Erratum

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This paper was unfortunately printed with two small errors. We would like to take this opportunity to correct these errors for all readers.

(1) On p. 679, Equation 12, which currently reads:

$$s\dot{V}_{O_2} = -118.18f_H + 0.29 , \quad (12)$$

should read:

$$s\dot{V}_{O_2} = 0.29f_H - 18.88 , \quad (12)$$

(2) On p. 676, the third sentence of the last paragraph, which currently reads:

Analysis of covariance (ANCOVA) was used to compare the values of the constants *a* (the slope) and *b* (the intercept) of the individual regressions within each group.

should read:

Analysis of covariance (ANCOVA) was used to compare the values of the constants *b* (the slope) and *a* (the intercept) of the individual regressions within each group.

The authors apologise for any inconvenience this may have caused.

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HEART RATE AND RATE OF OXYGEN CONSUMPTION OF EXERCISING MACARONI PENGUINS

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Summary

Twenty-four macaroni penguins (*Eudyptes chrysolophus*) from three groups, breeding males (*N*=9), breeding females (*N*=9) and moulting females (*N*=6), were exercised on a variable-speed treadmill. Heart rate (f_H) and mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) were recorded from the animals, and both f_H and $s\dot{V}_{O_2}$ were found to increase linearly with increasing treadmill speed. A linear regression equation described the relationship between f_H and $s\dot{V}_{O_2}$ for each individual. There were no significant differences in these regressions between breeding and moulting females. There were significant differences in these relationships between all females and breeding males. f_H and $s\dot{V}_{O_2}$ were recorded from five of these animals for a total of 24 h. When f_H was used to predict $s\dot{V}_{O_2}$ for the 24 h period using the derived regressions, the estimate was not significantly different from the measured values, with an average error of –2.1 %. When f_H was used to predict $s\dot{V}_{O_2}$ for the 5 min intervals used for the calibration in all 24 birds, the estimate was not significantly different from the observed values, and the average error was only +0.47 %. Since the $f_H/s\dot{V}_{O_2}$ relationship was the same during periods of the annual cycle when the animals were inactive/fasting and active/foraging, it seems reasonable that, as long as sex differences are taken into account, f_H can be used to predict the metabolic rates of free-ranging macaroni penguins all year round.

Key words: heart rate, oxygen consumption, macaroni penguin, *Eudyptes chrysolophus*, exercise, metabolic rate, fasting, foraging.

Introduction

Approximately 80 % of the avian biomass in the Antarctic region is made up of penguins (Croxall, 1984). In the Southern Ocean around the island of South Georgia, large resident populations of penguins (Woehler, 1993) are thought to be significant consumers of marine resources (Croxall et al., 1997). These resources include Antarctic krill (*Euphausia superba*), which is the major food item for several species including Antarctic fur seal (*Arctocephalus gazella*; Reid, 1995; Reid and Arnould, 1996) and gentoo penguin (*Pygoscelis papua*; Croxall et al., 1997) and which also has the potential to form a major fishery. Most models that estimate population food consumption are based on energetics (Croxall, 1995). Thus, accurate estimates of energy expenditure are essential. The energy budgets of individuals that are used to construct population energy budgets and food consumption rates should, ideally, be sensitive to changes in behaviour so that different classes of individual (e.g. adults, juveniles, breeders, non-breeders) can be reflected in the energy budget. To avoid the need to measure energy expenditures in all these classes of individual, it is important to be able to construct energy budgets from activity budgets. This requires the measurement of the energy expenditures associated with the major classes of activity, such as swimming, diving, feeding, walking, incubating and moulting.

Several studies have attempted to obtain the energy costs of specific activities in penguins, either in the laboratory (Pinshow et al., 1976; Butler and Woakes, 1984; Baudinette and Gill, 1985; Culik and Wilson, 1991) or in the field through the use of doubly labelled water (DLW) (Davis et al., 1983; Nagy et al., 1984; Costa et al., 1986; Nagy and Obst, 1992). The DLW technique can give a reasonably accurate estimate of energy expenditure for an animal and is relatively simple to use in the field. However, the estimate obtained is only an average for the duration of the experiment, which is limited to a few days in penguins by the biological half-life of the injected stable isotopes (Speakman, 1997, p. 224). Despite refinements to this technique (Davis et al., 1989; Culik and Wilson, 1991; Gales et al., 1993), it is still difficult to obtain estimates of rates of energy expenditure for specific activities such as foraging, sustained swimming or bouts of diving.

More recently, heart rate (f_H) has been used as a proxy for the measurement of metabolic rate (Owen, 1969; Nolet et al., 1992; Bevan et al., 1994; Bevan et al., 1995a; Boyd et al., 1995; Butler et al., 1995). Heart rate and rate of oxygen

consumption ($\dot{V}_{O_2}$) are related to each other, as illustrated by the Fick equation:

$$\dot{V}_{O_2} = f_H \dot{V}_s (Ca_{O_2} - C\bar{v}_{O_2}), \quad (1)$$

where $\dot{V}_{O_2}$ is rate of oxygen consumption, f_H is heart rate, $\dot{V}_s$ is cardiac stroke volume (the amount of blood pumped during one heart beat), Ca_{O_2} is the oxygen content of arterial blood and $C\bar{v}_{O_2}$ is the oxygen content of mixed venous blood.

If $\dot{V}_s(Ca_{O_2}-C\bar{v}_{O_2})$, the oxygen pulse, remains constant or varies systematically, then it is possible to demonstrate a simple relationship between $\dot{V}_{O_2}$ and f_H and, hence, to calculate the former from the latter (Butler, 1993). Studies have shown that this method is at least as robust as the DLW technique for most species, including gentoo penguins (Bevan et al., 1995c). Recent developments in technology with regard to electronic miniaturisation have made it possible to monitor and record f_H in free-ranging animals over long time periods (Woakes et al., 1995). Heart rate is then a feasible and useful technique for estimating metabolic rate for extended periods of up to a year at a resolution of just a few minutes. However, the success of this technique depends on the robustness of the relationship between f_H and $\dot{V}_{O_2}$. Ideally, validation is required at different times of year (Flynn and Gessaman, 1979) under differing social conditions (Holter et al., 1976), for both sexes and for all types of activities (Woakes and Butler, 1983) so that any appropriate corrections can be made.

The present study tests the hypothesis that heart rate can be used as a proxy of metabolic rate in macaroni penguins (*Eudyptes chrysolophus*). The aims are (i) to measure f_H and $\dot{V}_{O_2}$ of penguins walking on a treadmill and determine the relationship between these two variables, (ii) to develop and refine the statistical methods used to describe this relationship and the errors associated with its use, (iii) to determine whether the relationship is valid over an extended period in the laboratory and (iv) to determine whether this relationship is significantly different at different stages of the annual cycle when the animals may be experiencing different physiological stresses.

Materials and methods

Animals

Although the United Kingdom Animal (Scientific Procedures) Act 1986 does not apply to South Georgia, where this study was conducted, we were meticulous in following its provisions, especially those set out by the Home Office in the Official Guidance on the operation of the Act. As our benchmark, we followed guidance to researchers using similar methods in the United Kingdom. Our procedures also conformed to the Code of Ethics of Animal Experimentation in Antarctica.

All experiments were performed at the British Antarctic Survey base at Bird Island, South Georgia, during the austral summer of 1998/99. Twenty-four macaroni penguins were caught from the colonies on the north side of the island and transported back to the base. Animals were sexed using bill size measurements, a reliable method for this species (Williams and Croxall, 1991). Nine male (mean mass 3.89 kg) and nine female (mean mass 3.26 kg) penguins were used during the early part of the breeding season (late November to early January) and six further females (mean mass 4.25 kg) were used during the middle of their moult (late March). All penguins were kept overnight prior to the experiment in an outdoor enclosure, without food but with access to water, to ensure that they were post-absorptive.

Experimental apparatus

An open-circuit respirometry system similar to that used on gentoo penguins (Bevan et al., 1995c) was used to measure rates of oxygen consumption and carbon dioxide production. A Perspex respirometer was fixed to a variable-speed treadmill (model EG10, Powerjog, Sports Engineering Ltd). The respirometer was equipped with three fans in a side compartment that ensured good and rapid mixing of air. Brush-style draught excluders ensured a good fit between a wooden frame, fixed to the treadmill frame, and the treadmill belt, while foam rubber seals ensured an air-tight junction between the respirometer and the wooden frame. Air was drawn through the respirometer using an air pump (B105, Charles Austen) at approximately 60 l min^{-1}, measured using an electronic flowmeter (100 Flo-Sen, McMillan Co.) calibrated at the beginning, middle and end of the series of experiments using two 40 l min^{-1} variable-area flowmeters (Fisher Controls 1100). A subsample of the outlet air flow was passed, *via* a container of drying agent (silica gel), to an infrared CO_2 analyser (Servomex 1410) and then to a paramagnetic O_2 analyser (Servomex 570A). A solenoid valve (RS Components Ltd) switched between sampling outlet and ambient air. The atmospheric pressure was measured using an electronic barometer pressure transducer (Farnell Electronic Services), and the humidity and temperature of both the outlet and ambient air were continuously monitored using suitable sensors (Farnell Electronic Services). The O_2 and CO_2 analysers were calibrated with atmospheric air, nitrogen and a specially prepared mixture of 1 % CO_2 in N_2 (Air Products Ltd).

The output signals from the O_2 and CO_2 analysers, humidity and temperature sensors, barometer and flowmeter were passed to a purpose-built interface box that amplified the signals to a standard range of −10 V to +10 V. The amplified output voltages were passed to an analogue-to-digital converter unit (DAQPad-1200, National Instruments), then to a desktop computer (Viglen Genie Professional). The computer sampled the outputs at 1000 Hz, took a mean of these values and saved them to a file every 10 s with a program developed using a software package for automatic instrumentation (LabVIEW, National Instruments). The penguins were monitored on the treadmill using a closed-circuit television system to avoid any disturbance caused by observers.

Heart rate was sampled using a miniature heart rate data logger (HRDL) designed for abdominal implantation (Woakes et al., 1995). This device samples and stores heart rate every 15 s. For animals during the breeding season, the data logger was mounted externally to avoid the post-operative recovery time associated

with implantation of these devices. Moulting birds were implanted because the large amount of subcutaneous fat deposited as reserves for the moult made it impossible to obtain an electrocardiogram (ECG) free of interference if the device was mounted externally. The birds were implanted 10 days before the calibration experiments, which allowed a more than adequate recovery time (Bevan et al., 1995a). For external deployment, the electrodes of the HRDL were replaced with 20 gauge hypodermic needles; the complete unit weighed 25 g. The HRDL was attached to the dorsal feathers using adhesive tape, and the two needles were inserted subcutaneously. The most satisfactory position was for one electrode to be positioned at the back of the neck and the other underneath the left flipper. This position gave the best ECG signal, free of interference from other electrical activity such as that associated with muscle contraction. As well as storing the heart rate data, the HRDL also emitted a radio signal when an ECG signal was detected, and this was picked up using a receiver (877R, International) tuned to 115 MHz. This signal was monitored at all times and could be counted, using a hand tally counter, to calculate a heart rate as a back-up to the HRDL.

Protocol

Once equipped with an HRDL, the penguin was introduced into the respirometer. The bird was initially left for at least an hour to attain a resting metabolic rate, which was judged to occur when a stable rate of oxygen consumption had been observed for 25 min. After the resting measurement, the bird then walked on the treadmill at nine speeds, increasing in steps of 0.2 km h^{-1} from 0.2 to 1.8 km h^{-1}. The sequence of workloads was randomly assigned, although not every bird could walk at the highest speed. The penguins undertook three exercise periods, with a 30 min rest between them. In each period, the penguins walked for 30 min at each of three different speeds. At each speed, limb frequency was determined by counting the number of steps taken by the right leg over a period of 3 min. Measurements of heart rate, O_2 and CO_2 levels, humidity, temperature, pressure and flow were taken continuously throughout the duration of the experiment, but for calibration calculations the values from only the last 5 min of each rest and exercise period (when steady-state conditions had been achieved) were used. Heart rate was also counted from the radio signal for this 5 min period. After the final exercise period, the penguin was kept in the respirometer for at least one more hour to take a final resting rate in darkness. Five of the penguins remained in the respirometer overnight, for a total of 24 h, to gather more data to validate the findings from the calibration runs. These penguins walked for a further hour at 1 km h^{-1} the next morning. The room used for respirometry had large windows and adequate ventilation, and the conditions of light and temperature were therefore very similar to those in the natural state in the colony. The mean temperature inside the respirometer from all experiments was 7.88±0.25 °C, which was only slightly higher than the average temperature of 5.78±0.39 °C outside the room used for respirometry, from where the ambient air was sampled.

Analysis and calculations

The rate of oxygen consumption was calculated using the equations of Depocas and Hart (Depocas and Hart, 1957), modified by Withers (Withers, 1977):

$$\dot{V}_{CO_2} = \dot{V}_{STPD}\,\frac{F_{CO_2,out} - F_{CO_2,am}}{1-[1-(F_{CO_2,out}-F_{CO_2,am})/(F_{O_2,am}-F_{O_2,out})F_{CO_2,am}]} \quad (2)$$

$$\dot{V}_{O_2} = \frac{\dot{V}_{STPD}(F_{O_2,am}-F_{O_2,out}) - \dot{V}_{CO_2}F_{O_2,am}}{1-F_{O_2,am}} \quad (3)$$

where $\dot{V}_{STPD}$ is the flow rate of air through the respirometer (in ml min^{-1}) corrected for standard temperature and pressure, and $F_{O_2,out}$, $F_{O_2,am}$, $F_{CO_2,out}$ and $F_{CO_2,am}$ are the fractional concentrations of O_2 and CO_2 in outlet and ambient air, respectively.

Rate of oxygen consumption $\dot{V}_{O_2}$ is also commonly expressed as mass-specific $\dot{V}_{O_2}$ ($s\dot{V}_{O_2}$) to correct for the potential effects of individual differences in the mass of animals. A function based on the relationship between resting $\dot{V}_{O_2}$ and mass is used to modify $\dot{V}_{O_2}$ to give $s\dot{V}_{O_2}$.

The data obtained during the calibration experiments were used to derive an equation to estimate $s\dot{V}_{O_2}$ from heart rate. During the validation experiments, this equation was used to derive predicted values of $s\dot{V}_{O_2}$ over two time scales. First, $s\dot{V}_{O_2}$ was estimated for 30 min periods throughout the experimental period of 24 h to determine whether short-term changes in metabolic rate could be accurately predicted. Second, $s\dot{V}_{O_2}$ was estimated over the whole 24 h period to determine whether longer-term estimates of metabolic rate were accurate.

Statistical analyses

Statistical testing was performed with the spreadsheet package Excel (Microsoft) and the statistical packages Minitab (Minitab Inc.) and Systat (SPSS Inc.). Least-squares regressions were used to determine the relationships between two variables (e.g. f_H and $s\dot{V}_{O_2}$ for individual calibrations). Regression equations were compared using an analysis of variance general linear model (GLM) (Zar, 1984). Student's *t*-test was used to compare the significance of any difference between the means of two populations. Analysis of variance (ANOVA) with Tukey *post-hoc* testing was used when more than two populations were compared. Results were considered significant at $P<0.05$ and are quoted at the level at which they were found to be significant. All mean values are given ± S.E.M.

Results

Effects of mass

Resting $\dot{V}_{O_2}$ increased with body mass (in kg). Over the range of values for body mass (m_b) in this experiment, the relationship was best described by a linear equation:

$$\dot{V}_{O_2} = 12.11 m_b - 5.99\,, \quad (4)$$

($N=24$, $r^2=0.53$, $P<0.001$) ,where m_b is in kg and $\dot{V}_{O_2}$ is in ml min^{-1}.

A similar coefficient of determination was obtained when

Table 1. *Mean resting and maximum values of mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) for the three groups of macaroni penguins used in the calibration procedure*

Group	Mean resting f_H (beats min^{-1})	Mean resting $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Mean maximum f_H (beats min^{-1})	Mean maximum $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	*N*
Breeding males	85.36±4.41*	9.45±0.43$^{\Phi}$	162.97±4.68§	35.13±1.00	9
Breeding females	96.61±2.27$^{\omega}$	10.72±0.47	166.20±6.50‡	33.58±0.91	9
Moulting females	124.87±11.58*,$^{\omega}$	11.59±0.87$^{\Phi}$	193.00±10.93§,‡	33.63±0.58	6

Values are means ± S.E.M.
Significant differences (P<0.05) between pairs of groups are represented by the following symbols: *ωΦ§‡.

using the logarithms of both variables to investigate the power relationship between the two variables (r^2=0.52). The relationship between mass and resting $\dot{V}_{O_2}$ is often described by a power relationship (Schmidt-Nielsen, 1984), especially where the range of body mass is great (typically several orders of magnitude). In the present study, however, the ranges of body mass and resting $\dot{V}_{O_2}$ were relatively small and, since the aim is merely to correct for the potential effects of variation in mass, then the linear relationship can be used to derive $s\dot{V}_{O_2}$. As such, $s\dot{V}_{O_2}$ is merely $\dot{V}_{O_2}$ divided by the mass of the animal (in kg).

Calibrations

During rest periods, some penguins preened or investigated the respirometer, but most usually stood hunched, a typical behaviour observed in the colony, or occasionally lay prone (on the belly). For all individuals, the minimum values of f_H and $s\dot{V}_{O_2}$ occurred during these rest periods. When all resting values from all individuals were pooled into groups (breeding females, breeding males, moulting females), the mean values of resting f_H and $s\dot{V}_{O_2}$ were significantly different (ANOVA; for f_H, $F_{2,21}$=10.39, P<0.001, for $s\dot{V}_{O_2}$, $F_{2,21}$=3.47, P<0.05; Table 1). Further *post-hoc* Tukey tests showed that the resting f_H of breeding males and females were both significantly lower than that for moulting females (P<0.05). However, there was no difference in resting f_H between breeding and moulting females (P>0.05). Resting $s\dot{V}_{O_2}$ of males was significantly lower than that of moulting females (P<0.05), but there were no significant differences in resting $s\dot{V}_{O_2}$ between breeding and moulting females and between breeding males and females (P>0.05).

The fastest walking speed attained by the birds was usually 1.8 km h^{-1}, except for two of the moulting birds that could only sustain 1.6 km h^{-1}. Maximum values of f_H and $s\dot{V}_{O_2}$ occurred at either the fastest or second fastest walking speed. When the maximum values from each individual were pooled into groups and these mean maximum values were compared, there was no significant difference between maximum $s\dot{V}_{O_2}$ (ANOVA; $F_{2,21}$=0.97, P>0.05; Table 1), but there was a significant difference between maximum f_H (ANOVA; $F_{2,21}$=4.72, P<0.05). Further *post-hoc* Tukey tests showed that there was no difference in mean maximum f_H between breeding males and breeding females, but both these groups had a mean maximum f_H that was lower than that of moulting females.

Table 2. *Mean factorial increases in mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) from resting to maximum activity in three groups of macaroni penguins*

Group	Mean factorial increase in f_H	Mean factorial increase in $s\dot{V}_{O_2}$	*N*
Breeding males	1.95±0.10*	3.77±0.19‡	9
Breeding females	1.72±0.06	3.20±0.20	9
Moulting females	1.58±0.09*	2.98±0.22‡	6

Values are means ± S.E.M.
Significant differences (P<0.05) between pairs of groups are represented by the following symbols: *‡.

There was consistent variation between groups in factorial increases in f_H and $s\dot{V}_{O_2}$ from resting to maximum activity (Table 2). For both f_H (ANOVA; $F_{2,21}$=4.33, P<0.05 with the Tukey test) and $s\dot{V}_{O_2}$ (ANOVA; $F_{2,21}$=4.08, P<0.05 with the Tukey test), the factorial increase from resting to maximum activity was not significantly different between breeding males and breeding females but was significantly different between breeding males and moulting females.

When the results from all birds were pooled, both, f_H and $s\dot{V}_{O_2}$ increased linearly with treadmill speed (S) (Fig. 1). These relationships were both significant (P<0.001):

$$f_H = 33.48S + 109.15\,, \quad (5)$$

(N=24, r^2=0.99, P<0.001), where f_H is in beats min^{-1} and S is in km h^{-1}, and

$$s\dot{V}_{O_2} = 10.66S + 14.02\,, \quad (6)$$

(N=24, r^2=0.98, P<0.001), where $s\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1} and S is in km h^{-1}.

Heart rate and $s\dot{V}_{O_2}$ were well correlated in each individual (Fig. 2). These relationships were best described by a linear function, (Table 3; mean r^2=0.85±0.024, N=24), and the fit was not improved by $\log_e$-transformation of the data (mean r^2=0.81±0.030). Analysis of covariance (ANCOVA) was used to compare the values of the constants a (the slope) and b (the intercept) of the individual regressions within each group. This analysis showed no significant differences (P>0.05) between the slopes but did show significant differences between the intercepts within each group. Because the penguins selected were a random sample from a wider population, the intercepts were regarded as a random sample from a distribution of

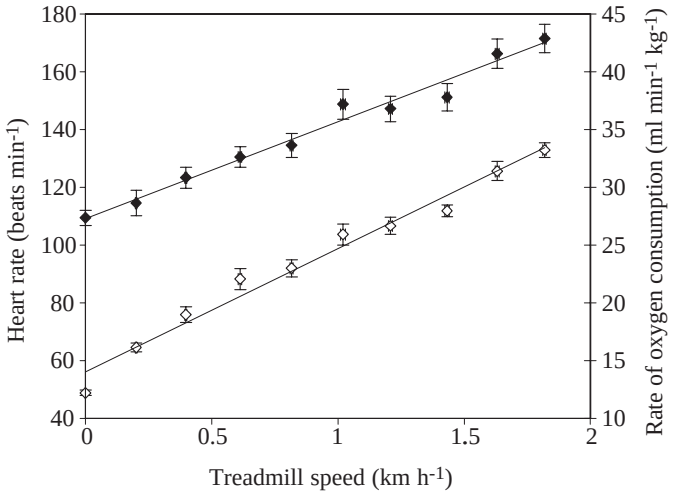

Fig. 1. Mean heart rate (f_H; open symbols) and mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$; filled symbols) of 24 macaroni penguins walking at different speeds on a treadmill. The error bars represent ±1 S.E.M. Details of the regression lines are given in the text.

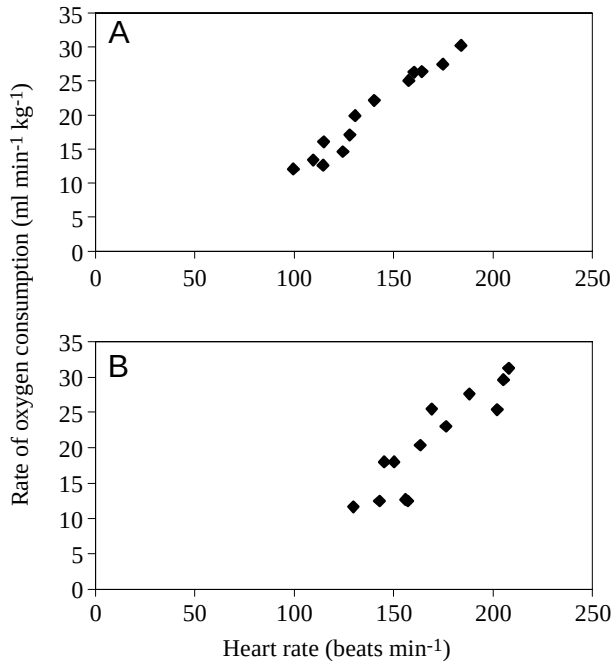

Fig. 2. Mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) as a function of heart rate (f_H) in two macaroni penguins, (A) a breeding female of mass 3.14 kg ($s\dot{V}_{O_2}$=0.23f_H–11.62; r^2=0.958, P<0.001) and (B) a moulting female of mass 3.99 kg ($s\dot{V}_{O_2}$=0.25f_H–20.93; r^2=0.812, P<0.001).

intercept values and a random-effects model was adopted. An additional random term was therefore introduced into the model by allowing the intercept to be entered as a random effect in an appropriate ANCOVA. The equations derived for each of the groups in the present study are:

$$s\dot{V}_{O_2} = 0.33 f_H - 18.77 , \quad (7)$$

Table 3. *Individual regression equations of mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$, ml min^{-1} kg^{-1}) against heart rate (f_H, beats min^{-1}) of the 24 macaroni penguins used in the calibration procedure*

Bird number	Group	Mass (kg)	*a*	*b*	r^2	*P*
cal04	BM	3.86	–19.795	0.308	0.972	<0.001
cal05	BM	3.70	–22.422	0.341	0.947	<0.001
cal06	BM	3.48	–16.514	0.303	0.938	<0.001
cal08	BM	3.23	–25.981	0.379	0.834	<0.001
cal09	BM	3.24	–14.857	0.351	0.966	<0.001
cal10	BM	3.20	–11.464	0.328	0.898	<0.001
cal12	BF	3.32	–22.499	0.298	0.858	<0.001
cal13	BF	3.23	–22.207	0.323	0.834	<0.001
cal16	BF	3.14	–11.618	0.230	0.958	<0.001
cal18	BF	3.23	–28.207	0.375	0.832	<0.001
cal19	BF	2.98	–17.530	0.312	0.924	<0.001
cal20	BF	2.88	–14.370	0.257	0.783	<0.001
cal21	BF	2.96	–25.247	0.415	0.928	<0.001
cal24	BM	4.56	–18.104	0.321	0.886	<0.001
cal25	BM	4.22	–19.925	0.321	0.627	<0.005
cal26	BF	3.62	–13.038	0.277	0.776	<0.001
cal27	BF	4.00	–20.146	0.354	0.709	<0.001
cal28	BM	4.34	–24.055	0.371	0.945	<0.001
n05	MF	3.99	–20.927	0.246	0.812	<0.001
n07	MF	4.57	–24.417	0.410	0.818	<0.001
x02	MF	3.91	–38.576	0.324	0.843	<0.001
n09	MF	4.10	–35.459	0.341	0.472	<0.05
h83	MF	4.40	–17.434	0.267	0.958	<0.001
c13	MF	4.56	–14.618	0.232	0.936	<0.001

The form of the equation is $s\dot{V}_{O_2}=a+bf_H$.

The three groups are breeding males (BM), breeding females (BF) and moulting females (MF)

for breeding males (N=9, r^2=0.91, P<0.001),

$$s\dot{V}_{O_2} = 0.30 f_H - 17.40 , \quad (8)$$

for breeding females (N=9, r^2=0.84, P<0.001) and

$$s\dot{V}_{O_2} = 0.27 f_H - 20.94 , \quad (9)$$

for moulting females (N=6, r^2=0.81, P<0.001), where $s\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1} and f_H is in beats min^{-1}.

The standard deviation (σ_0) of an estimate made using a regression equation can be used to calculate confidence intervals for the regression line (for definition, see Zar, 1984, p. 273). However, in the present case, the presence of intercept as a random factor leads to the introduction of an additional error term in the calculation of σ_0, and the equation used to calculate it must be modified:

$$\sigma_0 = \sqrt{d^2\left(\frac{1}{n_1}\right) + e^2\left[\frac{1}{n_2} + \frac{(X_i - \bar{X})^2}{\sum x^2}\right]} , \quad (10)$$

where d^2 is the error associated with the variation between penguins, n_1 is the number of penguins used in the calibration process, e^2 is the error associated with the scatter around the

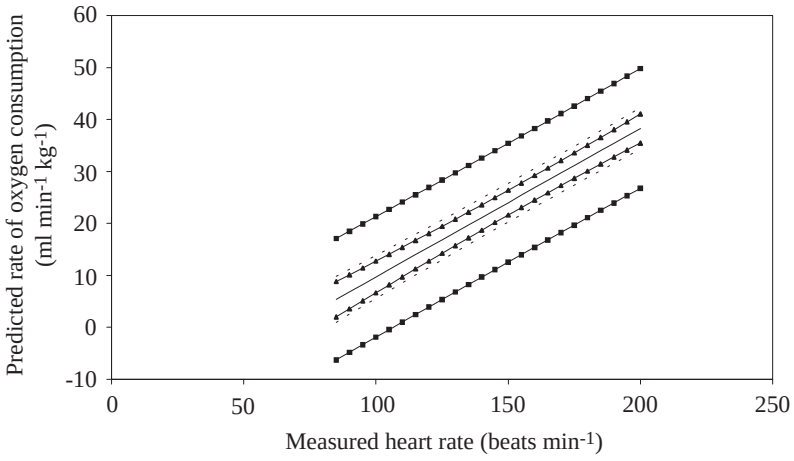

Fig. 3. Predicted mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) as a function of measured heart rate (f_H) obtained from 15 female macaroni penguins, showing 95 % confidence intervals (triangles) and 95 % prediction intervals (squares) for the predictions. The broken lines represent the 95 % prediction intervals when the rate of oxygen consumption is estimated from 10 000 measurements of heart rate spread between 10 individuals.

regression lines, n_2 is the total number of data points in the regression, $\bar{X}$ is the mean value of heart rate used in the regression, X_i is the value of heart rate for which σ_0 is to be estimated and $\sum x^2$ is the sum of all the squared values of heart rate used in the regression.

The values of d^2, the variance associated with the random distribution of intercepts, and e^2, the variance associated with the scatter of points around the individual regressions would tend to decrease if more penguins were used in the calibration process.

If the equation is to be used to estimate $s\dot{V}_{O_2}$ from an average value of f_H measured in the field, then a further additional error term is added (for definition, see Zar, 1984, p. 275) to account for the variation within the new individuals selected at random from the field. In the case of this model, another error term is once again introduced to account for the variation between the individuals selected at random from the field. The standard deviation σ_1 of an estimate of $s\dot{V}_{O_2}$ made from an average value of f_H taken from the field is given by:

$$\sigma_1 = \sqrt{d^2\left(\frac{1}{n_1}+\frac{1}{n_3}\right)+e^2\left[\frac{1}{n_2}+\frac{1}{n_4}+\frac{(X_i-\bar{X})^2}{\sum x^2}\right]}, \qquad (11)$$

where d^2 is the error associated with the variation between calibration penguins, n_1 is the number of penguins used in the calibration process, n_3 is the number of penguins from which the field value of heart rate was obtained, e^2 is the error associated with the scatter around the regression lines, n_2 is the total number of data points in the regression, n_4 is the number of data points from which the field value of heart rate was obtained, $\bar{X}$ is the mean value of heart rate used in the regression, X_i is the mean value of heart rate from the field from which σ_1 is to be estimated and $\sum x^2$ is the sum of all the squared values of heart rate used in the regression.

If the values of n_3 and n_4 are set to 1, then equation 11 can be used to produce 95 % prediction intervals for the regression (Fig. 3). As the values of n_1, n_3 and n_4 increase, σ_1 will tend to decrease as a proportion of the estimate (Fig. 4). The value of n_4 is dependent on the number of data points used to derive

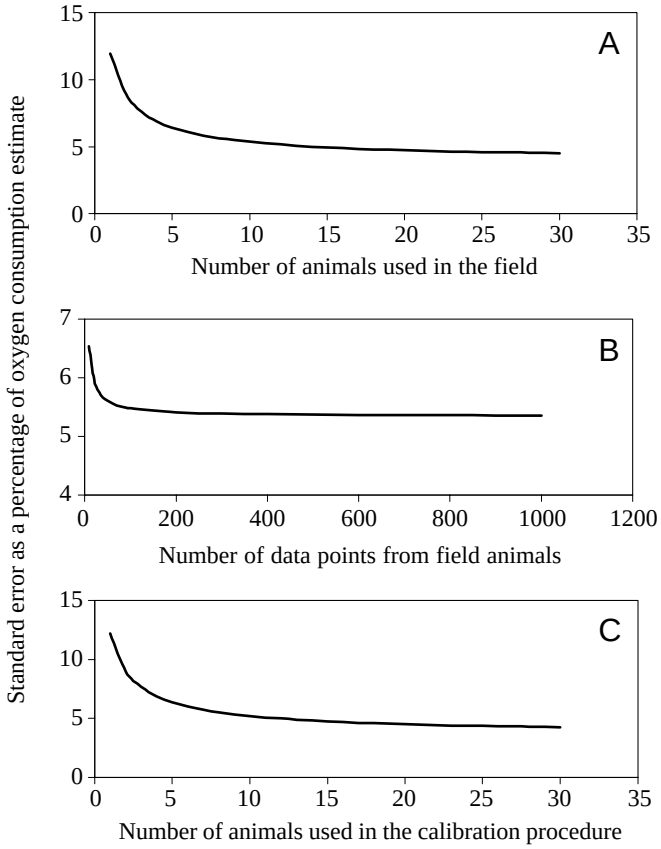

Fig. 4. Graphic representation of the effect on the standard error of the estimate of rate of oxygen consumption of changing four parameters; n_1 (the number of calibration animals), n_2 (the number of data points from calibration animals), n_3 (the number of field animals) and n_4 (the number of data points from field animals). Mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) and standard error were calculated with the heart rate arbitrarily fixed at 150 beats min^{-1} using equation 8 for breeding females. (A) n_1=9, n_2=117, n_3=variable, n_4=100 per field animal. (B) n_1=9, n_2=117, n_3=10, n_4=variable. (C) n_1=variable, n_2=13 per calibration animal, n_3=10, n_4=1000. Changing the value of heart rate or the equation used to calculate $s\dot{V}_{O_2}$ had no effect on the shape of the curves.

the average f_H and, since f_H can be recorded over periods of 5 min for several days at a time in the field, the value of the error term involving n_4 will tend towards zero, increasing the accuracy of the estimate of $s\dot{V}_{O_2}$. This also illustrates the importance of recording f_H from as many individuals as possible in the field so that n_3 is high and the value of that error term will also tend towards zero (Fig. 4).

Random-effects analysis of covariance can also be used to compare the three common equations and to determine whether they are similar. When the slopes and intercepts are treated as random effects, and hence would potentially take different values if the whole experiment were to be repeated, there was a significant difference between the slopes for breeding males and breeding or moulting females ($P<0.01$). However, there was no significant difference in the slope between breeding and moulting females ($P>0.05$). The individual breeding and moulting females were therefore considered together, and a new common regression equation for all females was derived (equation 12) which is still significantly different from the regression equation for males ($P<0.01$). Since σ_0 for all females is significantly greater than σ_0 for males (F-test $P<0.05$), the variance components for both groups must be calculated separately. If there had been no significant difference, then the data from all the individuals could have been used to calculate the variance for both common regressions. For all females:

$$s\dot{V}_{O_2} = -118.18 f_H + 0.29 , \quad (12)$$

($N=15$, $r^2=0.83$, $P=<0.001$),where $s\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1} and f_H is in beats min^{-1}.

Validations

Breeding female penguins were used to validate the procedure and to determine its accuracy in estimating rate of oxygen consumption from heart rate for macaroni penguins. The data obtained from the five penguins kept in the respirometer for 24 h were divided into 30 min segments. The mean f_H for each 30 min segment for each individual bird was used to estimate $s\dot{V}_{O_2}$ for that segment using a version of equation 12, modified by not including the calibration data for this individual (Fig. 5). Predicted $s\dot{V}_{O_2}$ was plotted against observed $s\dot{V}_{O_2}$; the regression line was significantly different from the line of equality. However, the mean of these estimates of $s\dot{V}_{O_2}$ was 15.29±0.51 ml min^{-1} kg^{-1}, which was not signifcantly different from the observed mean $s\dot{V}_{O_2}$ of 15.70±0.48 ml min^{-1} kg^{-1} (paired-sample t-test, $P>0.05$). Percentage error for these estimates is calculated by dividing the difference between the estimated and observed values by the observed value and multiplying by 100. The average percentage algebraic error of all the 30 min segments, which takes the sign of the difference into account, was 0.74 %. However, the range of this error was from −59.90 % to +114.52 %, so the average absolute error, which ignores the sign of the difference between the observed and estimated values, was 30.02 %.

However, in a study of this nature, it is more appropriate to look at the average oxygen consumption and error for the whole 24 h period for each individual bird (Table 4). There was again no significant difference between the means of the observed and predicted $s\dot{V}_{O_2}$ (paired-sample t-test, $P>0.05$), and the average algebraic error was −2.13 %. In this case, the average absolute error was 24.91 %. If equations 12 and 10 are used to calculate $s\dot{V}_{O_2}$ and σ_0 from the mean value of f_H obtained from the validation, the estimate of $s\dot{V}_{O_2}$ is 15.54±1.24 ml min^{-1} kg^{-1}. The average measured value of $s\dot{V}_{O_2}$ is within the 95 % prediction intervals for this estimate, and the algebraic error of this estimate is −1.03 %.

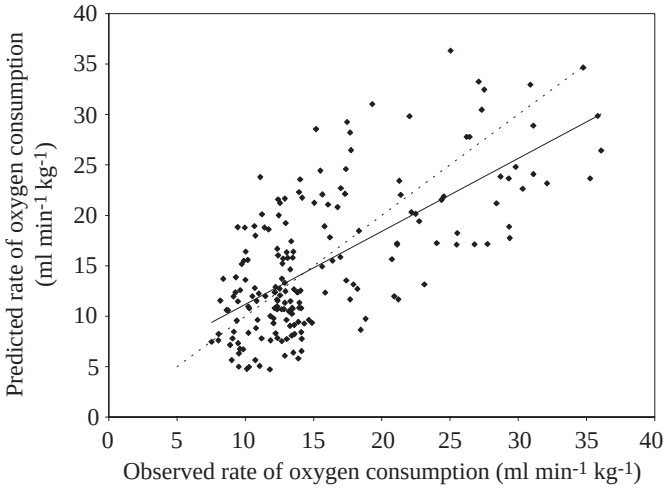

Fig. 5. Mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) estimated from heart rate (f_H) as a function of measured mass-specific rate of oxygen consumption at different exercise levels in five macaroni penguins. The broken line is the line of equality, and the solid line is the regression equation between the two variables described by the equation: estimated $s\dot{V}_{O_2}$=3.3904+0.3238 (measured $s\dot{V}_{O_2}$) ($r^2=0.46$, $P<0.001$). The two lines are significantly different.

As a further check on the validity of the f_H method, the data used for the calibrations were examined. For each animal, $s\dot{V}_{O_2}$ was estimated using modified versions of equations 7 and 12 for males and all females, respectively; the constants for the slope and intercept were calculated using the data from the other members of its group. This was repeated for each animal, and average observed and predicted values of $s\dot{V}_{O_2}$ were calculated for each animal. Again, if predicted $s\dot{V}_{O_2}$ is plotted against observed $s\dot{V}_{O_2}$ then the regression line between the two variables is significantly different from the line of equality (not shown). However, within both groups and for all the individuals, there was no significant difference between the estimated and measured $s\dot{V}_{O_2}$ (paired-sample t-test $P>0.05$; Table 5).

Discussion

The accuracy and usefulness of f_H for determining metabolic rate in free-ranging predators has already been demonstrated for a number of different species. Previous studies have shown that f_H is at least as accurate as the DLW technique (Nolet et al., 1992; Bevan et al., 1994; Bevan et al., 1995c; Boyd et al.,

Table 4. *Mean mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) obtained from five macaroni penguins over 24 h with estimates of $s\dot{V}_{O_2}$ derived from a previously calibrated relationship between $s\dot{V}_{O_2}$ and f_H*

Bird	Observed 24 h f_H (beats min^{-1})	Observed 24 h $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Predicted 24 h $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Predicted $s\dot{V}_{O_2}$ minus observed $s\dot{V}_{O_2}$	Algebraic error (%)	Absolute error (%)
Cal12	128.56	14.33	18.01	3.68	25.69	25.69
Cal19	108.89	14.35	11.99	–2.35	–16.41	16.41
Cal20	144.77	17.13	22.47	5.35	31.23	31.23
Cal26	109.50	16.28	12.06	–4.22	–25.95	25.95
Cal27	110.50	16.45	12.30	–4.15	–25.24	25.24
Mean	120.45	15.71	15.37	–0.34	–2.13	24.91
S.E.M.	7.11	0.58	2.11	2.03		

Table 5. *Mean mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) and heart rate (f_H) obtained from 24 macaroni penguins kept in a respirometer for an 8 h session of rest and exercise, with estimates of $s\dot{V}_{O_2}$ derived from a previously calibrated relationship between $s\dot{V}_{O_2}$ and f_H*

Group	Average observed f_H (beats min^{-1})	Average observed $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Average predicted $s\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	Average predicted $s\dot{V}_{O_2}$ minus observed $s\dot{V}_{O_2}$	Average algebraic error (%)	Average absolute error (%)	*N*
Males	119.91±2.56	20.99±0.72	21.00±0.98	0.01±1.42	1.44	15.12	9
Females	139.92±5.95	21.11±0.24	21.12±1.80	0.01±1.76	–0.11	24.44	15
All	132.42±4.19	21.06±0.30	21.08±1.13	0.01±1.17	0.47	20.94	24

Values are means ± S.E.M.

1995) and that, for gentoo penguins, the relationship between f_H and $s\dot{V}_{O_2}$ is the same when exercising by walking or by swimming (Bevan et al., 1995c). Following on from that work, the present study had three main aims: first, to determine and validate the $f_H/s\dot{V}_{O_2}$ relationship for macaroni penguins to determine whether f_H can be used as a technique for measuring the rate of energy expenditure of these animals in the field; second, to determine whether this relationship is valid for different sexes and stages of the penguins' yearly physiological cycle; and finally to develop further the statistical methods used to derive these relationships and to determine and minimise the errors associated with using them to estimate field metabolic rates (FMRs).

Resting $s\dot{V}_{O_2}$ determined in the present study is 15 % lower than the FMR of 12.24 ml O_2 min^{-1} kg^{-1} for incubating macaroni penguins estimated using the DLW technique (Davis et al., 1989). This seems reasonable since conditions inside the respirometer are likely to be less stressful than those in the colony. Even though the temperature in the respirometer was only 2.1 °C higher than the external temperature, the penguins were protected from wind-chill factors, so thermoregulation could be partly responsible for raised $s\dot{V}_{O_2}$ in the field. Comparison with resting $s\dot{V}_{O_2}$, obtained by respirometry in other penguin species, suggests that the resting rates of macaroni penguins in the present study are comparable with those of other species (Fig. 6). Resting metabolic rate (RMR) decreases exponentially with increasing body mass (m_b) (equation 13), and the values from the present study fall very close to this line:

$$\mathrm{RMR} = 14.79 m_b^{0.24} \quad (13)$$

(r^2=0.65, P<0.001). FMR measured using the DLW technique for moulting birds was 20 ml O_2 min^{-1} kg^{-1} (Davis et al., 1989), which is 73 % higher than the value for resting metabolic rate of moulting birds in the present study, but the value of FMR was taken from the field and, hence, is again likely to be higher than that measured in the laboratory.

Maximum rates of $s\dot{V}_{O_2}$ measured in the present study are nearly 50 % lower than estimates made of FMR for foraging penguins using the DLW technique (65.67 ml O_2 min^{-1} kg^{-1}; Davis et al., 1989) and up to 30 % lower than estimates of metabolic rate for penguins swimming at average speed made using respirometry (4.2×RMR; Culik et al., 1994). This supports the proposal that estimates of energy expenditure made using the DLW technique for diving and swimming birds and mammals may not be of acceptable accuracy (Bevan et al., 1995b; Boyd et al., 1995). Analysis of f_H data from free-ranging macaroni penguins will indicate whether this is a possibility. The DLW technique is useful, but there is potential for bias in estimates made using it if the assumptions inherent in its implementation are not upheld and, to use it to give activity-specific energy expenditures, further assumptions must be made.

The maximum walking speed attained by the penguins in the

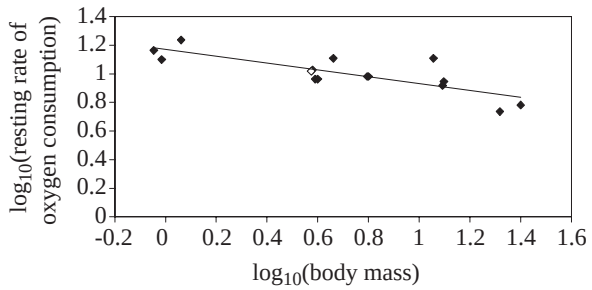

Fig. 6. Log_{10}(resting mass-specific rate of oxygen consumption) ($s\dot{V}_{O_2}$, $ml\,min^{-1}\,kg^{-1}$) as a function of log_{10}(body mass) (kg) of different penguin species (filled symbols). The equation of the regression line is $log_{10}(\text{resting } s\dot{V}_{O_2})=1.17–0.24log_{10}m_b$ (r^2=0.65, P<0.001), where m_b is body mass. Data are from gentoo (*Pygoscelis papua*) (Dumonteil et al., 1994; Bevan et al., 1995c), Humboldt (*Spheniscus humboldti*), white flippered (*Eudyptyla*) (Pinshow et al., 1976), little blue (*Eudyptyla minor*) (Stahel and Nicol, 1982; Stahel et al., 1984), king (*Aptenodytes patagonicus*) (G. Froget, P. J. Butler, A. J. Woakes, unpublished data; Barre and Roussel, 1986), Adélie (*Pygoscelis adeliae*) (Pinshow, 1976) emperor (*Aptenodytes forsteri*) (Pinshow et al., 1976; Le Maho et al., 1976) and macaroni (*Eudyptes chrysolophus*) (Brown, 1984) penguins. The open symbol is from the macaroni penguins in the present study.

present study was usually 1.8 $km\,h^{-1}$. When compared with data for other penguins walking on treadmills, this value is lower than those reported for Adélie (3.7 $km\,h^{-1}$), emperor (2.8 $km\,h^{-1}$), white flippered (2.7 $km\,h^{-1}$) (Pinshow et al., 1976) and king (2.6 $km\,h^{-1}$, G. Froget, P. J. Butler, A. J. Woakes and Y. Handrich, unpublished data) penguins, but faster than the 1.5 $km\,h^{-1}$ recorded for gentoo penguins in a very similar experimental situation (Bevan et al., 1995c). The reasons for this are unclear. It is possible that the penguins used in the earlier studies were trained to walk on the treadmill, but it is more likely that these species are in general better-adapted to walking than macaroni penguins. Emperor and Adélie penguins may have to walk up to 100 km over ice to reach their colonies (Pinshow et al., 1976), and king penguins may also have to walk long distances to their colonies. Macaroni penguins typically have to walk no further than a few hundred metres from landing areas on the shore to their nest sites (Marchant and Higgins, 1990). This journey is usually over steep rocky ground, and the penguins tend to hop rather than walk. On the treadmill, however, the macaroni penguins could not be induced to hop, even at high speeds or inclines of 22 °, and seemed untroubled when walking steadily. Gentoo penguins walk only slightly greater distances than macaroni penguins (Marchant and Higgins, 1990), so their lower maximum speed may have a similar explanation.

As with emperor, Adélie and white flippered penguins, $s\dot{V}_{O_2}$ and f_H both increased linearly with treadmill speed. There was no initial sharp increase from resting rates followed by a steady increase, as was seen in other studies (Nolet et al., 1992; Bevan et al., 1994; Bevan et al., 1995c), suggesting that this initial increase is due to the change in attitude from lying or sitting to standing and walking. Gentoo penguins, geese and albatrosses tend to sit when resting, whereas macaroni penguins usually remain standing. The starting of the treadmill and accompanying noise may startle some species, while others may never be comfortable on the treadmill, so leading to raised $s\dot{V}_{O_2}$ and f_H. The macaroni penguins seemed relatively untroubled on the treadmill and rarely tried to escape or jump out of the respirometer.

The relationships between f_H and $s\dot{V}_{O_2}$ for all individuals were well described by both linear and curvilinear regressions. However, the linear regressions provided a slightly better fit. A curvilinear relationship between f_H and $s\dot{V}_{O_2}$ has been found in other species (e.g. emu, Grubb et al., 1983; gentoo penguin, Bevan et al., 1995c; black-browed albatross, Bevan et al., 1994), although in these cases a linear relationship also gave a reasonable fit. Within each group, analysis of covariance showed that the slopes were the same but the intercepts significantly different. In this case, random-effects analysis of covariance was used to derive group relationships and standard errors. However, if both the slopes and the intercepts are significantly different within a group, then all the data should be pooled, and a simple regression should be derived from these pooled data (Hawkins et al., 2000). This represents a case in which the individual variation is even greater than that in the present study, and hence the regressions will be less accurate, with more error associated with their implementation in estimations of $s\dot{V}_{O_2}$.

In the present study, random-effects analysis of covariance was also used to show that, despite a great deal of individual variation, there was no significant difference in the relationship between f_H and $s\dot{V}_{O_2}$ between breeding and moulting females. However, there was a significant difference in the relationship between f_H and $s\dot{V}_{O_2}$ between breeding males and all females. Differences in the $f_H/s\dot{V}_{O_2}$ relationship between sexes have not been demonstrated previously. However, it is not always clear whether the sex of the animals used in similar experiments had been determined. Failure to determine the relationship in different sex classes and at different times of the breeding season has often been cited as a possible weakness in the f_H technique (Holter et al., 1976; Flynn and Gessaman, 1979; Woakes and Butler, 1983). The present study shows that these are legitimate concerns and, wherever appropriate, sex differences must be taken into account when using the heart rate method. Male macaroni penguins have a significantly greater body mass and body size than females. It is possible that this sexual dimorphism in body mass and size is involved in creating the difference in the relationship between f_H and $s\dot{V}_{O_2}$. However, it seems unlikely that the difference is due simply to body mass and size itself. Mass was corrected for in the analysis, and moulting female penguins weigh significantly more than breeding female penguins, although no difference in the relationship between f_H and $s\dot{V}_{O_2}$ was found when these two groups were compared.

Moulting penguins undergo extreme physiological stress (Brown, 1985). Even though the mean values of f_H were significantly higher for moulting females than breeding females while at rest and at maximum walking speed, and $s\dot{V}_{O_2}$

was significantly higher at rest than in moulting females, there was no significant difference in the relationship between f_H and $s\dot{V}_{O_2}$. Moulting a complete set of feathers is an energetically costly exercise, and metabolic rates are expected to be elevated (Brown, 1985).

Analysis of the errors associated with estimating $s\dot{V}_{O_2}$ from f_H gives a more quantitative description of the assertion that increasing the numbers of animals and data points in both the calibration process and field work is essential in minimising the error of the estimate (Nolet et al., 1992; Bevan et al., 1994; Bevan et al., 1995c; Boyd et al., 1995). Fig. 4 shows that increasing the number of animals from the field continues to increase the accuracy of the estimate up to approximately 20 animals; beyond this, gain is minimal. Similarly, if the number of data points per field animal for 10 animals increases above approximately 10, then there is very little improvement in accuracy. This means that, in this example, with 10 animals from the field, it would be possible to estimate the cost of an activity with only 10 data points. This would mean that the animals need only perform an activity once for 50 min or 10 times for 5 min on each occasion, representing a very fine scale of resolution for energetic measurements. Fig. 4 also shows the benefits of increasing the number of calibration animals used. We assume that the animals used in the calibration accurately represent the variability in the population, but the more animals used, the more accurately we can model this variability. One restriction of the f_H technique is that, to use a regression relationship, it is only statistically sound to use values of f_H from the field from within the range of values found in the calibration procedure. To extrapolate the relationship beyond these boundaries is not valid since other physiological changes could be occurring of which we are unaware.

Validation

The equation derived to relate f_H to $s\dot{V}_{O_2}$ was applied to two sets of data to assess the accuracy of the relationship for macaroni penguins. When the data from the individual validations carried out over a period of 24 h were examined, the relationship between observed and estimated $s\dot{V}_{O_2}$ was significantly different from equality, but using f_H to estimate $s\dot{V}_{O_2}$ for the whole 24 h period underestimated $s\dot{V}_{O_2}$ by only 2.13 %. When the data from the calibration runs only were examined, the relationship between observed and estimated $s\dot{V}_{O_2}$ was again significantly different from equality, tending to underestimate $s\dot{V}_{O_2}$ at the highest levels of activity (Fig. 5), as was also seen with gentoo penguins (Bevan et al., 1995c). However, since the magnitude of these underestimations can be quantified, it is possible to compensate for it when considering activities recorded from the field which have a corresponding high average heart rate. When the full range of heart rates from the calibration runs is considered, then f_H overestimated $s\dot{V}_{O_2}$ by only 0.47 %. It seems, therefore, that f_H can provide a good estimate of mean $s\dot{V}_{O_2}$ over a range of activities in macaroni penguins. The absolute errors in these validations are relatively large (25 % for individual 24 h validations and 21 % for the calibration runs), but this is to be expected since there is considerable individual variation, which we have modelled and accounted for. Validations of the DLW technique show a similar pattern: the technique is as accurate as f_H, but the mean absolute errors can be as large as 42 % (Bevan et al., 1995c).

Table 6. *Method for detection of significant differences between two estimates of $s\dot{V}_{O_2}$ associated with different activities in the same group of animals*

Bird	$\dot{V}_{O_2}$ estimate of activity a, V_a	$\dot{V}_{O_2}$ estimate of activity b, V_b	Difference, D	Variance of difference, σ^2_D
1	$V_{a,1}\pm\sigma^2_{a,1}$	$V_{b,1}\pm\sigma^2_{b,1}$	$D_1=V_{a,1}-V_{b,1}$	$\sigma^2_{D1}=\sigma^2_{a,1}+\sigma^2_{b,1}$
2	$V_{a,2}\pm\sigma^2_{a,2}$	$V_{b,2}\pm\sigma^2_{b,2}$	$D_2=V_{a,2}-V_{b,2}$	$\sigma^2_{D2}=\sigma^2_{a,2}+\sigma^2_{b,2}$
3	$V_{a,3}\pm\sigma^2_{a,3}$	$V_{b,3}\pm\sigma^2_{b,3}$	$D_3=V_{a,3}-V_{b,3}$	$\sigma^2_{D3}=\sigma^2_{a,3}+\sigma^2_{b,3}$
n	$V_{a,n}\pm\sigma^2_{a,n}$	$V_{b,n}\pm\sigma^2_{b,n}$	$D_n=V_{a,n}-V_{b,n}$	$\sigma^2_{Dn}=\sigma^2_{a,n}+\sigma^2_{b,n}$

$s\dot{V}_{O_2}$, mass-specific rate of oxygen consumption.
σ, standard deviation.
See text for further explanation.

The regressions derived can be used to predict values of $s\dot{V}_{O_2}$ for birds in the field from values of f_H that are the average of many data points from several animals. It is easy to compare average heart rates for different activities, but less obvious how the values of $s\dot{V}_{O_2}$ predicted from the regression equations may be compared. One way in which this could be achieved is by examining the distribution of differences between the averages for each activity for each animal. For example, an average f_H is recorded from each of n penguins for two activities a and b, and for each mean value of f_H a value of $s\dot{V}_{O_2}$ is calculated using equation 7 or 12 as appropriate. σ^2_1 for each estimate should be calculated, but omitting the error term involving n_3 since, in this case, each penguin is considered individually and is not representing the whole population. For each individual, the difference between the $s\dot{V}_{O_2}$ value for activities a and b (D), and σ^2_D, the variance of D, which is the sum of the two variances for the two $s\dot{V}_{O_2}$ values, are calculated (Table 6). Next $\bar{D}$, which is simply the mean of all n values of D and $\sigma^2_{\bar{D}}$ (equation 14) are calculated, and the value of $\bar{D}$ is then compared with zero using equation 15 to obtain a Z-statistic. A simple approximate normal test will determine whether $\bar{D}$ is significantly different from zero. If it is, then the mean estimates of $s\dot{V}_{O_2}$ for the two activities are significantly different.

$$\sigma^2_{\bar{D}}=\left(\frac{1}{n}\right)^2\left(\sum_1^n \sigma^2_D\right), \tag{14}$$

where $\sigma^2_{\bar{D}}$ is the variance of $\bar{D}$, the mean difference, n is the number of individuals and σ^2_D is the variance of D for each individual.

$$Z=\frac{\bar{D}-0}{\sqrt{\sigma^2_{\bar{D}}}}, \tag{15}$$

where $\bar{D}$ is the mean of the differences for each individual and $\sigma^2_{\bar{D}}$ is the variance of $\bar{D}$.

In conclusion, the present study has shown for the macaroni penguin that, provided data are obtained from as large a group as possible and f_H is not applied to individual animals, f_H can be used accurately to estimate mean metabolic rate, even for animals in different physiological states. There were differences between the sexes in the way that f_H and $s\dot{V}_{O_2}$ was related, and this should be taken into account during any calibration process for this species. Provided that this precaution is taken then, through the long-term monitoring of f_H, it is possible to determine the metabolic rates of free-ranging animals over several months or years, yet still with a fine enough time resolution to determine the energetic costs of different activities.

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