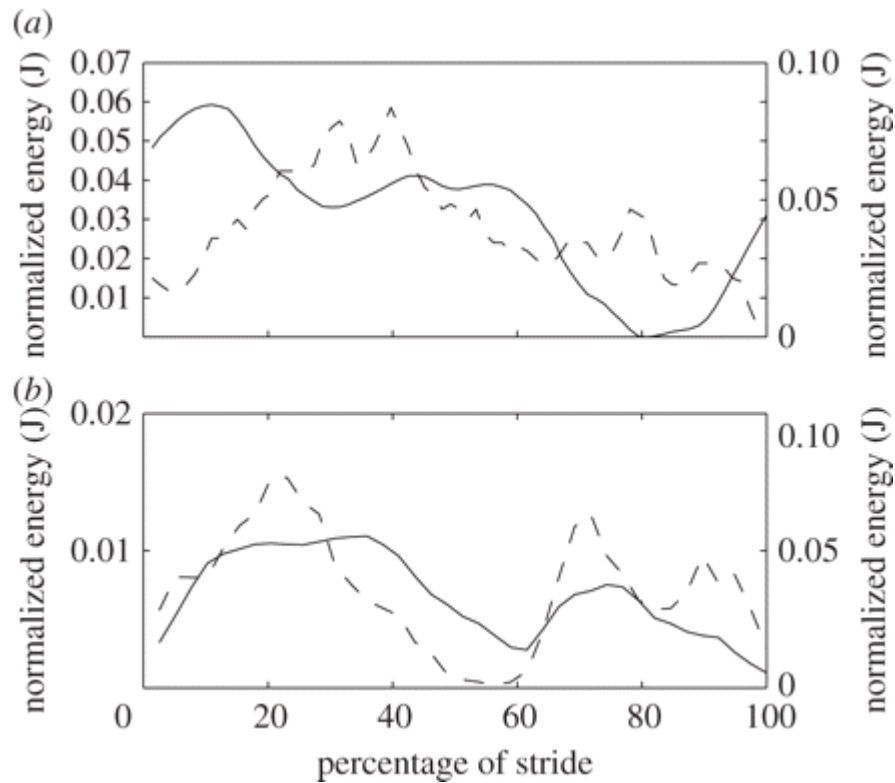

Figure 4: Examples of typical COM energy fluctuations for (*a*) walking at 0.50 m s^{-1} and (*b*) grounded running at 1.11 m s^{-1}. The solid lines and right *y*-axis represent potential plus vertical kinetic energy, and the dashed lines and left *y*-axis the horizontal kinetic energy of the COM. (Nudds et al., 2011).

Along with this mechanical explanation between the walk-run transition, another unit-less variable known as duty factor (DF), the proportion of time of a stride cycle when one foot is on the ground, is used. A DF > 0.5 corresponds to walking, while a DF < 0.5 is related to aerial running [25]. Still, many argue that a shift in DF is not clear enough to describe the walk-run transition, especially in the transition between grounded and aerial running seen in birds [26]. In some species, this decrease in duty factor towards aerial running occurs gradually [22], while in others the transition is more abrupt [27].

Other kinetic parameters commonly investigated during locomotion studies are stride frequency (SF), stride length (SL), swing phase (t_{swing}) and stance phase (t_{stance}). Stride frequency is the number of strides taken in unit time. In order for an animal to increase its speed, it must either increase its stride length, its stride frequency or both [25]. Stride length is the distance travelled in a stride, seen defined as a complete cycle of movements, i. e. from one foot leaves the ground until it again touches and leaves the ground [25]. Swing phase, the time that one foot is off the ground, is more or less even throughout all speeds, while stance phase, which is the time one foot is in contact with the ground, decreases with increasing speed. All these variables are normally quantified in studies of the biomechanics of locomotion, including in the treadmill studies of Svalbard ptarmigan [1, 2]. Indeed, the birds display a lowered cost of locomotion when adopting an aerial running mode during summer when they are able to reach their top running speeds [2]. Furthermore, male birds have been shown to run with an increased locomotor performance and a lower cost of locomotion at high speeds than females [3]. This may be due to differences in morphology, specifically the shorter leg length in females compared to males, and sexual selection for improved male performance, but the exact mechanisms still remain unclear [3].

Problems and hypotheses

The process of making the birds run in the lab, however, may not coincide with reality. On Svalbard, the birds navigate through uneven, sometimes rocky terrain during summer, and often on snow in winter. The purpose of the present study was to investigate the biomechanics of locomotion in ptarmigans during overground running on a natural substrate, without the influence of the mechanically driven treadmill, in order to give some insights to the extent to which artificial laboratory conditions represent a reliable model for ptarmigan locomotion in the field.
In addition to investigate kinematics, studies have also been conducted of the energetics of the Svalbard ptarmigan's locomotion, both during the birds' lean summer months, and fat winter months [1, 2]. In these studies, indirect calorimetry

recordings of O_2-uptake were used to assess metabolic rate during running, and for this method to be useful it is a requirement that the birds work aerobically. To validate this, I also investigated the blood gas chemistry of summer- and winter-adapted birds running on a treadmill, and measured blood plasma lactate levels for the same speeds at which the birds were running in the study by Lees et. al., using the very same individuals. The main goals of this study were:

1. To compare laboratory and semi-field studies of biomechanical parameters of birds running on a treadmill and birds running outside on a natural substrate, to investigate to what extent running in a more natural environment coincide with the artificial conditions of treadmill-running.

2. Validate the use of indirect calorimetry as a method of measuring energy expenditure in running birds by investigating their blood gas chemistry and blood plasma lactate levels at different speeds, to conclude to what extent the birds work aerobically.

II: Materials and Methods

Biomechanics of locomotion

Animals

10 individuals – 6 females and 4 males, all juveniles that were hatched and raised in captivity at the animal research facilities of the Department of Arctic and Marine Biology, University of Tromsø (69°, 46'N) (mean body mass ± s.e. = 0.465 ± 0.015 kg (females), = 0.556 ± 0.024 kg (males))– were used for the outside running trials. The trials were carried out in August and December of 2010, and the birds were yearlings hatched during the summer of 2010. Between experiments, all birds were housed in double outdoor cages (2x90x90cm) at the animal research facility, where they were exposed to natural climate conditions and light cycles at Tromsø, with ad libitum access to high quality feed (Prydfugl Rype Komplett, TKM αNO00000324 C) and water or snow.
All use of animals had been approved by the Norwegian National Animal Research Authority (approval no. 2636).

Running

Birds were run several times back and forth in a running tunnel on a grass field outside the animal facility, in order to study running biomechanics on a natural substrate. The tunnel measured 6.0 m length/0.5 m height/0.5 m width and consisted of wood and netting that allowed visual inspection. One end of the tunnel was closed, while the other was open, making catching easier and motivating the birds to run in that direction. The back of the mid-section was lined with marks every 20cm, as a scale for later data analysis. A camera (Sony Handycam HDR-SR12E, SONY Japan) was placed away from the tunnel in lateral view, zooming in to fit the entire middle section with the markers. To make the birds run, a broomstick was used to tap the side of the cages, provoking the running. As they ran, the camera

took high-speed footage of each run. Each bird was allowed to rest 5 minutes prior to running, and ran continuously for no more than 30 minutes, or until at least 15-20 acceptable runs at various speeds determined/chosen by the birds, were filmed. Acceptable films were defined as trials where the birds ran in the middle of the runway, and contained at least 3 continuous stride cycles, one stride cycle being foot on ground-foot off ground-foot on ground.

All experiments were conducted at the same time of day, for a total period of 8 hours a day for two-three weeks, in summer (August/September) and winter (December). In addition to run on level ground the birds were allowed to run on inclines.

Tracker analysis

Videos of acceptable running trials were converted from a Sony .mte format to Quickplayer .mov files, and analysed using TRACKER software v. 4.0 (Open Source Physics, Cabrillo, CA, USA) by tracking the foot nearest the camera over a minimum of 3 continuous strides (fig. 5). Stride frequency, stride length, swing and stance time, duty factor and average speed were the parameters calculated.
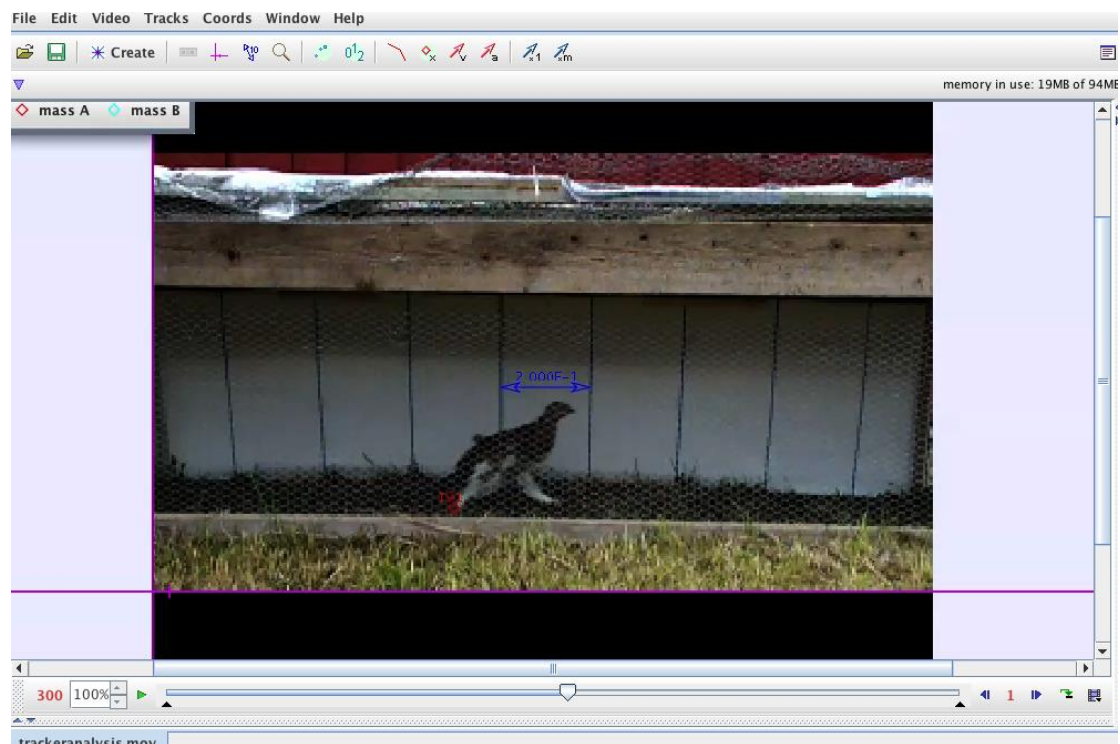
Figure 5: Screencapture of video analysis using Tracker software v. 4.0 (Open Source Physics, Cabrillo, CA, USA). The blue marker in the back adjusts analysis to the actual measurements taken (in this case, 20 cm between each bar). Markers were placed on the foot nearest to the camera (red dots) when foot was on ground, and at foot take-off, then touch down again. One stride consists of "foot on-foot off-foot on", and this was done for a minimum of 3 strides. Additionally, a marker was placed on the birds beak at the first and last foot on, to calculate the distance and time run, thus calculating average speed.

Stride frequency is the number of strides taken by the bird over a certain time period measured in Hz. In this case the number of strides taken by the bird during the time spent running from the analysis started to it ended.

Stride length is the distance between two successive placements of the same foot, consisting of two step lengths.

Swing time is measured as the time used by the foot in the air during each stride.

Likewise, stance time is the time used by this foot on ground during each stride.

Together swing and stance time make up the period of one stride.

Duty factor was calculated as the fraction of one stride cycle where the foot is on the ground.

Lastly, running speed was calculated by dividing the distance *(x)* ran by the bird, by the time *(t)* used to run this distance.

Blood chemistry analysis

Animals

Indoor treadmill trials were conducted according to the same exact protocol as employed in Lees et al., 2010, for energetic study, and using the very same birds, all males, (9 birds in winter (mean body mass $\pm$ s.e. = 0.615 ± 0.1042 kg), and 8 birds in summer (mean body mass $\pm$ s.e. = 0.703 ± 0.0629 kg)). These individuals were kept in cages inside the department (90x90cm), under controlled thermoneutral conditions [28] and simulated light cycles of Tromsø (69°46'N) for summer and winter birds. The birds had ad libitum access to (Prydfugl Rype Komplett, TKM αNO00000324 C) and water.

Blood sampling and blood gas analysis

The birds were run on a treadmill (Bremshey Trail Sport, Finland) at various speeds in winter (0, 0.8, 2.7, 3.3 and 4.5 km/h)/(0, 0.22, 0.75, 0.92 and 1.25 m/s^{-1}) and summer (0, 1.8, 4.5 and 6.8 km/h)/(0, 0.5, 1.25 and 1.89 m/s^{-1}) for 5 minutes, each bird running only at one speed per day. Animals were collected individually for their respective trials, straight from the cage and immediately placed in a Perspex® chamber (30 x 26 x 61.7cm) placed on the treadmill edges, through which air was pulled at a fixed flow rate of ~52 l min^{-1}, by use of a vacuum pump. The birds were left to rest for initially 5 minutes before the running or control (0 km/h), to allow them to settle after the initial handling [1]. Then the birds ran (or rested as in case of the control run at 0 km/h) for 5 min.

After each run or control, the birds were immediately secured by wrapping them in a blanket with either their right or left wing exposed, and a blood sample was taken from the exposed branchial vein in one of the two wings within 1-3 min of end of running, using a 1ml heparinized syringe. The blood was then promptly (within max. 15 sec of sampling), injected into a blood analyzing machine (Radiometer ABL 700 (Bergmann Diagnostika AS, Oslo, Norway)) for analysis of plasma O_2-tension (pO_2), plasma pCO_2-tension (pCO_2), hematocrit (Hct) and pH. The analyzer was calibrated as per usual protocol (Radiometer ABL 700 User Manual) prior to analysis and running of the birds. Care was taken to prevent blood from coming into contact with air. The remaining blood was transferred to Eppendorff tubes and then on ice for up to 2 hours, sentrifuged and plasma was extracted, to be frozen at -78 degrees for later lactate assay.

Lactate analysis

Analyses were carried out using a lactate assay kit (BioVision, Inc.; Mountain View, CA, USA) 18 (winter) and 10 (summer) months after sampling. A standard curve was made using lactate standard, and the plasma was then thawed and used to prepare the lactate samples as per standard protocol (BioVision). After a 30 minute

13

incubation at room temperature, the samples were measured spectrophotometrically at a wavelength of 570nm in a colorimetric assay using SpectraMax® PLUS384 (Molecular Devices, Sunnyvale, CA, USA) and sample readings were applied to the standard curve, to give the amount of lactate in each of the samples. Lactate concentration (mM/l) in each of the samples were plotted against their respective collection speeds. The amount of plasma only allowed for duplicates to be tested.

Data analysis

The data from the kinematics trials included 10 birds, 6 females and 4 males, which each gave a different amount of runs each. Because of this uneven amount of trials per individual, a linear mixed model approach was used with a p-value of 0.05 (95% confidence level) set as threshold. Since the term individual*speed was found insignificant, and thus showed no signs of pseudoreplication, we ran an analysis of covariance (ANCOVA) for the samples. We then checked if the interaction term gender*speed was significant, and since the interaction term was found insignificant, we removed it and the ANCOVA was re-run without it.

Lactate and blood gas data were displayed as mean + s.d.

Test for statistical analysis were conducted using SPSS v.19.0 (SPSS inc, Chicago, IL, USA), while plots were made using Sigmaplot v.12 (Systat Software Inc., Chicago, IL, USA).

III: Results

Biomechanics

Although experiments were carried out both during summer and winter, the winter trials yielded no usable data material as the birds would not run steadily enough, or even at all even though provoked to do so. Likewise, running on inclines proved to be impossible, as the birds either stayed put when provoked or started taking off. Our data is solely based on the data from the summer trials and it is this data I have compared to the treadmill studies by Nudds et al. [2].

The same kinematic parametres (stride frequency, stride length, duty factor, swing time and stance time) that were quantified in the initial study [2] were measured while the birds ran overground.
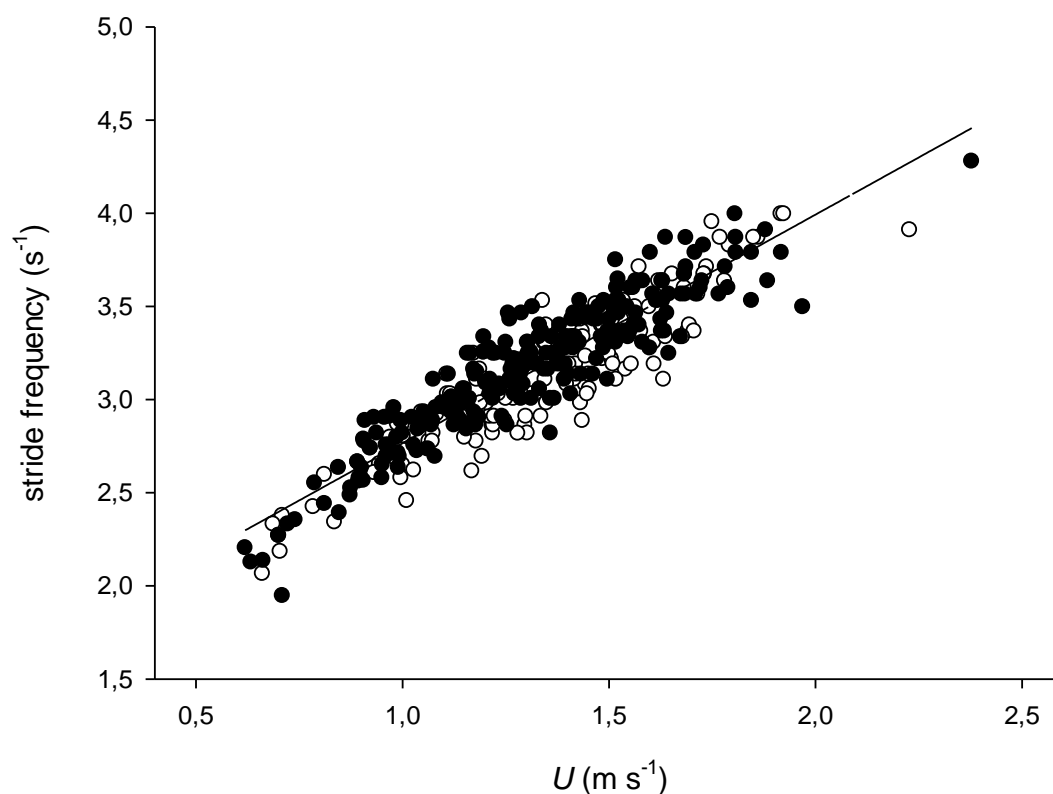

Figure 6: Stride frequency (SF) plotted against running speed (U) for males (open circles) and females (filled circles). The regression line fitted through the data is derived from the linear regression output, and is SF = 1.54 + 1.23U (n = 389, r^2= 0.840, p < 0.0001)

Stride frequency displayed an expected and typical linear relationship with running speed (U) (fig. 6). The regression line was described by SF = 1.54 + 1.23U (n = 389, r^2= 0.840, p < 0.0001) for both genders pooled together. There were no obvious gender differences, although statistics comparison showed that gender was an important factor describing stride frequency when plotted against U ($F_{397,2}$ = 24.88, p = 0.0001). Their respective regression lines were given by SF_F = 1.59 + 1.21U (n = 240, r^2 = 0.850, p < 0.0001) and SF_M = 1.46 + 1.24U (n = 149, r^2 = 0.826, p < 0.0001).
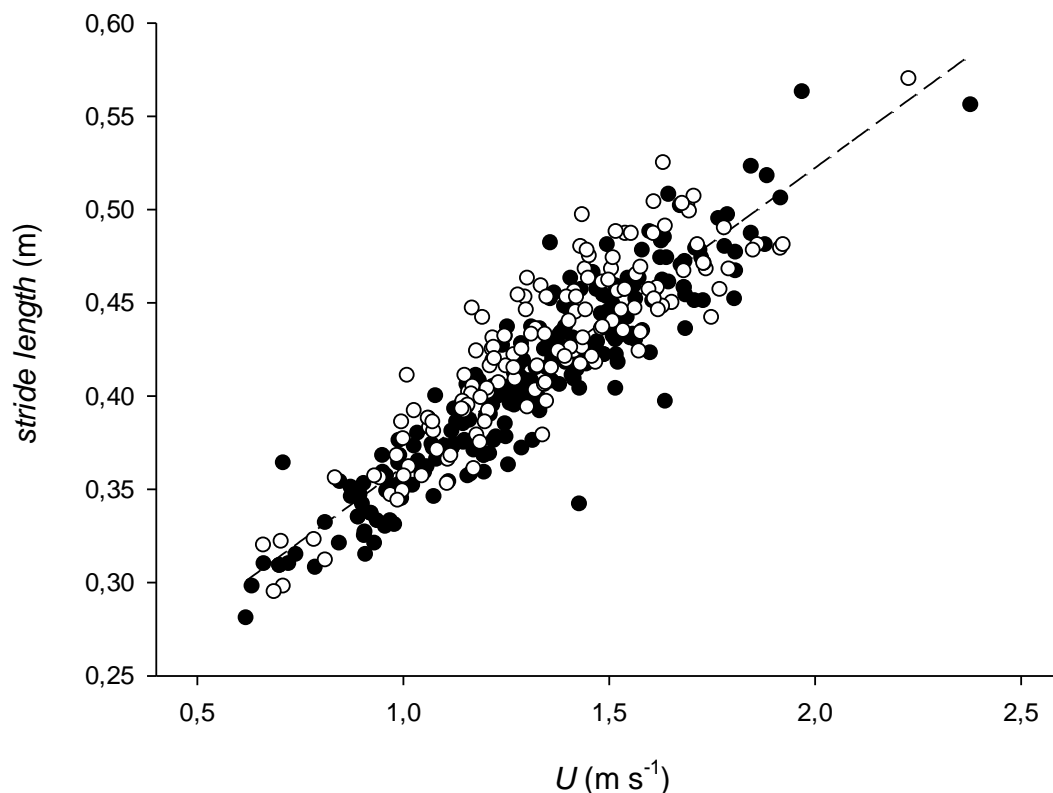

Figure 7: Stride length (SL) plotted against running speed (U) for males (open circles) and females (filled circles). The regression line fitted through the data is derived from the linear regression output, and is SL = 0.20 + 0.16U (n = 389, r^2= 0.831, p < 0.0001).

Also, stride length (SL) showed a close linear relationship with U, with SL = 0.20 + 0.16U (n = 389, r^2= 0.831, p < 0.0001, (fig. 7). Again, the increase in SL with increasing U were different for the genders ($F_{397,2}$= 32.91, p = 0.0001). Their respective regression lines were given by SF_F = 0.20 + 0.16U (n = 240, r^2= 0.858, p < 0.0001) and SF_M = 0.22 + 0.16U (n = 149, r^2= 0.788, p < 0.0001).
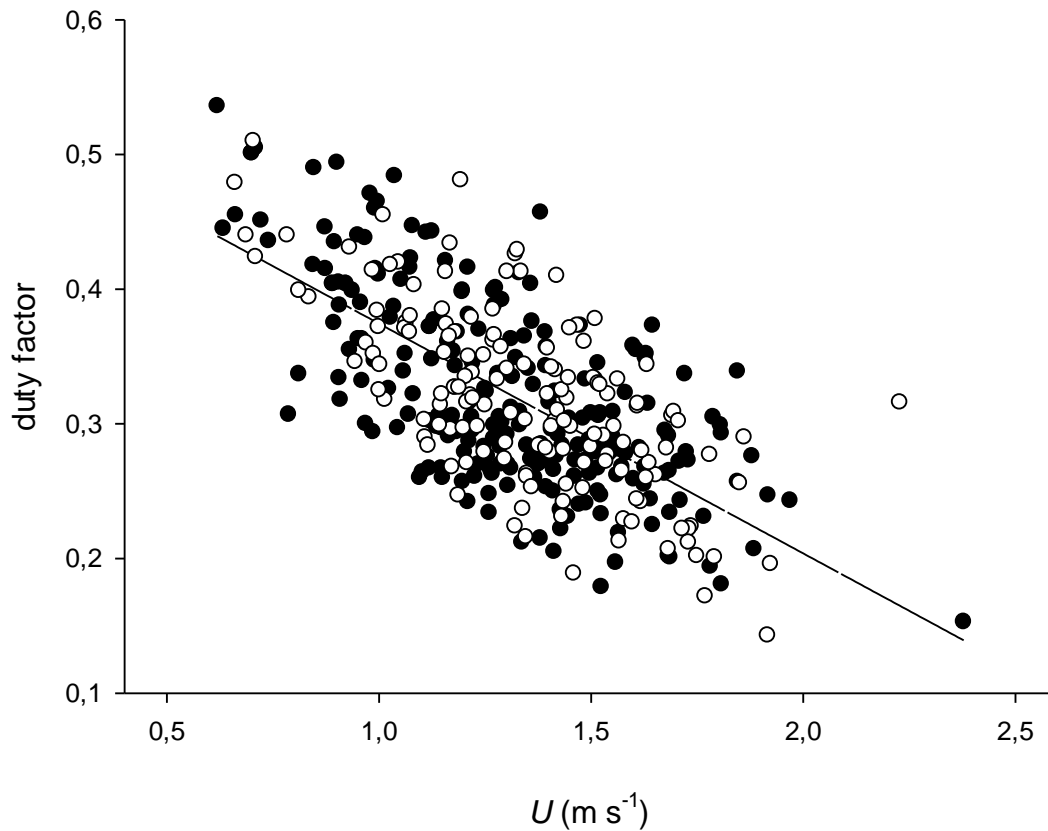
Figure 8: Duty factor (DF) plotted against running speed (U) for males (open circles) and females (filled circles). The regression line fitted through the data is derived from the linear regression output, and is DF = 0.55 – 0.17U (n = 389, r^2= 0.470, p < 0.0001).

Duty factor (DF) was inversely related to U (fig. 8), as expected, described by the equation DF = 0.55 – 0.17U (n = 389, r^2= 0.470, p < 0.0001). There were no significant differences between genders in the relationship between DF and U ($F_{397,2}$= 0.465, p = 0.496).
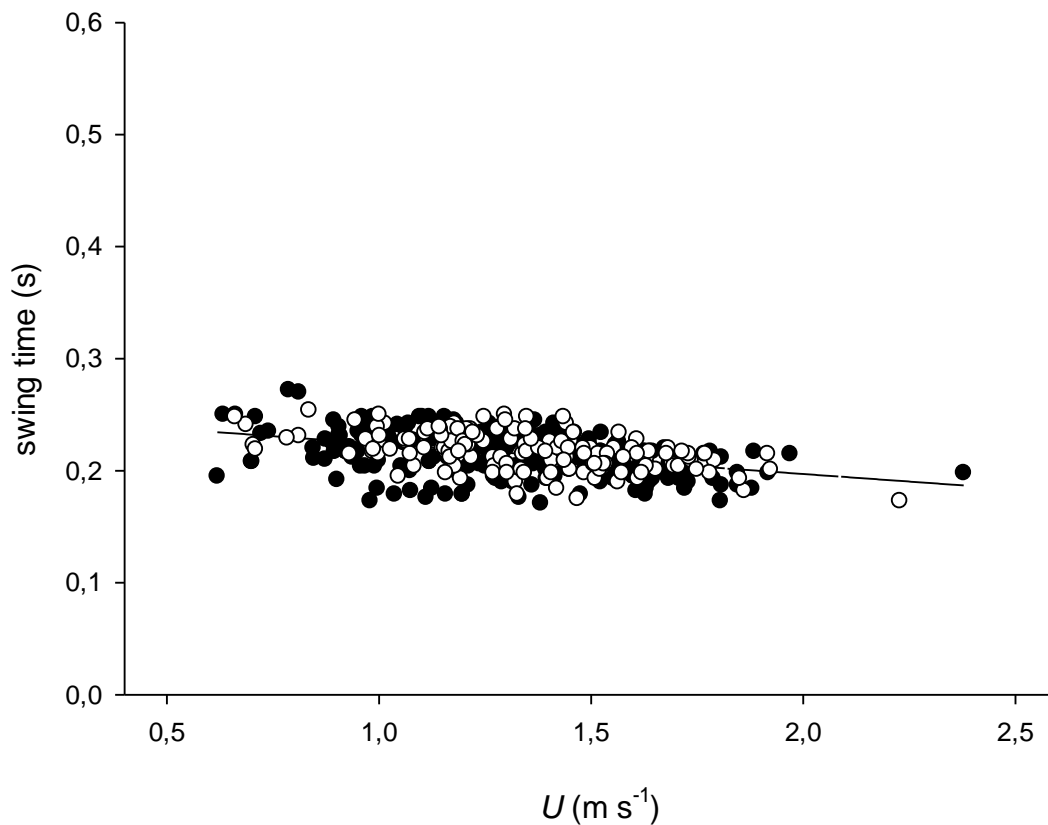
Figure 9: Swing time (t_{swing}) plotted against running speed (U) for males (open circles) and females (filled circles). The regression line fitted through the data is derived from the nonlinear regression output, and is $t_{swing} = 0.25 - 0.03U$ ($n = 389$, $r^2 = 0.185$, $p < 0.0001$).

In accordance with previous studies and that of the treadmill, swing time (t_{swing}) did not change much during the various speeds and remained around 0.2-0.25 s (fig. 9). The relation is described by the equation $t_{swing} = 0.25 - 0.03U$ ($n = 389$, $r^2 = 0.185$, $p < 0.0001$). There were significant differences between genders in the relationship between t_{swing} and U ($F_{397,2} = 6.698$, $p = 0.010$). Their respective regression lines were given by $SF_F = 0.25 - 0.03U$ ($n = 240$, $r^2 = 0.156$, $p < 0.0001$) and $SF_M = 0.26 - 0.03U$ ($n = 149$, $r^2 = 0.250$, $p < 0.0001$).
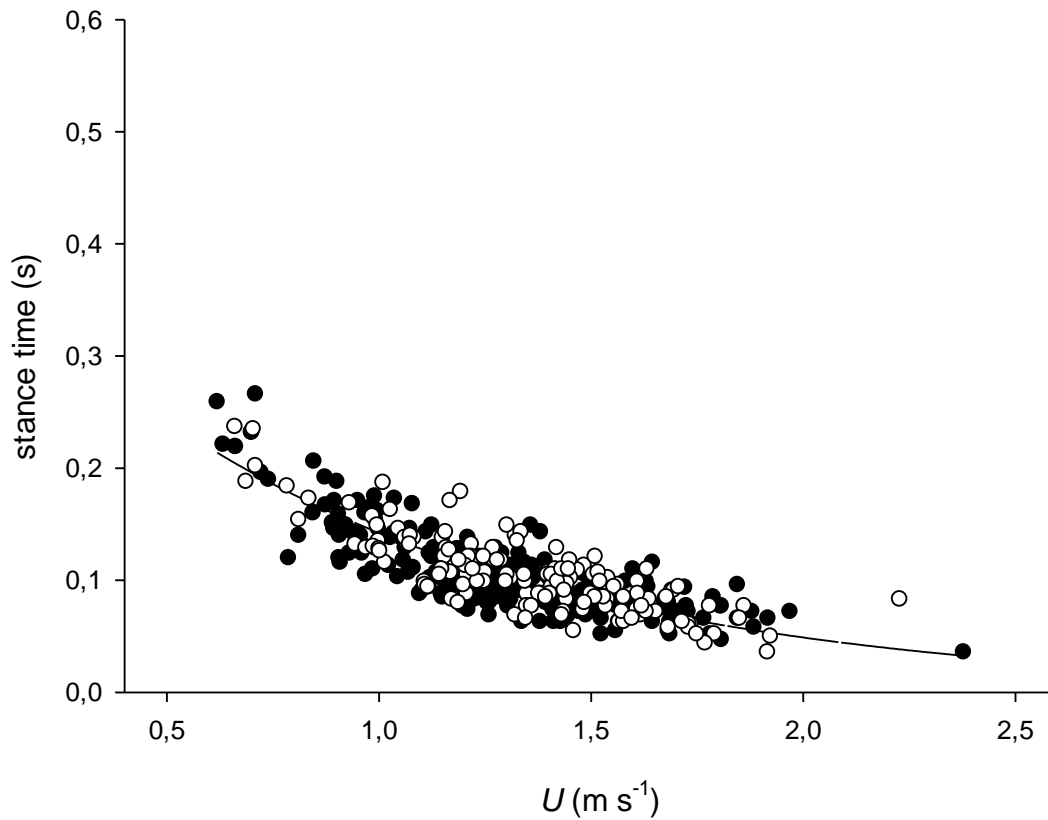
Figure 10: Stance time (t_{stance}) plotted against running speed (U) for males (open circles) and females (filled circles). The regression line fitted through the data is derived from the nonlinear regression output, and is $t_{stance} = 0.41e^{-1.07U}$ ($n = 389$, $r^2 = 0.721$, $p < 0.0001$).

Stance time (t_{stance}) decreased with running speed (fig. 10), as shown by the equation $t_{stance} = 0.41e^{-1.07U}$ ($n = 389$, $r^2 = 0.721$, $p < 0.0001$). There were no significant differences in the relationship between t_{stance} and U ($F_{397,2} = 2.973$, $p = 0.085$).
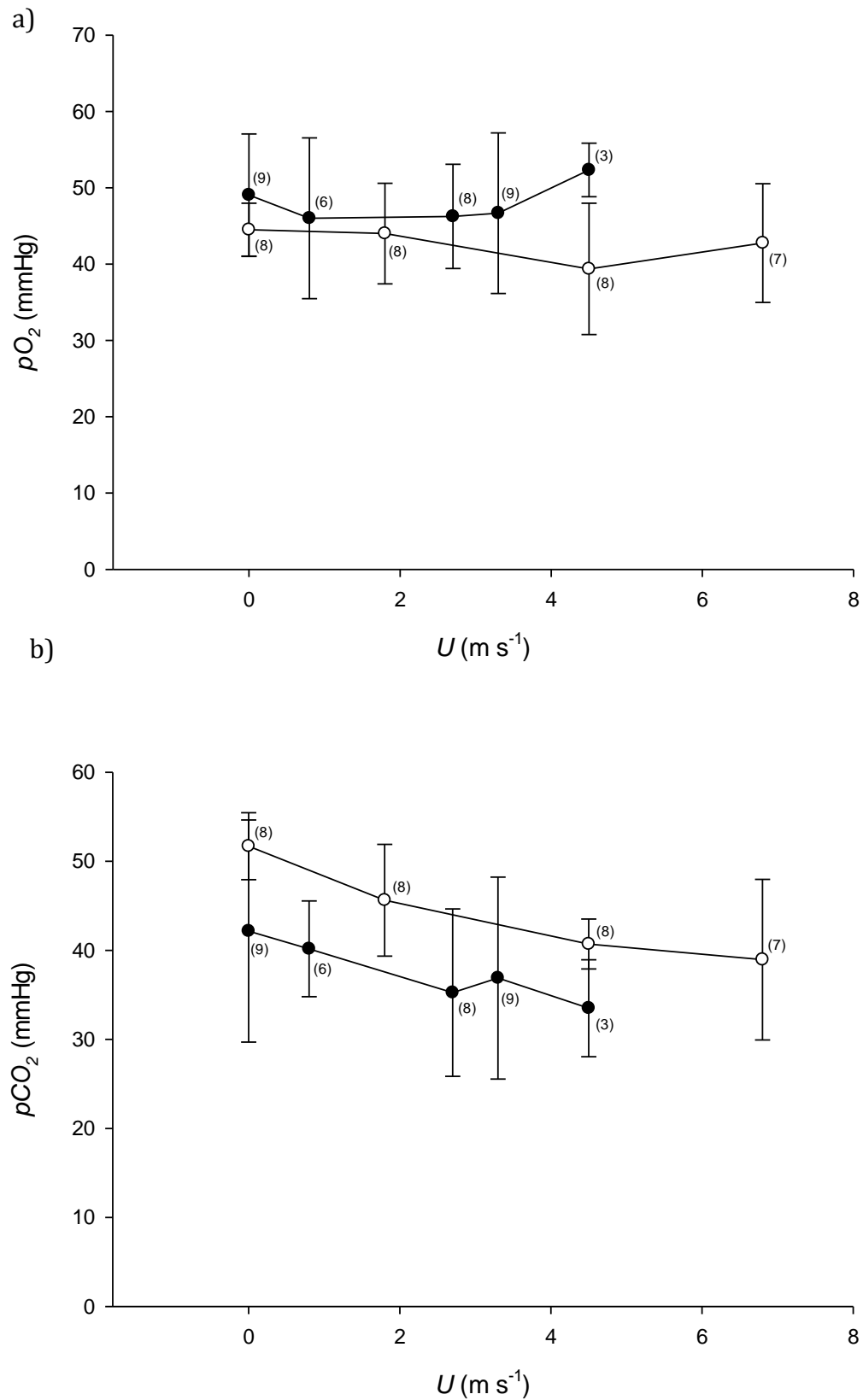
Blood gases and lactate

a)

b)

Figure 11: Plasma venous O$_2$ tension (pO$_2$) (a) and plasma venous CO$_2$ tension (pCO$_2$) (b) in mmHg plotted against speed (*U*). Open circles display results for summer and filled circles display results for

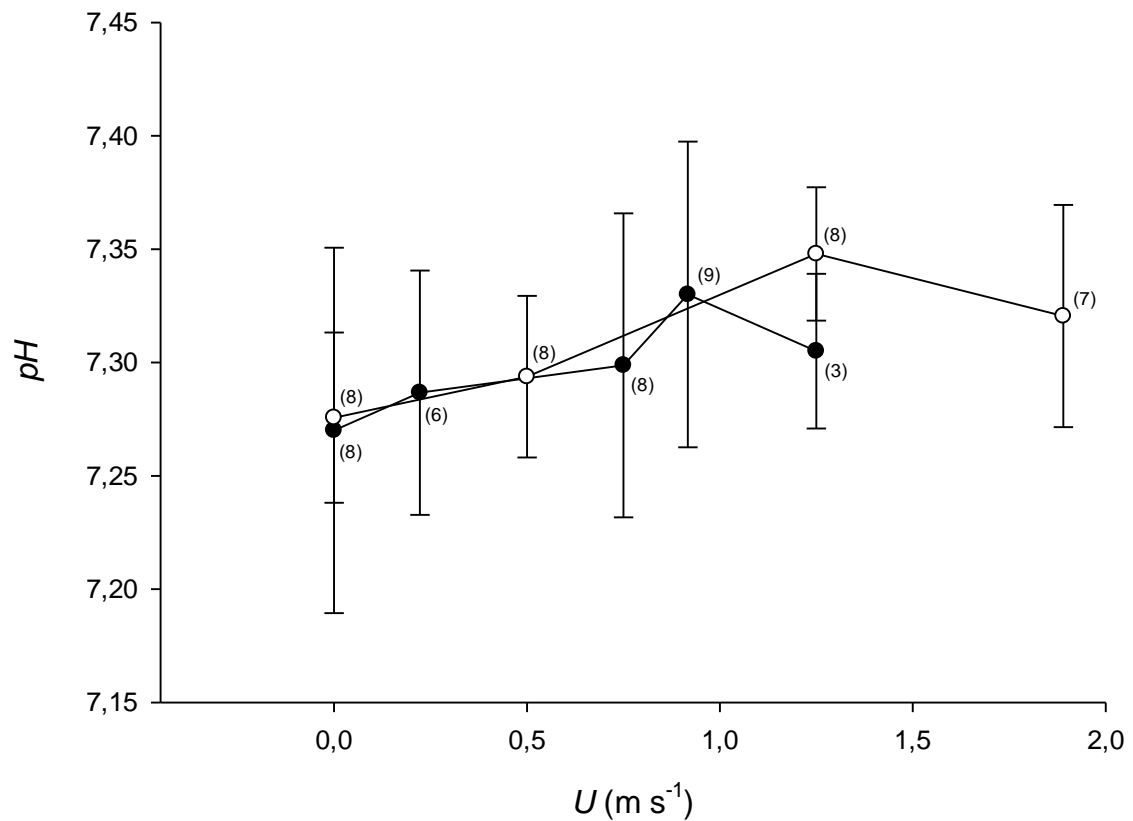winter. Data points are displayed as means ± s.d. Numbers in parantheses indicate number of individuals used (n).

Figure 12: Blood venous pH values plotted against speed (*U*). The open circles display summer data while filled circles display winter data. Data points are displayed as means ± s.d. Numbers in parantheses indicate number of individuals used (n).

From figure 11 (a) one can see that pO_2 showed a somewhat stable course with increasing speed, both during winter and summer. At the same time, there was a tendency for pCO_2 to decrease with increasing speed during both seasons (fig. 11, b), while pH tended to increase (fig. 12).
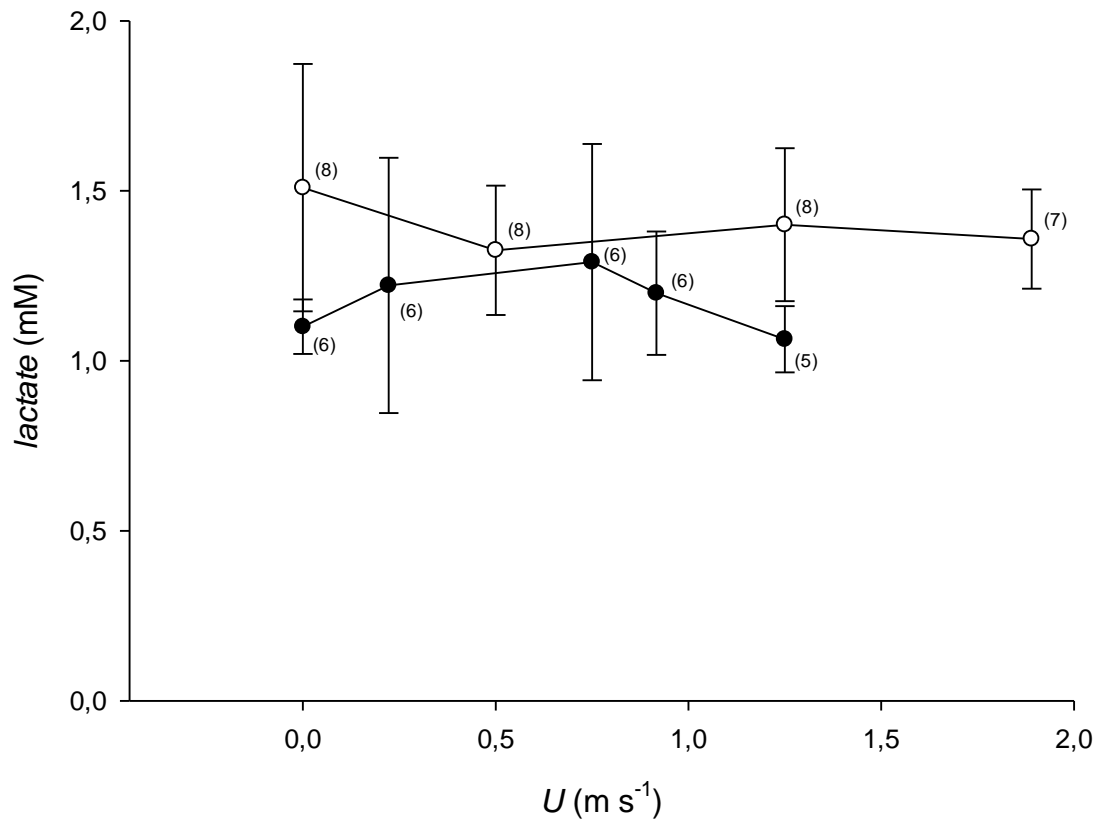
Figure 13: Plasma venous lactate levels (mM) plotted against speed (*U*). Open circles display winter data, while filled circles display summer data. Data points are displayed in mean ± s.d. Numbers in parantheses indicate number of individuals used (n).

Mean plasma lactate levels remained between 1 and 2mM for all speeds used (fig. 13), both during winter and summer trials.

IV: Discussion

Biomechanics

In order to better visualize the comparison between my studies and that of Nudds et al. [2], I have made a combine plot of their and mine regression lines for all the kinematic parameters (fig. 14). Note that in these regression plots we have used both females (n=6) and males (n=4), while their study used only males (n=6) [2], since I either found that differences between genders were non-significant, or in those cases where a significant gender effect was observed (stride frequency, stride length and swing time), this was very small (see figs. 6, 7 and 9).
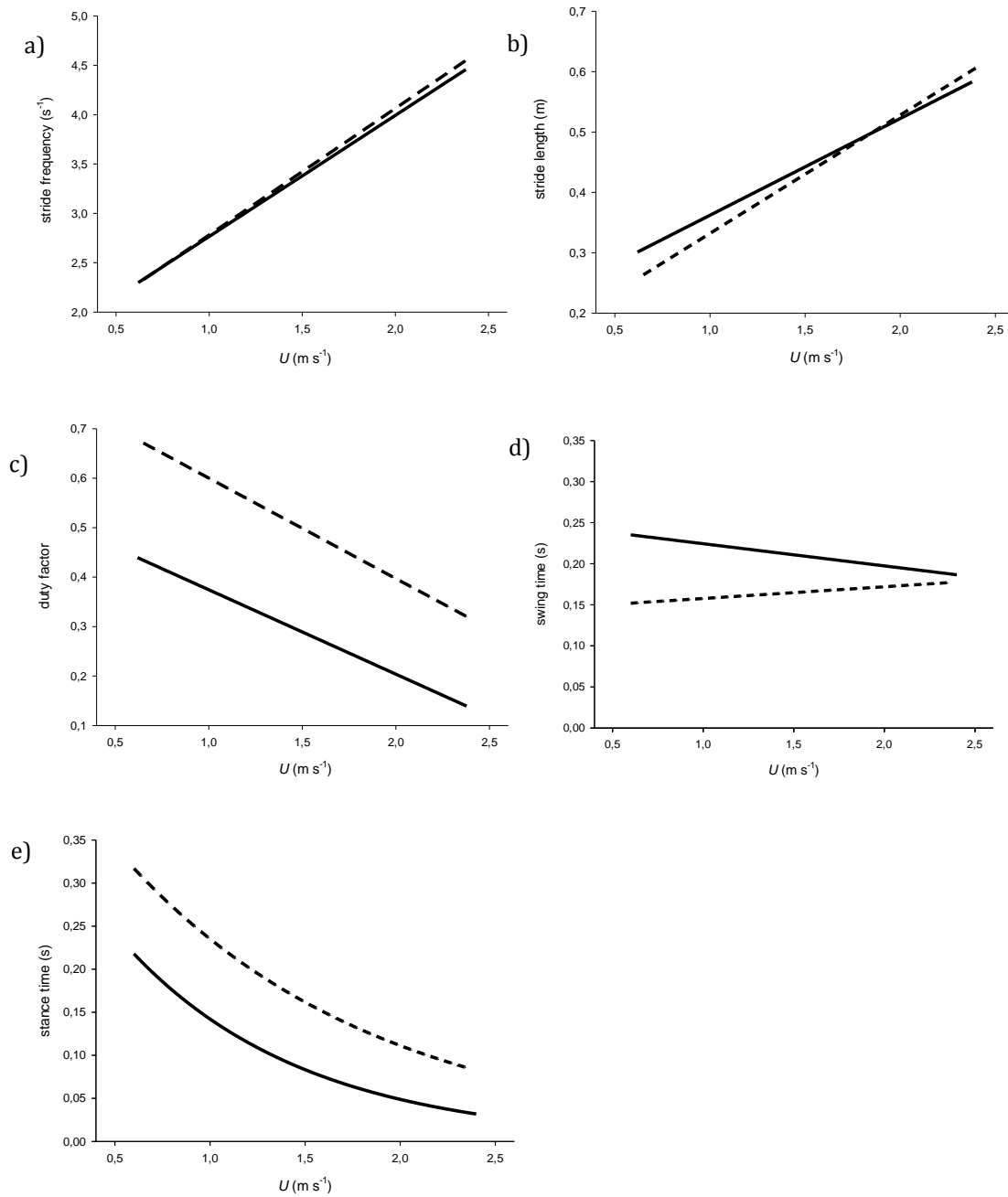
Figure 14: Comparison regression lines between my studies (solid lines) and those of Nudds et al. (2011) (dotted lines) for stride frequency (a), stride length (b) and duty factor (c), swing time (d) and stance time (e), plotted against speed (*U*) – within the range of speeds that were represented in both studies.

Comparing the present study's results with those of Nudds et al. [2], we can see more or less the same qualitative trends for all parameters.

There are, however, differences in values, and in particular for duty factor (fig. 14, c) and stance time (fig. 14, e). Thus, while Nudds' values for duty factor were in consent

24

with other studies from running birds on treadmill [22], my data displayed much lower values. For most part, DF of birds running overground were well below the 0.5 value at which gait changes into aerial running [25], and this for all speeds – even walking speeds. They imply the birds had little ground contact throughout the various gait regimes in my study. It should be noted that these two parametres are related, since low stance times will naturally give a lower duty factor as seen by the mathematical relationship

DF = stance time/ (stance time + swing time)

Conclusion from this study was that it was difficult to judge at what speeds gait changes occured, between walking and grounded running, and between grounded running and aerial running since DF values (which are typically used to differentiate between gaits) were always < 0.5, implying aerial running. Based on DF from the treadmill study, the transitional speeds for walking/grounded and grounded/aerial were judged to be at 0.75-1.0 and 1.5-1.67 m s^{-1}, respectively [2]. Judging from our duty factor values, the birds running on grass were aerial way before the 1.5 m s^{-1}-mark, which seems very unlikely judging from previous studies. Explaining why the foot contact with the ground is so short, we tried to look into the running pattern of the birds when they run outside versus them running on a treadmill. Such inspection showed that the legs seemed to be lifted higher when the birds were walking/running outside, probably due to a less flat terrain and vegetation obstacles compared to on the level treadmill. This would cause the foot to spend more time in the air for a given stride cycle, i.e. aerial, than it would on the treadmill, which implies that locomotion on terrain gives other biomechanics than on the treadmill. It has been suggested that the need for stability, in other words being able to maintain the same kinematics while locomoting in uneven terrain, require animals to adjust limb parametres as necessary to avoid stumbling or falling and return the system to a steady periodic motion [29]. In humans, this can be done by adjusting leg stiffness [30, 31] and leg contact angle [32], thereby maintaining similar COM motions over surfaces of varying compliance.

Another possible theory may be the effects of the treadmill itself, and the friction created between the foot and the substrate. The moving band of the treadmill might leave the foot on the moving belt as it moves, thereby actively elongating the stance time by causing the foot to stay in contact with the band for a longer period of time. Outside, in the grassy terrain, we don't have this effect, and the foot leaves the ground earlier. A moving band might also cause the foot to make contact with the substrate earlier than on natural ground, also increasing the duration of foot contact with the substrate. This all leads to the foot spending a substantial longer time of the stride cycle on the band, leading to a higher stance time and thus a larger duty factor. Indeed, in one study comparing overground and treadmill running in humans, it was found that the runners tended to stretch out the lead leg and let the support foot move with the belt backwards, leading to an increased time of support and thus a longer stance time [33]. Although these findings were seen in human subjects, both birds and humans locomote bipedally and there would be no reason they should differ too much in this aspect.

The active effect of the treadmill may also have an impact on stride length, as one would think such an elongation of the contact with the leg would produce a longer stride length on the treadmill. However, our results correlate very well with the treadmill results for stride length.

Stride frequency is also very well correlated with the treadmill results, if not a little bit higher in our studies (fig. 14, a). Usually larger birds like the ostrich and emu tend to increase stride frequency as a mean of achieving greater speed, more so than smaller birds like the quail and kiwi, which increase speed mainly by increasing stride length relative to their size [23]. These differences have also been found to be related to posture, where a more upright posture, like that of the ostrich, leads to shorter strides than the more "horizontal", crouched posture of the quail [34]. The Svalbard ptarmigan utilizes a more upright posture and judging from the videos of the birds running on the treadmill and those of them running outside on the grass, it seems like they run more upright in the latter case than on the treadmill (fig. 15).
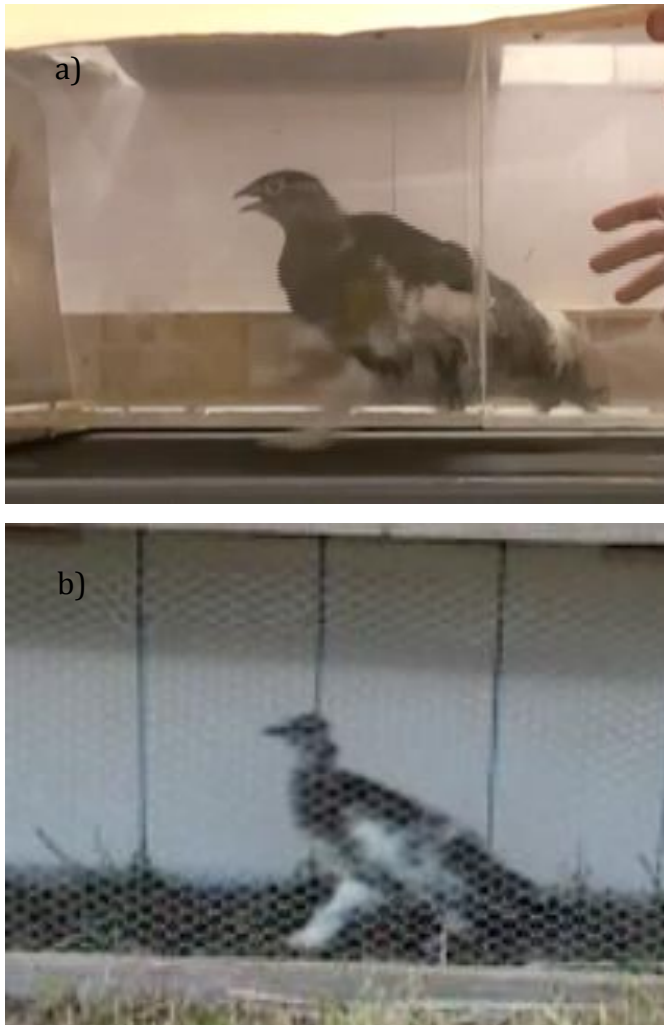
Figure 15: Screenshots of ptarmigan running on (a) treadmill and (b) grass.

One reason for this might, again, come from the need for stability when moving around. The Svalbard ptarmigan's natural habitat consists of uneven and partly rocky terrain, both summer and winter, with the latter season adding the problem of snow and slippery ice. Birds, like the Svalbard ptarmigan, possess several interesting mechanical features in their locomotor system (fig. 16). They have a lower center of gravity due to the fact that their COM is located under the hip near the knee, and this should confer a higher level of stability [35].
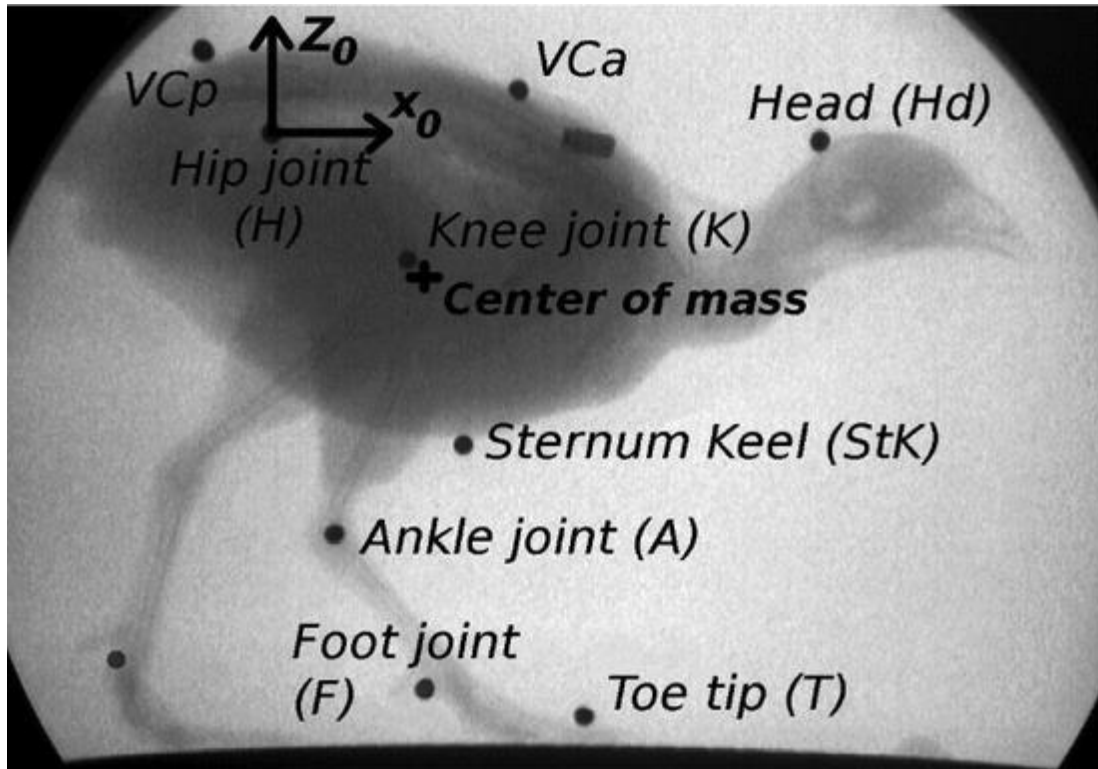
Figure 16: (Side view) X-ray image of quail with lead balls to mark vertebral column anterior point (VCa), vertebral column posterior point (VCp), hip joint (H), knee joint (K), ankle joint (A), foot joint (F), toe tip (T), head point (Hd), and sternum keel point (StK). The center of mass is located approximately between the knees. Lead balls were stuck along one leg only to avoid confusion during the coordinate-extraction process. Coordinate frame is fixed to the hip joint. X_0 is in the direction of motion. Z_0 is the vertical axis. (from Hugel et al. 2011.)

Another point where treadmill studies may deviate from overground studies is in the intrastride variations observed on the treadmill caused by energy transfer between the runner and the treadmill [36]. This happens because the vertical component of the ground reaction force (GRF) increases friction between the belt and the supporting surface, resisting sliding movements, while the horizontal component of the GRF opposes the movement of the belt during the terminal stance. The force and the horizontal velocity emounts to mechanical power, in essence the rate of energy transfer between the runner and the treadmill, and all this is caused by speed variations between different treadmills, and it may very well contribute to the many conflicting results seen in the studies between treadmill and overground running biomechanics.

Errors in judging the time when the foot is on or off the ground during analyzing - mainly putting on the foot markers - poses yet another problem that may account for the conflicting results seen. If there is a delay in the prediction of foot-on/-off,

one might overestimate the stance phase, and the opposite happens when one is too quick [36]. This may very well be the case in my study, were the birds ran in the grass, which may have impaired detailed visibility when analyzing movies.

The accomodation to running, both on the treadmill and overground, may have yet an effect of the differences. In my study and those of Lees et al. [1], birds were trained to run for weeks prior to the experiments on the treadmill. As I ran my bloodsampling experiments right after they ended their experiments, the birds was well accustomed to run on the treadmill. For the birds running overground they were trained for a few days prior to filming, mostly to see which individuals could be used for the trials. These birds were also used for treadmill experiments indoors just prior to the outside experiments [2]. It has been experienced that trained runners may adjust better to the mechanical differences in surface between treadmill and running overground, than novice runners, indicating that training and experience most likely can influence results [36].

Running stability in uneven terrain is another plausible explanation to the differences between the treadmill and overground running. Stability is defined as the system's ability to reduce a deviation in the COM trajectory caused by onetime perturbation [37]. Unlike humans, birds do not adjust leg stiffness to accomodate for varying substrates [22]. Adjusting leg stiffness reduces the perturbations experienced by the runner when changing underlayers, and is most caused by inherent properties of the animals muscoskeletal system [30]. This means the runner can maintain similar running kinematics (i.e. same SF, stance time etc.) despite the difference in forward speed. If not adjusted, the ground contact time and displacement of COM will increase as surface stiffness decreases. As stated, birds cannot use this strategy for coping with surfaces of different properties and heights. Instead, they utilize a crouched leg posture both able of shortening and lengthening, providing a greater robustness for changes in terrain height, like that typically experienced by the Svalbard ptarmigan in its natural environment.

This is done by lengthening the leg before foot touchdown, thus coping with a large amount of distrurbances [37], for example as demonstrated by bird in fig. 15 (b. Also, by running upright, the Svalbard ptarmigan aligns its sternal mass, which is significantly increased during winter due to fat accumulation, with their COM, and

this may improve their mechanical advantage. The findings of a more upright posture were also seen on the treadmill during the winter experiments when the birds were at their fattest [1]. The birds, although at this time they weren't able to reach the aerial phase of running, were found to overall locomote more economically per unit body mass during winter than summer, despite being twice as heavy. The theory of posture influencing the mechanics of running were found as the most plausible argument that the birds could carry this excess weight as economically as they did [1].

Blood gases and lactate

From the results we got from the blood gas sampling (fig. 11 and 12) and lactate (fig. 13), there was nothing to indicate that the birds were relying on anaerobic metabolism during the range of speeds tested (the same speeds used during the calorimetry experiments by Lees et al., [1].

Lactate levels, which were probably the most interesting factor in this respect, were all under 2 mM, with the exception of a few outliers, and showed no clear relation to speed. Higher intensity of mechanical work will increase the production of lactic acid, causing its concentration in the blood to rise, and the animal will rely on anaerobic metabolism. The anaerobic threshold is the body's inability to rid itself of excess lactic acid that has accumulated in the blood, and a threshold of 4mM is set as a typical approximate for aerobic capacity [38]. Thus, my data indicate that the birds were relying on aerobic metabolism throughout [26]. On this basis it is concluded that the use of indirect calorimetry was appropriate for the range of speeds employed by Lees et al. [1]. The declining levels of pCO_2 (fig. 11) with increasing speed, accompanied by an increase in pH (fig. 12) might indicate that the birds effectively are getting rid of more CO_2 in the expired air as they run faster, and that this may be tuned to increase with increased activity. Indeed, birds have a very effective respiratory system to begin with more so than mammals [39], and this supports the theory that locomotion and respiration may be coupled in order to reduce energy expenditure [40]. Getting rid of excess pCO_2 will also lower the acidity

and thus the pH effects of possible increases in lactate concentration, which may be one beneficial consequence of the observed speed-related changes in both pCO_2 and pH.

V: Conclusion

The results of my study imply that there is indeed a difference in locomotor mechanics between treadmill and overground running in these birds. Running outside on a natural substrate gives data that would represent a more natural situation for studying locomotion, as opposed to the artificial environment of the treadmill. This concern DF and stance time in particular, which were significantly lower overground than on the treadmill. This does not mean that the results of the treadmill experiments on Svalbard ptarmigan, on which my study was based, are non-valid, since they revealed between-season, between-gender and between-age class results that presumably apply both in the "treadmill" and the natural situation. Use of treadmill is not an ideal way of studying natural locomotion in the Svalbard ptarmigan, which underlines that supplementary field experiments are required to fully understand the locomotion energetics and biomechanics of these birds. Nevertheless the treadmill remains an important tool to study locomotion energetics and biomechanics due to the close control of exercise conditions that it offers, but its limitations, such as those revealed in the present study, must be appreciated!

Adknowledgements

References

1. Lees J, Nudds R, Stokkan KA, Folkow L, Codd J. Reduced metabolic cost of locomotion in Svalbard rock ptarmigan (*Lagopus muta hyperborea*) during winter. *PLoS ONE* [Internet]. 2010 [cited 2013 May 8]; **5**: e15490. Available from: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0015490

2. Nudds RL, Folkow LP, Lees JJ, Tickle PG, Stokkan K A, Codd JR. Evidence for energy savings from aerial running in the Svalbard rock ptarmigan (*Lagopus muta hyperborea*). Proc Biol Sci [Internet]. 2011 Sep; [cited 2013 May 8]; 278(1718): 2654-61. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3136837/pdf/rspb2010274 2.pdf

3. Lees JJ, Nudds RL, Folkow LP, Stokkan KA, Codd JR. Understanding sex differences in the cost of terrestrial locomotion. Proc Biol Sci [Internet]. 2012 Feb; [cited May 16]; 27(1729): 826-32. Available from: http://rspb.royalsocietypublishing.org/content/279/1729/826.full.pdf+htm l

4. Lees JJ, Stokkan KA, Folkow LP, Codd JR. Locomotor development in the Svalbard rock ptarmigan (Lagopus muta hyperborea). Polar Biol [Internet]. 2012 Jun; [cited May 16]; 35(6): 867-74. Available from: http://link.springer.com/article/10.1007/s00300-011-1131-x

5. Lees J, Folkow L, Stokkan KA, Codd J. 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. J Exp Biol [Internet]. 2013 Apr; [cited May 16]; 216(pt 8): 1355-63. Available from: http://jeb.biologists.org/content/216/8/1355.full.pdf+html

6. Pedersen ÅØ, Lier M., Routti H, Christiansen HH, Fuglei E. Co-feeding between Svalbard rock ptarmigan *(Lagopus muta hyperborea)* and Svalbard reindeer *(Rangifer tarandus platyrhynchus)*. Arctic [Internet]. 2006 Mar;

[cited 2013 May]; 59(1): 61-4. Available from:

http://www.jstor.org/stable/40512768

7. Mortensen A, Unander S, Kolstad M, Blix AS. Seasonal changes in body composition and crop content of Spitzbergen ptarmigan *Lagopus mutus hyperboreus*. Ornis Scandinavica [Internet]. 1983 Jun; [cited 2013 May 8]; 14(2): 144-8. Available from: http://www.jstor.org/stable/3676018

8. Steen JB, Unander S. Breeding biology of the Svalbard rock ptarmigan *Lagopus mutus hyperboreus*. Ornis Scandinavica [Internet]. 1985 Oct; [cited 2013 May 16]; 16(3): 191-7. Available from: http://www.jstor.org/stable/3676630

9. Stokkan KA, Mortensen A, Blix AS. Food-intake, feeding rhythm, and body-mass regulation in Svalbard rock ptarmigan. Am J Physiol [Internet]. 1986 Aug; [cited 2013 May 8]; 251(2 Pt. 2): R264-R267. Available from: http://ajpregu.physiology.org/content/251/2/R264.full.pdf+html

10. Lindgård K, Stokkan KA. Daylength control of food intake and body weight in Svalbard ptarmigan *Lagopus mutus hyperboreus*. Ornis Scandinavica [Internet]. 1989 Sep; [cited 2013 May 16]; 20(3): 176-80. Available from: http://www.jstor.org/stable/3676910

11. Mortensen A, Blix AS. Seasonal changes in resting metabolic rate and mass-specific conductance in Svalbard ptarmigan, Norwegian rock ptarmigan and willow ptarmigan. Ornis Scandinavica [Internet]. 1986 Mar; [cited 2013 May 8]; 17(1): 8-13. Available from: http://www.jstor.org/stable/3676746

12. Mortensen A, Blix AS. Seasonal changes in energy intake, energy expenditure, and digestibility in captive Svalbard rock ptarmigan and Norwegian willow ptarmigan. Ornis Scandinavica [Internet]. 1989 Mar; [cited 2013 May 16]; 20(1): 22-8. Available from: http://www.jstor.org/stable/3676703

13. Reierth E, Stokkan KA. Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). J Biol Rhythms [Internet]. 1998 Oct; [cited 2013 May 8]; 13(5): 393-402. Available from: http://jbr.sagepub.com/content/13/5/393.full.pdf+html

14. Reierth E, Van't Hof TJ, Stokkan KA. Seasonal and daily variations in plasma melatonin in the high-arctic Svalbard ptarmigan (*Lagopus mutus hyperboreus*). J Biol Rhythms [Internet]. 1999 Aug; [cited 2013 May 8]; 14(4): 314-9. Available from: http://jbr.sagepub.com/content/14/4/314.full.pdf+html

15. Stokkan KA, Harvey S, Klandorf H, Unander S, Blix AS. Endocrine changes associated with fat deposition and mobilization in Svalbard ptarmigan (*Lagopus mutus hyperboreus*). Gen Comp Endocrinol [Internet]. 1985 Apr; [cited 2013 May 16]; 58(1): 76-80. Available from: http://www.sciencedirect.com/science/article/pii/0016648085901376

16. Lindgård K, Näslund S, Stokkan KA. Annual changes in body mass in captive Svalbard ptarmigan: role of changes in locomotor activity and food intake. J Comp Physiol B [Internet]. 1995 Dec; [cited 2013 May 8]; 165(6): 445-9. Available from: http://link.springer.com/article/10.1007/BF00261298

17. Tickle PG, Richardson MF, Codd JR. Load carrying during locomotion in the barnacle goose (*Branta leucopsis*): the effect of load placement and size. Comp Biochem Physiol A Mol Integr Physiol. 2010 Jul; 156 (3), 309-17.

18. Claessens LPAM. The skeletal kinematics of lung ventilation in three basal bird taxa (emu, tinamou and guinea fowl). J Exp Zool [Internet]. 2009 Oct; [cited 2013 May 8]; 311A(8): 586-99. Available from: http://onlinelibrary.wiley.com/doi/10.1002/jez.501/pdf

19. Cavagna GA, Heglund NC, Taylor CR. Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. Am J Physiol [Internet]. 1977 Nov; [cited 2013 May 8]; 233(5): R243-R261. Available from: http://ajpregu.physiology.org//content/233/5/R243

20. Kram R, Taylor CR. Energetics of running: a new perspective. Nature [Internet]. 1990 Jul; [cited 2013 May 16]; 346(6281) 265-7. Available from: http://www.nature.com/nature/journal/v346/n6281/pdf/346265a0.pdf

21. McMahon TA, Valiant G, Frederick EC. Groucho running. J Appl Physiol [Internet]. 1987 Jun; [cited 2013 May 8]; 62(6): 2326-37. Available from: http://jap.physiology.org/content/62/6/2326.full.pdf+html

22. Gatesy SM, Biewener AA. Bipedal locomotion—effects of speed, size and limb posture in birds and humans. J Zool. 1991 May; 224(1): 127–47.

23. Abourachid A. Kinematic parameters of terrestrial locomotion in cursorial (ratites), swimming (ducks), and striding birds (quail and guinea fowl). Comp Biochem Physiol A Mol Integr Physiol [Internet]. 2001 Dec; [cited 2013 May 8]; 131:113-9. Available from: http://www.sciencedirect.com/science/article/pii/S1095643301004718

24. Taylor CR. Force development during sustained locomotion: a determinant of gait, speed and metabolic power. J Exp Biol [Internet]. 1985 Mar; [cited 2013 May 8]; 115: 252-62. Available from: http://jeb.biologists.org/content/115/1/253.full.pdf+html

25. Alexander, RM. Terrestrial locomotion. In: Goldspink G, Alexander RM editors. Mechanics and energetics of animal locomotion. London: Chapman & Hall; 1977. p 168-203.

26. Rubenson J, Heliams DB, Lloyd DG, Fournier PA. Gait selection in the ostrich: mechanical and metabolic characteristics of walking and running with and without an aerial phase. Proc Biol Sci [Internet]. 2004 May; [cited 2013 May 8]; 271(1543), 1091-9. Available from: http://www.jstor.org/stable/4142537

27. Verstappen M, Aerts P. Terrestrial locomotion in the black-billed magpie. I. Spatio-temporal gait characteristics. Motor Control. 2000 Apr; 4(2):150-64.

28. Stokkan KA, Lindgård K, Reierth E. Photoperiodic and ambient temperature control of the annual body mass cycle in Svalbard ptarmigan. J Comp Physiol B [Internet]. 1995 Oct; [cited 2013 May 16]; 165(5): 359-65. Available from: http://link.springer.com/article/10.1007%2FBF00387306

29. Daley MA, Usherwood JR, Felix G, Biewener AA. Running over rough terrain: guinea fowl maintain dynamic stability despite a large unexpected change in substrate height. J Exp Biol [Internet]. 2006 Jan; [cited 2013 May

8]; 209 (Pt 1): 171-87. Available from:

http://jeb.biologists.org/content/209/1/171.full.pdf+html

30. Ferris DP, Louie M, Farley CT. Running in the real world: adjusting leg stiffness for different surfaces. Proc R Soc Lond B Biol Sci [Internet]. 1998 Jun; [cited 2013 May 8]; 265: 989-94.* Available from:

http://rspb.royalsocietypublishing.org/content/265/1400/989.full.pdf

31. Ferris DP, Liang K, Farley CT. Runners adjust leg stiffness for their first step on a new running surface. J Biomech [Internet]. 1999 Aug; [cited 2013 May 8]; 32: 787-94. Available from:

http://www.sciencedirectec.com/science/article/pii/S0021929099000780

32. Seyfarth A, Geyer H, Günther M, Blickhan R. A movement criterion for running. J Biomech [Internet]. 2002 May; [cited 2013 May 16]; 35(5): 649-55. Available from:

http://www.sciencedirect.com/science/article/pii/S0021929001002457

33. Nelson RC, Dillman CJ, Lagasse P, Bickett P. Biomechanics of overground versus treadmill running. Med Sci Sports [Internet]. 1972 [cited 2013 May 8]; 4(4): 233-40. Available from: http://www.setantacollege.com/wp-content/uploads/Journal_db/biomechanics%20of%20overground%20versus%20treadmill%20running.pdf

34. Abourachid A. Bipedal locomotion in birds: the importance of functional parameters in terrestrial adaptation in Anatidae. Can J Zool [Internet]. 2000 Nov; [cited 2013 May 8]; 78(11):1994-8. Available from:

http://www.nrcresearchpress.com/doi/pdf/10.1139/z00-112

35. Hugel V, Hackert R, Abourachid A. Kinematic modeling of bird locomotion from experimental data. IEEE Trans Robot [Internet]. 2011 Apr [cited 2013 May 8]; 27(2): 185-200. Available from:

http://ieeexplore.ieee.org/stamp/stamp.jsp?tp=&arnumber=5722043

36. Scache AG, Blanch PD, Rath DA, Wrigley TV, Starr R, Bennell K L. A comparison of overground and treadmill running for measuring the three-dimensional kinematics of the lumbo-pelvic-hip complex. Clin Biomech [Internet]. 2001 Oct; [cited 2013 May 8]; 16(8): 667-80. Available from:

http://www.sciencedirect.com/science/article/pii/S0268003301000614

37. Blum Y, Birn-Jeffery,A., Daley MA, Seyfarth A. Does a crouched leg posture enhance running stability and robustness? J Theor Biol [Internet]. 2011 Jul; [cited 2013 May 8]; 281: 97-106. Available from: http://www.sciencedirect.com/science/article/pii/S002251931100230X

38. Yoshida T, Chida M, Ichioka M, Suda Y. Blood lactate parameters related to aerobic capacity and endurance performance. Eur J Appl Physiol Occup Physiol [Internet]. 1987; [cited May 16]; 56(1): 7-11. Available from: http://link.springer.com/article/10.1007/BF00696368

39. Calder WA, Schmidt-Nielsen K. Panting and blood carbon dioxide in birds. Am j Physiol [Internet]. 1968 Aug; [cited May 16]; 215(2): 477-82. Available from: http://ajplegacy.physiology.org/content/215/2/477.full.pdf

40. Baudinette RV. The energetics and cardiorespiratory correlates of mammalian terrestrial locomotion. J Exp Biol [Internet]. 1991 Oct; [cited 2013 May 16]; 160: 209-31. Available from: http://jeb.biologists.org/content/160/1/209.full.pdf+html