Predicting the rate of oxygen consumption from heart rate in barnacle geese *Branta leucopsis*: effects of captivity and annual changes in body condition

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SUMMARY

Quantifying a relationship between heart rate (f_H) and rate of oxygen consumption ($\dot{V}_{O_2}$) allows the estimation of $\dot{V}_{O_2}$ from f_H recordings in free-ranging birds. It has been proposed that this relationship may vary throughout an animal's annual cycle, due to changes in physiological status. Barnacle geese, *Branta leucopsis*, provide an ideal model to test this hypothesis, as they exhibit significant intra-annual variability in body mass, body composition and abdominal temperature, even in captivity. Heart rate data loggers were implanted in 14 captive barnacle geese, and at six points in the year the relationship between f_H and $\dot{V}_{O_2}$ was determined. The $f_H/\dot{V}_{O_2}$ relationship was also determined in seven moulting wild barnacle geese to examine whether relationships from captive animals might be applicable to wild animals. In captive barnacle geese, the $f_H/\dot{V}_{O_2}$ relationship was significantly different only between two out of the six periods when the relationship was determined (late September–early October and November). Accounting for changes in physiological parameters such as body mass, body composition and abdominal temperature did not eliminate this difference. The relationship between f_H and $\dot{V}_{O_2}$ obtained from wild geese was significantly different from all of the relationships derived from the captive geese, suggesting that it is not possible to apply calibrations from captive birds to wild geese. However, the similarity of the f_H and $\dot{V}_{O_2}$ relationship derived during moult in the captive geese to those during the remainder of the annual cycle implies it is not unreasonable to assume that the relationship between $f_H/\dot{V}_{O_2}$ during moult in the wild geese is indicative of the relationship throughout the remainder of the annual cycle.

Key words: heart rate, cardiovascular, barnacle goose, rate of oxygen consumption, stroke volume.

INTRODUCTION

Efficient use of energy is a key determinant of fitness (Brody, 1945; Kleiber, 1961; Butler et al., 2004). Therefore, information about the energy expenditure of free-ranging animals holds the key to understanding major aspects of their behaviour (Nagy et al., 1978; Nagy et al., 1984; Froget et al., 2001; Tolkamp et al., 2002). Using heart rate (f_H) as an indicator of the rate of oxygen consumption ($\dot{V}_{O_2}$) has proved to be an increasingly popular tool when investigating the energy expenditure of free-ranging animals (Malhotra et al., 1963; Owen, 1969; Holter et al., 1976; Flynn and Gessaman, 1979; Gessaman, 1980; Butler, 1991; Butler, 1993; Bevan et al., 1995; Green et al., 2001; Butler et al., 2002; Froget et al., 2002). The method is based on the relationship between $\dot{V}_{O_2}$ and f_H and relies on accurate calibrations between the two (Butler, 1993). The ideal approach would be to calibrate this relationship in each animal that is to be used in the field. However, the disturbance associated with calibration, the difficulty of obtaining animals and the potential interference with selected individuals usually makes this impractical (Green et al., 2001; Green and Frappell, 2007). In most cases, therefore, this relationship has been established in one group of animals and then applied to a different group of free-ranging animals, taking into account the associated inter- and intra-individual variability (e.g. Froget et al., 2001; Green et al., 2001). Most animals used in calibration experiments have been free-ranging individuals from the same population to minimise potential sources of error (Green et al., 2001; Green et al., 2005). However, studies on wild animals may want to reduce the number of individual animals used,

particularly if populations are of conservation concern. It may be possible to use captive animals from zoos or other sources for calibration experiments but differences in metabolism have been identified between wild and captive birds (McKechnie et al., 2006), suggesting that this possibility needs to be explored further.

The usefulness of the f_H method depends on the established relationship with $\dot{V}_{O_2}$ being similar under different conditions or at least for any differences to be quantified and taken into account (Butler et al., 2002). For example, Froget et al. found that the relationship between $\dot{V}_{O_2}$ and f_H in king penguins (*Aptenodytes patagonicus*) was influenced by variation in body condition and season (Froget et al., 2001). Similarly, Holter et al. noted that the relationship was influenced by season in white-tailed deer (*Odocoileus virginisanus*) (Holter et al., 1976). It appears, therefore, that determination of the $f_H/\dot{V}_{O_2}$ relationship when animals are in different physiological states is essential, and may explain some of the temporal differences observed in a number of studies (e.g. Holter et al., 1976; Gessaman, 1980).

The $f_H/\dot{V}_{O_2}$ relationship has been previously determined in captive barnacle geese *Branta leucopsis* (Nolet et al., 1992; Ward et al., 2002) (see also Butler and Woakes, 1980). For this species, there was no difference in calibrations between f_H and $\dot{V}_{O_2}$ carried out on different individuals, 10 years apart (Nolet et al., 1992; Ward et al., 2002). However, the experiments in both studies were performed at similar times in the annual cycle. Recent work on captive barnacle geese has demonstrated that they undergo changes in body mass, body composition and abdominal temperature similar to those of

wild geese (Portugal et al., 2007; Portugal, 2008; Portugal et al., 2009). For example, despite *ad libitum* access to food, captive geese lost approximately 25% of their body mass during their annual wing moult, while simultaneously experiencing an increase in the resting rate of metabolism of approximately 80% (Portugal et al., 2007). These findings are comparable with those found for wild moulting waterfowl (see Hohman et al., 1992). Therefore, captive barnacle geese present an ideal model to investigate how naturally occurring seasonal changes in physiological variables such as body mass, body composition and abdominal temperature may interact and influence the relationship between f_H and $\dot{V}_{O_2}$. We hypothesised that any intra-annual variability in the $f_H/\dot{V}_{O_2}$ relationship could be attributed to quantifiable variation in one or more of these traits.

The primary aim of this work was to construct a model from physiological variables to predict $\dot{V}_{O_2}$ in the geese. More specifically, we aimed to: (1) calibrate the relationship between f_H and $\dot{V}_{O_2}$ at points of significance during the annual cycle of a species that shows substantial intra-annual variability in several traits; (2) quantify any variability in the $f_H/\dot{V}_{O_2}$ relationship between seasonal periods, through the inclusion of physiological variables such as body mass, body composition and abdominal temperature; and (3) calibrate the relationship between f_H and $\dot{V}_{O_2}$ in wild barnacle geese, and compare the relationship with that of captive geese.

MATERIALS AND METHODS
Captive birds
A captive population of 44 barnacle geese (*Branta leucopsis* Bechstein) obtained as three-week-old goslings was maintained under natural light in a large outdoor aviary (30 m×20 m) at The University of Birmingham, UK. The goslings were obtained from Bentley Wildfowl Park (Sussex, UK), which has held a self-sustaining population of this species since 1982. The birds were two years old at the time of study. The geese were fed with a 50–50 diet (Lilico, Surrey, UK) of mixed poultry corn (4% fat, 12% protein and 71% carbohydrate) and poultry growers pellets (3% fat, 16% protein and 61% carbohydrate). Food and water were available *ad libitum*. All regulated procedures were performed by UK Home Office licensed personnel in possession of a Personal License, and working under the auspices of a corresponding Project License, as set out in the Animals (Scientific Procedures) Act 1986.

Fourteen birds out of the flock of 44 were trained to walk on a treadmill for 2–3 weeks prior to the first experiments to build up physical condition and become fully accustomed to the experimental surroundings and the handler. Training consisted of walking the birds at previously determined speeds for 2–3 h a week (see Ward et al., 2002). Sampling periods were selected based on year round body mass data, collected from the same captive flock of barnacle geese the previous year (see Portugal et al., 2007). Points chosen included the maximum and minimum body masses recorded in the year, and periods of body mass increase and decrease, in particular, those associated with wing moult. Sampling periods were, therefore, February, May, late July–early

August, mid August–early September, late September–early October and November 2006 (see Table 1 for sample sizes). Sample sizes varied for each respirometry period, as the two periods during moult were very close to each other. At this time, we chose to maximise the number of seasonal periods rather than the number of birds used in all sampling periods, and so not all birds were used in all periods.

Moult score
A 5-point classification moult score system was used to assign the stage of moult in both the wild and captive geese (e.g. Bridge, 2004; Portugal et al., 2007). Moult score was defined as: (1) pre-wing moult; (2) primaries and secondaries remain, new primary pin visible; (3) all primaries and secondaries missing; (4) new primaries visible well beyond primary coverts and secondaries visible beyond secondary coverts (small flight feathers covering the primaries and secondaries); and (5) post wing-moult.

Respirometry
In order to minimise the duration of the sampling periods, two respirometry systems (equipment from ADInstruments, Colorado Springs, CO, USA) were used simultaneously to record $\dot{V}_{O_2}$. Full information and particulars on the respirometry set-up, equipment and analysis, is given in detail in Portugal et al. (Portugal et al., 2007) (see also Green et al., 2001; Wilson et al., 2006). The temperature for all respirometry experiments was within the thermoneutral zone for barnacle geese (Ward et al., 2002).

Prior to the walking periods, each goose was rested overnight between the hours of 23:00 h and 07:00 h (GMT) to measure resting $\dot{V}_{O_2}$ (see Portugal et al., 2007). Food (not water) was withheld 8 h prior to placing the birds in the respirometer for overnight experiments. Water was offered to the birds once the overnight sampling period was complete and before walking began. Birds were always walked between 07:30 h and 10:30 h (GMT) for each period. Random number tables were used to determine the order of treadmill speeds so that the geese did not become accustomed to a repeated pattern of speeds. Each bird was exercised at up to seven different speeds (0.5–3.5 km h^{-1}). This speed range included the lowest speed available on the treadmill and the highest speed the birds could maintain for sufficient time to allow for gas equilibration (10–20 min). $\dot{V}_{O_2}$ was calculated as a 5 min average of the steady-state values observed. Birds were monitored constantly, and data were excluded if the birds showed evidence of fatigue and were unable to maintain station within the respirometer for a sufficient length of time to allow for gas equilibration. On some occasions, gas equilibration did not occur at 0.5 km h^{-1} due to the birds not walking at a constant speed. When this occurred, the data were not included in further analyses. All geese that were involved in respirometry experiments during the late July–early August period were observed to have a moult score between 2 and 4 and were considered to be in peak wing moult.

Table 1. Sample sizes and means (± s.e.m.) of each physiological variable measured for each sampling period in 14 captive barnacle geese

Period	N	Body mass (kg)	Lean body mass (kg)	Total body fat content (kg)	Body temperature (°C)
Feb	6	2.02±0.11	1.84±0.10	0.18±0.01	38.5±0.34
May	6	1.83±0.14	1.58±0.12	0.25±0.01	38.5±0.27
Jul–Aug	12	2.02±0.07	1.72±0.24	0.30±0.01	39.1±0.18
Aug–Sep	7	1.61±0.06	1.55±0.05	0.16±0.00	38.7±0.20
Sep–Oct	10	1.78±0.02	1.42±0.01	0.36±0.00	37.7±0.39
Nov	6	1.82±0.22	1.62±0.08	0.19±0.01	38.4±0.29

f_H

f_H and abdominal temperature were measured using a miniature heart rate data logger (HRDL), which has been successfully used with this species on previous occasions (Butler et al., 1998; Ward et al., 2002). HRDLs (55 mm×25 mm×7 mm) were programmed to record f_H every 5 s. The implantation procedure and further information on the HRDLs are given in detail elsewhere (e.g. Butler et al., 1998; Green et al., 2005).

Physiological variables

Five other physiological and morphological variables were recorded alongside f_H and $\dot{V}_{O_2}$ in each sampling period. Each variable was measured from the same individual geese and taken at the same time as the respirometry experiments. The variables were body mass (BM), lean body mass (LBM), total body fat (TBF), structural size (SS) and abdominal temperature (T_{ab}). Tarsus length was used as an indicator of SS, as previous studies on wild barnacle geese have shown it to be the best indicator of SS, and a principal component analysis showed tarsus length in the captive geese to be significantly correlated with other structural body parts (Larsson et al., 1998; Portugal, 2008). TBF and LBM were estimated using the isotope dilution technique (Speakman et al., 2001), which has previously been shown to be a good measure of TBF content in birds (Kerstel et al., 2006; Eichorn and Visser, 2008) (for details, see Portugal, 2008).

Wild birds

Data were obtained from seven adult barnacle geese of the breeding population at Ny-Ålesund, in the high Arctic (79°55′, 11°56′E) on the west coast of the island of Spitsbergen. The birds were captured in corral nets during the post-breeding season moult, during the last two weeks of July and the first week in August when they were flightless. Geese were weighed and housed outdoors as a group, with food and water available *ad libitum*.

Respirometry

The same respirometry system used for the captive birds was used to measure $\dot{V}_{O_2}$ in the wild geese, with the following modifications. Air was pulled by a downstream sealed pump at a flow of about 30 l min⁻¹, controlled by a mass flow controller (Aalborg, Orangeburg, NY, USA). The excurrent air was sub-sampled (100 ml min⁻¹), passed through a drying column and analysed by the O_2 and CO_2 analysers. The recorded traces for O_2 and CO_2 were averaged every 5 s, corrected to instantaneous values as if the system had been sealed assuming a first-order linear system (see Frappell et al., 1989) and subsequently averaged every 2 min. Calculation of $\dot{V}_{O_2}$ was determined as previously described [see Appendix in Frappell et al. (Frappell et al., 1992)]. As with the captive geese, birds were held in the respirometer overnight to measure resting rates of metabolism (Portugal et al., 2007). At 08:00 h (EST) birds were offered water, before commencement of walking on the treadmill. The protocol for walking, including the range of speeds, was the same as that for the captive geese.

Heart rate

f_H was monitored using a customised heart rate transmitter system (25 g, POLAR a3, Polar Electro Oy, Finland). The heart rate transmitter was attached dorsally to the feathers using lightweight paper tape, and custom-made brass electrodes were inserted under the skin. The transmitter unit had a functional range of approximately 1 m, and the receiver unit was placed on top of the respirometer to ensure reception. The outputs were collected at 200 Hz (Powerlab, ADInstruments).

Statistical analysis

As stated, the overall aim was to construct a single model of physiological variables (f_H, BM, TBF, LBM, SS and T_{ab}) to predict $\dot{V}_{O_2}$ in the geese throughout their annual cycle. Captive geese were examined first, using general linear mixed models (GLM) (Zar, 1984) in Minitab to establish the relationship between f_H and $\dot{V}_{O_2}$ within each of the six sampling periods.

To compare sampling periods, a novel approach was adopted that accounted for the unbalanced design, which resulted from having different sets of individuals in some periods. The slope of the relationship between f_H and $\dot{V}_{O_2}$ for each individual in each sampling period was treated as the dependent variable. We examined the effects of sampling period (fixed effect) with goose ID as a random factor and the physiological variables (f_H, BM, LBM, TBF, SS and T_{ab}) as covariates. A model of best-fit was achieved by firstly including all possible main effects and all possible second degree interactions in the model. Each non-significant term was then removed, one iteration at a time, by backwards elimination (Green et al., 2005). Non-significant main effects were kept in the model if the variable in question was part of a statistically significant interaction. Our objective was to create a single model in which both sampling period and the interaction between sampling period and slope were not significant effects.

Five 'sets' of models were investigated. Model set one used the slopes from the relationships between f_H and $\dot{V}_{O_2}$, firstly using 'whole animal' values of $\dot{V}_{O_2}$ and subsequently $\dot{V}_{O_2}$ divided by BM (kg), LBM (kg), TBF (kg) and SS (tarsus length, mm). Model set two repeated this analysis but, in this case, each of these measurements (except SS) was also divided by tarsus length as an indicator of structural size, and re-run with $\dot{V}_{O_2}$ divided by the resultant structurally corrected value. In model sets one and two, a variable was not included as a covariate if it had been used to correct $\dot{V}_{O_2}$. Model set three examined the exponent by which values of $\dot{V}_{O_2}$ were mass corrected. In model sets one and two, values of $\dot{V}_{O_2}$ were corrected to an exponent of 1. However, data from experiments studying resting $\dot{V}_{O_2}$ in the captive geese (Portugal et al., 2007) showed that this exponent may change throughout the season. Therefore, this set re-constructed every analysis from model sets one and two and re-analysed them with mass exponents ranging from 0.5 to 1.5 inclusive in increments of 0.1. Model set four took the best model from sets one to three and investigated cases where differences existed between sampling periods. These *post-hoc* comparisons were made by comparing each sampling period in a pairwise fashion, with the appropriate sequential Bonferonni corrections applied (Holm, 1979; Shaffer, 1986). Those sampling period comparisons, which indicated no significant interaction between sampling period and f_H were pooled into groups, until the calibration regression lines from all sampling periods within one group were not significantly different from each other. Finally, model set five compared the calibration regression lines from the captive geese with that of the wild barnacle geese, studied during their annual wing moult in mid July–early August. For all statistical tests used α=0.05, with α adjusted for the number of tests when applying Bonferroni corrections.

RESULTS

GLM revealed f_H and $\dot{V}_{O_2}$ to be significantly related in each of the individual sampling periods of the captive geese (Fig. 1, Table 2). Considerable variation could be observed in both slope and intercept and the range of both f_H and $\dot{V}_{O_2}$ values between the different sampling periods.
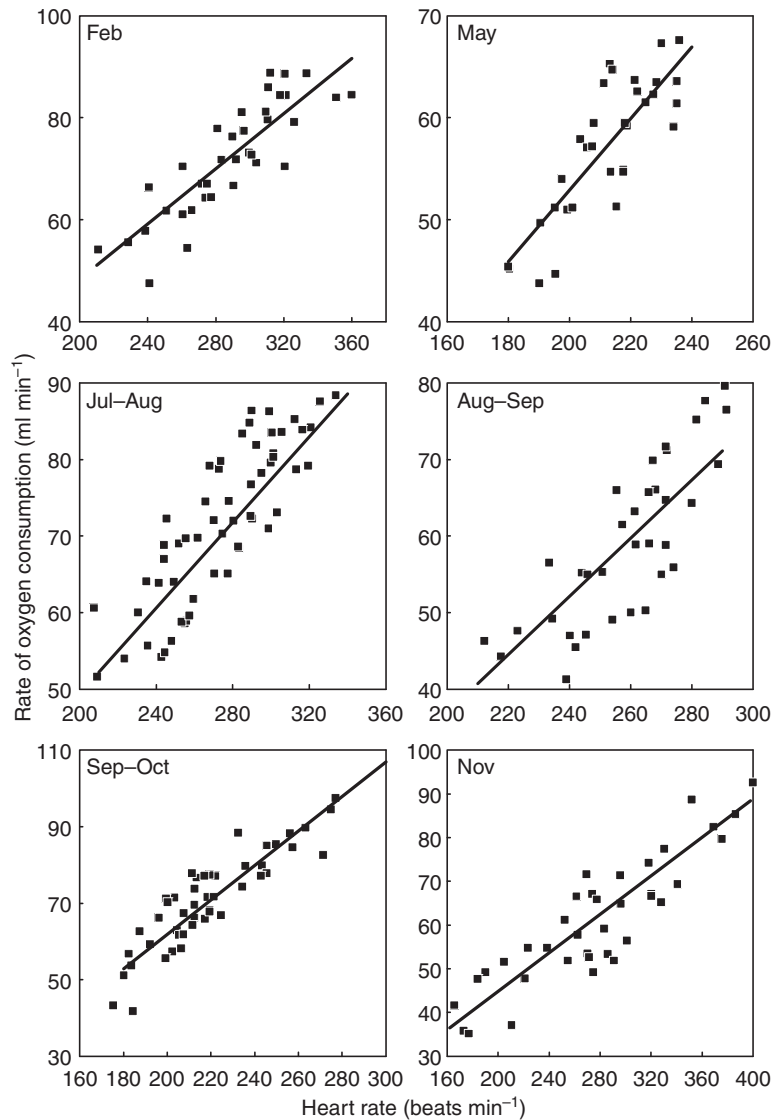
Fig. 1. Rate of oxygen consumption as a function of heart rate during six sampling periods throughout the year for captive barnacle geese. Rate of oxygen consumption is presented as that for the whole-animal data. Details of the regression lines and sample sizes are presented in Tables 1 and 2.

Model set one

This set of analyses aimed to determine what correction factor might be applied to $\dot{V}_{O_2}$ to improve the relationship with f_H and examined differences between sampling periods. Each analysis in this set was run with either whole-animal values of $\dot{V}_{O_2}$ or $\dot{V}_{O_2}$ corrected by BM, LBM, TBF, T_{ab} or SS. Of note was: (1) in four of the models, there was a non-significant interaction between f_H and BM but BM remained a significant main effect in all of the models; and (2) none of the GLMs was successful in removing sampling period as a significant effect.

Model set two

This set of analyses also incorporated whole-animal values of $\dot{V}_{O_2}$ along with values of $\dot{V}_{O_2}$ corrected to BM, LBM and TBF. However, in this instance, each value of BM, LBM and TBF had in turn been corrected according to structural size by dividing by tarsal length. Each analysis in model set one was thus re-run with the structurally corrected values of BM, LBM and TBF applied to correct $\dot{V}_{O_2}$. Key results from this set of analyses were: (1) structurally correcting values of BM, LBM and TBF had no significant effect on the results from model set one; and (2) thus, none of the models from this set

of analyses was successful in removing sampling period as a significant main effect.

Model set three

This set examined the exponent by which values of $\dot{V}_{O_2}$ were mass corrected, by re-analysing all of the models from sets one and two with mass exponents ranging from 0.5 to 1.5 (inclusive) in increments of 0.1. Of note was: (1) as with model set two, altering the mass exponent did not change the overall results nor which main effects were significant nor the significant interactions; and (2) in summary, changing the mass exponent did not improve the accuracy (based on the R^2) of the model nor did it remove sampling period as a significant interaction.

At this point, none of the analyses had been successful in removing sampling period as a significant influence on $\dot{V}_{O_2}$, and so it was not possible to create one single calibration line that could be constructed from all six sampling periods and applied to year round data sets. For further analysis, $\dot{V}_{O_2}$ corrected for body mass (mass-specific $\dot{V}_{O_2}$) was chosen as it had the greatest effect on reducing variability between sampling periods. The model with mass corrected $\dot{V}_{O_2}$ had the highest R^2 and this approach was the most

Table 2. Rate of oxygen consumption as a function of heart rate in barnacle geese

Captive	Period	Equation of the line	R^2	$F_{\text{degrees of freedom}}$	P
Whole-animal	Feb	$\dot{V}_{O_2}=0.27\ (\pm0.02),\ f_H-5.62\ (\pm6.6)$	0.87	$F_{1,29}=139.6$	$P<0.001$
	May	$\dot{V}_{O_2}=0.35\ (\pm0.04),\ f_H-17.1\ (\pm8.7)$	0.79	$F_{1,26}=73.31$	$P<0.001$
	July–Aug	$\dot{V}_{O_2}=0.28\ (\pm0.02),\ f_H-6.64\ (\pm6.6)$	0.88	$F_{1,44}=140.72$	$P<0.001$
	Aug–Sep	$\dot{V}_{O_2}=0.38\ (\pm0.04),\ f_H-39.1\ (\pm10.7)$	0.86	$F_{1,28}=86.23$	$P<0.001$
	Sep–Oct	$\dot{V}_{O_2}=0.22\ (\pm0.02),\ f_H-22.7\ (\pm6.34)$	0.83	$F_{1,28}=82.95$	$P<0.001$
	Nov	$\dot{V}_{O_2}=0.22\ (\pm0.02),\ f_H-0.83\ (\pm5.3)$	0.88	$F_{1,30}=130.4$	$P<0.01$
Mass-specific	Feb	$\dot{V}_{O_2}=0.14\ (\pm0.01),\ f_H-3.23\ (\pm3.8)$	0.90	$F_{1,29}=109.2$	$P<0.001$
	May	$\dot{V}_{O_2}=0.20\ (\pm0.02),\ f_H-9.52\ (\pm5.3)$	0.91	$F_{1,26}=61.17$	$P<0.001$
	July–Aug	$\dot{V}_{O_2}=0.14\ (\pm0.01),\ f_H-2.24\ (\pm3.6)$	0.94	$F_{1,44}=111.7$	$P<0.001$
	Aug–Sep	$\dot{V}_{O_2}=0.22\ (\pm0.02),\ f_H-22.72\ (\pm6.3)$	0.83	$F_{1,28}=82.95$	$P<0.001$
	Sep–Oct	$\dot{V}_{O_2}=0.26\ (\pm0.01),\ f_H-16.31\ (\pm2.8)$	0.96	$F_{1,37}=398.5$	$P<0.001$
	Nov	$\dot{V}_{O_2}=0.12\ (\pm0.01),\ f_H+0.117\ (\pm3.0)$	0.91	$F_{1,30}=120.8$	$P<0.001$
Wild					
Whole-animal	–	$\dot{V}_{O_2}=1.92\ (\pm0.25),\ f_H-181.13\ (\pm38)$	0.67	$F_{1,24}=12.90$	$P<0.001$
Mass-specific	–	$\dot{V}_{O_2}=1.04\ (\pm0.14),\ f_H-99.51\ (\pm21.1)$	0.60	$F_{1,24}=109.3$	$P<0.001$
Captive					
Mass-specific	Pooled line	$\dot{V}_{O_2}=0.20\ (\pm0.01),\ f_H-9.31\ (\pm3.7)$	0.79	$F_{1,182}=257.6$	$P<0.001$

Data are a result of general linear model analysis, values are presented as whole-animal and mass-specific values of rate of oxygen consumption (± s.e.m.), and relate to Fig. 2 for captive geese and Fig. 3 for wild birds.

parsimonious, in that it included the least additional variables and hence error terms (Fig. 2; Table 2).

Model set four
This set compared the slope of the relationship between f_H and mass specific $\dot{V}_{O_2}$ between sampling periods in a pairwise fashion. The purpose of this analysis was to derive the fewest sampling periods that were not significantly different from one another. The key results of these analyses were: (1) only late September–early October and November (regression lines 5 and 6 in Fig. 2) were significantly different from each other; and (2) the result of these analyses was two regression lines, late September–early October, and then a second regression line incorporating February, May, late July–early August, mid August–early September and November (Table 2). All
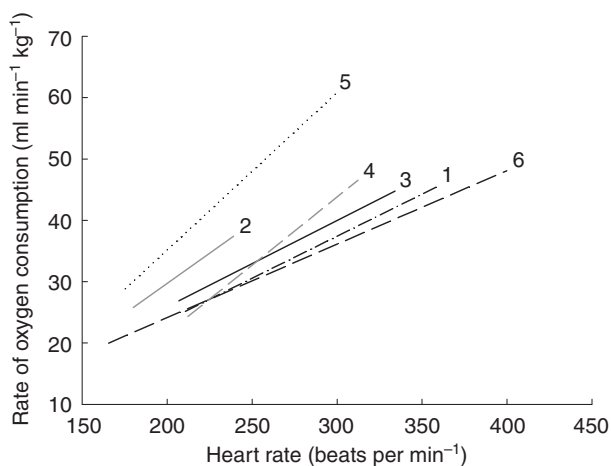

Fig. 2. Mass-specific rate of oxygen consumption as a function of heart rate during six sampling periods throughout the year in captive barnacle geese. Periods were: February (1, dot–dash black line), May (2, grey line), late July–early August (3, black line), mid August–early September (4, large-dashed grey line), late September–early October (5, small-dotted black line) and November (6, large-dashed black line). Details of the regression lines are presented in Table 2.

regression lines within this group were not significantly different from each other, so the data were combined.

Model set five
This final stage of analysis compared wild and captive geese. There was a significant relationship between f_H and $\dot{V}_{O_2}$ in the wild geese, and a simple GLM model (Table 2) revealed f_H to explain 67% and 60% of the variance for whole-animal and mass-specific $\dot{V}_{O_2}$, respectively. The slope of the relationship between f_H and $\dot{V}_{O_2}$ for each individual in each period for the captive geese was compared to that for the wild birds. For this, mass-specific $\dot{V}_{O_2}$ was used again (Fig. 3). The wild goose data were then also compared in the same manner with the two regression lines constructed on the captive geese in model set four. Key results from this analysis were: (1) all of the regression lines from the six sampling periods in the captive geese were significantly different from that of the wild birds; (2) the two regression lines constructed in model set four were also significantly different from that of the wild geese; and (3) using whole-animal values of $\dot{V}_{O_2}$ did not change these conclusions.

DISCUSSION
Using f_H to predict $\dot{V}_{O_2}$ in barnacle geese
Many authors have previously highlighted the need to establish the relationship between f_H and $\dot{V}_{O_2}$ at various stages throughout the annual cycle (e.g. Bevan et al., 1995; Green et al., 2001; Butler et al., 2002). Here, for the first time, we provide this relationship from six points in the annual cycle, for a species that exhibits significant seasonal changes in body mass, body composition and abdominal temperature, even when in captivity (Portugal et al., 2007; Portugal, 2008; Portugal et al., 2009).

For each sampling period in the captive barnacle geese, with both whole-animal and mass-specific values of $\dot{V}_{O_2}$, there was a significant relationship between f_H and $\dot{V}_{O_2}$, and the amount of variability explained by the GLMs (70%) is consistent with that found by Ward et al. (Ward et al., 2002). Apart from the mid August–early September sampling period (Table 2), including the effect of body mass by calculating mass-specific $\dot{V}_{O_2}$, as opposed to whole-animal, values greatly improved the accuracy of the models. During mid August–early September, the captive geese are
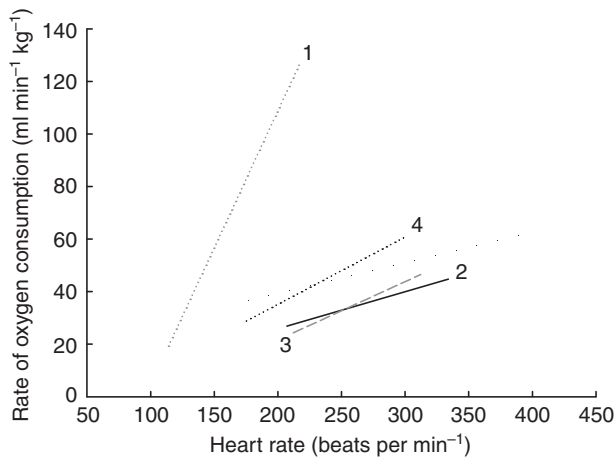
Fig. 3. Mass-specific rate of oxygen consumption as a function of heart rate for seven wild birds recorded during moult (1, small-dotted grey line, see Table 2 for details). For comparison, the relationship between rate of oxygen consumption and heart rate from captive birds is shown, from a similar period in the annual cycle: late July–early August (2, black line), mid August–early September (3, dashed grey line) and late September–early October (4, dotted line).

completing their annual wing moult, resulting in different individuals being at slightly different stages of moult completion and thus in different physiological states. Therefore, the effect of body mass and body fat on the $f_H/\dot{V}_{O_2}$ relationship may be more complex then that at other points in the annual cycle.

The suggested effects of body fat (adipose tissue) on rate of energy expenditure during exercise are somewhat contradictory. Fat is metabolically inactive but can elevate overall metabolic rate by increasing the energy demands of other tissues, i.e. energy is required from lean tissues to heat and transport the fat tissue (e.g. Froget et al., 2001; Nudds and Bryant, 2001), and also increase the cost of locomotion (the net cost of transport). However, our results suggest that the changes in the cardiovascular system in response to gains in body fat may be systematic, as the relationship between f_H and $\dot{V}_{O_2}$ did not change significantly between five out of the six of the sampling periods, despite significant changes in body mass, body composition and total body fat (Portugal et al., 2007; Portugal, 2008; Portugal et al., 2009).

Rates of $\dot{V}_{O_2}$ achieved at high speeds during treadmill exercise in late September–early October are greater than those recorded at any other point in the year in the captive geese. During strenuous exercise, both running and flying, birds will dissipate excess heat from the legs and feet or through panting (Bech and Nomoto, 1982). Froget et al. noted that during treadmill walking, king penguins were exposed to an unnatural situation, i.e. performing intense exercise in their thermoneutral zones or possibly even above it (Froget et al., 2002). Therefore, it could be possible that although the captive geese were in their thermoneutral zone, running in the treadmill experiments in late September–early October, when they have the highest relative body percentage of fat during their annual cycle (Portugal, 2008), caused the birds to be heat stressed, resulting in a change in the relationship between f_H and $\dot{V}_{O_2}$.

Alternatively, in wild barnacle geese, Bishop et al. suggested that a selective perfusion of tissues vital for support of locomotor activity could possibly occur as a physiological strategy to maximise flight performance (Bishop et al., 2002). Captive geese may undergo the same physiological change so that during late September–early

October they are better suited for flight as opposed to leg-powered locomotion. As a result, the potential increase in blood flow to the legs and feet to dissipate heat during treadmill exercise may influence the overall cardiovascular system and, in turn, the relationship between f_H and $\dot{V}_{O_2}$.

Comparing captive and wild geese

The $f_H/\dot{V}_{O_2}$ relationship in the captive geese that is most similar (although still significantly different) to that of the wild geese is the relationship determined during late September–early October. This was also the only calibration equation in the captive geese that was significantly different from that of any other sampling period (November). Late September–early October is the migratory period of the wild birds. Much evidence gathered from studying captive geese suggests they undergo similar patterns of physiological and behavioural change to those of their wild conspecifics (Butler and Woakes, 2001; Portugal et al., 2007; Portugal, 2008; Portugal et al., 2009). However, the difference in the $f_H/\dot{V}_{O_2}$ relationships between the captive and wild geese suggests though that there is a limitation to the similarities between them nonetheless, which is likely to be linked to the difference in physical fitness between the two. However, it may be possible that in the autumn, the captive geese are also undergoing similar adjustments to their cardiovascular system in preparation for their 'autumn migration' as the wild geese would be undertaking (Butler et al., 1998). Just prior to their autumn migration, wild barnacle geese exhibit hypertrophy of their hearts in preparation for the long migratory flight southwards to their wintering grounds (Bishop et al., 2002). Data on year round body mass, body composition and abdominal temperature suggest these factors at least are responding in the same manner as wild birds in the autumn period (Butler and Woakes, 2001; Portugal et al., 2007; Portugal, 2008; Portugal et al., 2009).

Regardless of what correction factor was applied to $\dot{V}_{O_2}$, the significant difference in the $f_H/\dot{V}_{O_2}$ relationship between captive and wild barnacle geese persisted. The wild moulting barnacle geese had significantly lower f_H for a given $\dot{V}_{O_2}$. This was corroborated by data from overnight resting $\dot{V}_{O_2}$ and f_H experiments. Resting $\dot{V}_{O_2}$ during moult was not significantly different between captive and wild geese. However, resting f_H was significantly lower in the wild geese (50 beats min^{-1}) in comparison with 75 beats min^{-1} in the captive geese (Portugal, 2008). This is more than likely related to the greater overall physical fitness of the wild geese in comparison with their captive conspecifics. Generally, the more athletic, or fitter, the individual, the lower its heart rate for a given $\dot{V}_{O_2}$, both during rest and exercise (Henderson et al., 1927; Bock et al., 1928; Butler and Turner, 1988; Bjornstad et al., 1993). As fitness improves, the heart enlarges, ventricular stretching is enhanced and blood volume increases, resulting in an increased stroke volume, allowing a reduction in the heart rate (Beswick and Jordan, 1961; Margaria, 1963; Butler, 1993; McPhee et al., 2003). Butler and Turner compared various morphological aspects of the cardiovascular and locomotor system in trained and untrained captive tufted ducks, *Aythya fuligula* (Butler and Turner, 1988). Trained tufted ducks had increased maximal swimming velocities, lower heart rates, higher oxygen pulses, more capillaries in their locomotor muscles, and these capillaries were present at a higher density. Maximal $\dot{V}_{O_2}$ ($\dot{V}_{O_2,max}$) was greater in the trained ducks but there was no change in maximum f_H. The greater $\dot{V}_{O_2,max}$ was the result of larger cardiac stroke volume in the trained birds, giving greater cardiac output, and increased oxygen extraction related to the increase in capillary density. Pelsers et al. also noted that intracellular transportation of fatty acids from the capillary network to the mitochondria in the muscles is

better developed in wild barnacle geese (Pelsers et al., 1999). Long-term captive geese had lower concentrations of fatty acid binding proteins and citrate synthase in the pectoral muscle than those of wild pre-migratory geese (Pelsers et al., 1999).

In addition to this difference, wild birds had much higher $\dot{V}_{O_2}$ than captive birds during exercise, despite the range of speeds being identical in the two sets of experiments. We suggest that this may be due to the wild birds being unfamiliar with the experimental setup and unaccustomed to being handled or running on the treadmill. As a result the wild birds expended more energy to move at the same speeds as the captive birds. The captive birds each had several training sessions, and observations of the exercise sessions suggested that the wild birds were less likely to run rhythmically or consistently than the captive birds. This lack of familiarity and inconsistency in gait may also explain the lower R^2 values for the $f_H/\dot{V}_{O_2}$ relationships in wild birds (Table 2) (Butler and Turner, 1988; Bishop and Bulter, 1995).

Application to free-ranging animals

As all six of the $f_H/\dot{V}_{O_2}$ calibration lines from the captive geese were significantly different from the relationship derived for the wild geese, it would not be possible to apply calibrations obtained from captive birds to f_H data recorded in wild geese. However, what the calibration lines from captive birds do demonstrate is that the relationship between f_H and $\dot{V}_{O_2}$ determined during the flightless phase of wing moult is not significantly different from those derived during most of the year (the exception being late September–early October). This suggests that it may be possible to apply the calibration between f_H and $\dot{V}_{O_2}$ established during moult in the wild geese to the rest of the annual cycle. Caution, however, should be used when applying this calibration to f_H data recorded in the autumn period. In any case, during late September, wild barnacle geese undertake 4–5 flights as part of their southward migration. The $f_H/\dot{V}_{O_2}$ relationship of barnacle geese during flight is significantly different from that obtained from walking geese (Butler and Woakes, 1980; Nolet et al., 1992; Bishop and Butler, 1995; Bishop et al., 2002). Therefore, the $f_H/\dot{V}_{O_2}$ calibrations from the captive and wild geese in this study would not be suitable for converting f_H values during flight into $\dot{V}_{O_2}$ during late September.

LIST OF ABBREVIATIONS

BM	body mass
f_H	heart rate
GLM	general linear model
HRDL	heart rate data logger
LBM	lean body mass
SS	structural size
T_{ab}	abdominal body temperature
TBF	total body fat
$\dot{V}_{O_2}$	rate of oxygen consumption

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