

HEART RATE AS AN INDICATOR OF OXYGEN CONSUMPTION: INFLUENCE OF BODY CONDITION IN THE KING PENGUIN

G. FROGET^{1,2,*}, P. J. BUTLER¹, Y. HANDRICH² AND A. J. WOAKES¹

¹School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK and ²Centre d'Ecologie et Physiologie Energétiques, C.N.R.S., 23 rue Becquerel, 67087 Strasbourg Cedex 02, France

*e-mail: g.froget@bham.ac.uk

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Summary

The use of heart rate to estimate field metabolic rate has become a more widely used technique. However, this method also has some limitations, among which is the possible impact that several variables such as sex, body condition (i.e. body fat stores) and/or inactivity might have on the relationship between heart rate and rate of oxygen consumption. In the present study, we investigate the extent to which body condition can affect the use of heart rate as an indicator of the rate of oxygen consumption.

Twenty-two breeding king penguins (*Aptenodytes patagonicus*) were exercised on a variable-speed treadmill. These birds were allocated to four groups according to their sex and whether or not they had been fasting. Linear regression equations were used to describe the relationship between heart rate and the rate of oxygen consumption for each group. There were significant differences between the regression equations for the four groups.

Good relationships were obtained between resting and active oxygen pulses and an index of the body condition of

the birds. Validation experiments on six courting king penguins showed that the use of a combination of resting oxygen pulse and active oxygen pulse gave the best estimate of the rate of oxygen consumption \dot{V}_{O_2} . The mean percentage error between predicted and measured \dot{V}_{O_2} was only +0.81 % for the six birds.

We conclude that heart rate can be used to estimate rate of oxygen consumption in free-ranging king penguins even over a small time scale (30 min). However, (i) the type of activity of the bird must be known and (ii) the body condition of the bird must be accurately determined. More investigations on the impact of fasting and/or inactivity on this relationship are required to refine these estimates further.

Key words: heart rate, oxygen consumption, penguin, *Aptenodytes patagonicus*, exercise, metabolic rate, foraging, fasting, body condition.

Introduction

The balance between the energetic cost and the profitability, in terms of energy gained, of a given activity is one of the factors that influences the behaviour of animals, especially while foraging (Boyd, 1999). Information about the energy expenditure of free-ranging animals therefore holds the key to understanding major aspects of their behaviour in the natural environment.

In early studies, it proved very difficult to measure the energy expenditure associated with different behaviours in the field. Doubly labelled water (DLW) is still widely used to measure field metabolic rate (Speakman, 1997). The main limitation of the DLW method is that it is used to calculate the mean metabolic rate between the time of the first blood sample after the injection of DLW and the time of the second blood sample at the end of the experimental period. Without a detailed time budget, it is therefore not possible to determine the costs associated with specific activities. Also, the duration of the experimental period is limited by the biological half-life of ¹⁸O. As an alternative, heart rate (f_H) has been proposed as

an indicator of the rate of oxygen consumption (\dot{V}_{O_2}) using the relationship between the variables described in the Fick equation (equation 1; Owen, 1969; Butler, 1993):

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

where \dot{V}_{O_2} is the rate of oxygen consumption, f_H is the heart rate, V_S is the cardiac stroke volume, Ca_{O_2} is the oxygen content of arterial blood and $C\bar{V}_{O_2}$ is the oxygen content of mixed venous blood. $Ca_{O_2} - C\bar{V}_{O_2}$ is also referred to as the tissue oxygen extraction; $V_S(Ca_{O_2} - C\bar{V}_{O_2})$ is the oxygen pulse expressed in ml O_2 heart beat⁻¹.

If we assume that V_S and $Ca_{O_2} - C\bar{V}_{O_2}$ remain constant, or that both vary systematically, then f_H is directly related to \dot{V}_{O_2} (Butler, 1993). This technique has already been successfully used to monitor continuously the rate of energy expenditure in several species (Bevan et al., 1994; Boyd et al., 1999; Butler et al., 1995; Woakes et al., 1995). The advantages of this technique include a monitoring period that can be substantial (new data-logging techniques have been developed that enable

heart rate to be monitored over many months; Woakes et al., 1995), the recorded heart rate can be divided into small time units to allow energy expenditure to be studied with fine time resolution and the cost of specific activities may be determined if they are identified.

However, the use of heart rate to estimate rate of energy expenditure also has its limitations. First, the method requires calibration for each species studied. Second, several variables have been found to influence the relationship between f_H and \dot{V}_{O_2} , such as seasonal variations (Holter et al., 1976) and type of activity, e.g. swimming, running and flying in birds (Nolet et al., 1992; Butler et al., 2000). However, Bevan et al. (Bevan et al., 1995) found that the f_H/\dot{V}_{O_2} relationship is similar in running and swimming gentoo penguins (*Pygoscelis papua*).

Unlike smaller penguins, such as gentoo and Adélie penguins (*Pygoscelis adeliae*), in which males and females alternate foraging and chick guarding on a daily basis during the chick-rearing period, king penguins (*Aptenodytes patagonicus*) alternate between relatively long periods of inactivity while fasting ashore and periods of high-level activity while foraging at sea (Fig. 1; Stonehouse, 1960; Barrat, 1976). The duration of these fasting periods decreases through the breeding cycle (see Fig. 1), which makes the king penguin an ideal model in which to study how variation in body condition could influence the oxygen uptake/heart rate relationship of the bird (Bishop, 1997).

To test the influence of physiological factors (such as the impact of fasting and/or inactivity) on this relationship, f_H and \dot{V}_{O_2} were measured in king penguins resting and exercising at different levels of activity on a treadmill. These experiments were performed on breeding king penguins either at the beginning or at the end of a fasting period.

The aims of the present study were (i) to calibrate f_H against \dot{V}_{O_2} (ii) to validate the use of f_H as a predictor of \dot{V}_{O_2} so that f_H can be used to determine the foraging metabolic rate of free-ranging king penguins and (iii) to test the hypothesis that variations in body composition or fat stores affect the relationship between f_H and \dot{V}_{O_2} .

Materials and methods

The study was divided into two separate protocols. Protocol 1 was used to determine the \dot{V}_{O_2}/f_H relationship for king penguins walking at different speeds on a treadmill. The second protocol used this relationship to estimate \dot{V}_{O_2} from f_H monitored over a 56 h period, and the derived estimate was then compared with \dot{V}_{O_2} measured continuously by respirometry during the same experimental period. Protocol 1 is referred to as the calibration experiment and protocol 2 as the validation experiment (Nolet et al., 1992; Bevan et al., 1995).

Animals

The experiments were carried out on Possession Island (Crozet Archipelago) over the Austral summer of 1998–1999. Twenty-two breeding king penguins *Aptenodytes patagonicus*

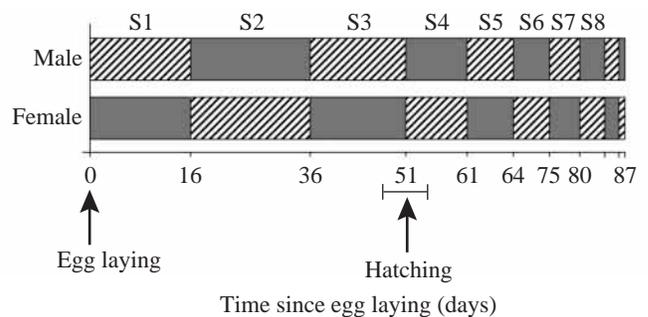


Fig. 1. Breeding cycle of an average king penguin on Crozet Island (adapted from Barrat, 1976). Incubation or chick rearing/guarding shifts ashore (hatched areas) are alternated with foraging shifts at sea (filled areas) and decrease in duration through the cycle. S_x denotes the shift number.

(Miller, 1778) were used for the calibration experiments and six courting king penguins for the validation experiments. Limited time available in the field and the tendency of king penguins to be less likely to desert their nest while incubating a small chick determined the stage of the breeding cycle at which birds were taken. Males were captured either at the beginning or at the end of their second incubation shift (S3 in Fig. 1), and females were captured at the beginning or at the end of their third foraging shift (S5 in Fig. 1). This enabled different fasting durations to be obtained. It was therefore possible to regroup these penguins into four categories depending on the sex and the physiological state of the animal, i.e. whether the bird was near departure after fasting for a long period or was just returning from a foraging trip. These four groups were: departing females, arriving females, departing males and arriving males. Sex was determined either by the song (Jouventin, 1982) or by the behaviour (such as mating or egg laying). All birds for the calibration experiment were rearing a newly hatched chick and were caught either at the beginning or at the end of a period ashore. The birds were released at the end of the experiment and resumed their previous breeding activity. For the validation experiment, birds engaged in courtship were caught on the beach. All birds were weighed, and measurements of their flipper length, bill length and foot length were taken according to standard techniques (Stonehouse, 1960).

Protocol 1: calibration on the treadmill

On the day of their capture, birds were weighed and their stomach contents retrieved and weighed using the 'water off-loading technique' (Wilson, 1984). The stomach content of each bird was kept in a refrigerator during the experiment, and the bird was re-fed with this prior to its release. After capture, the birds were allowed to rest overnight before being equipped with an externally mounted heart rate data logger placed in a cylindrical container (11 cm long, 4 cm diameter, mass 65 g). The logger was mounted externally rather than implanted to avoid the need for any post-operative recovery time. Electrode leads were made of silver-plated copper wire connected to stainless-steel safety pin electrodes. These were placed

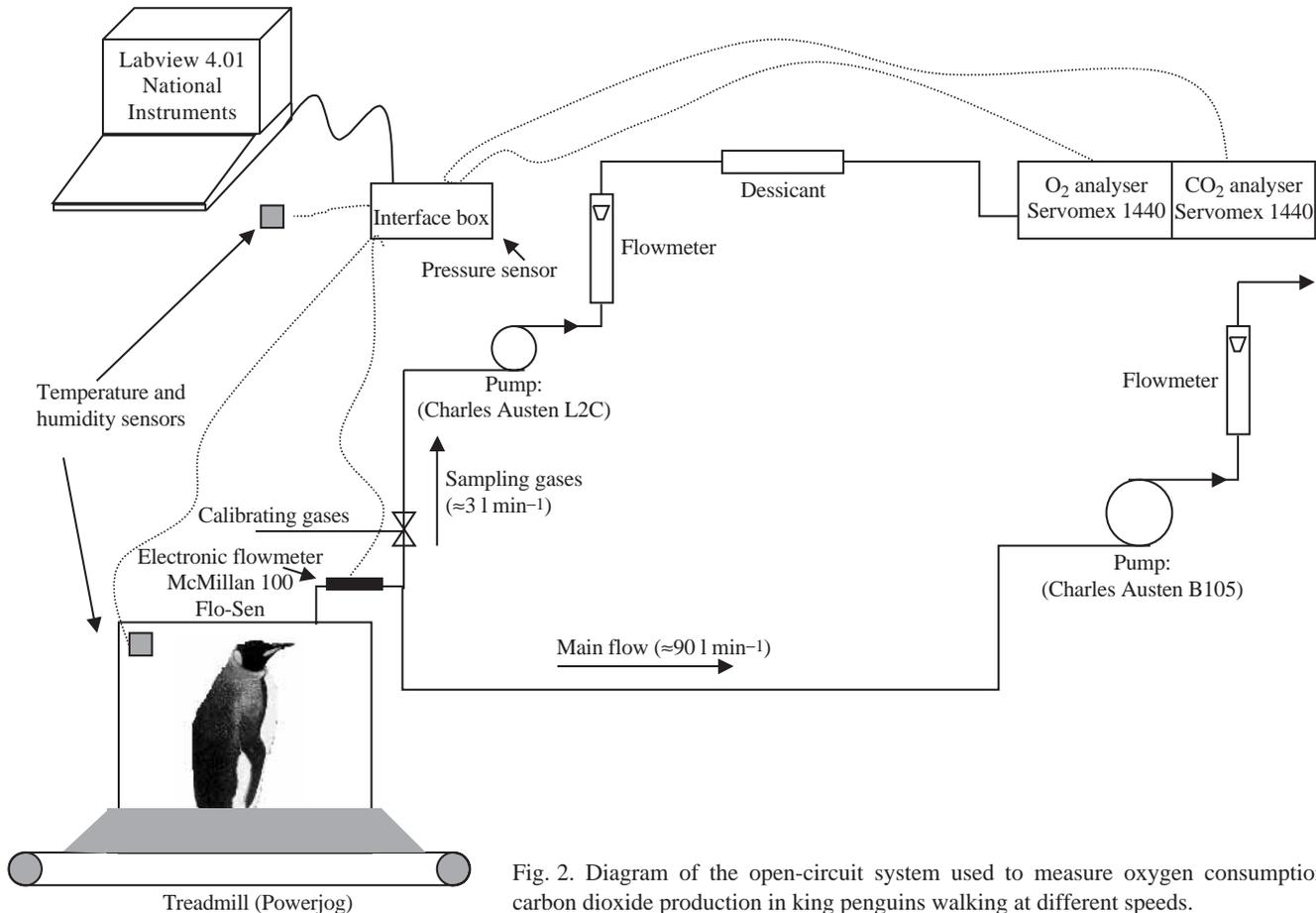


Fig. 2. Diagram of the open-circuit system used to measure oxygen consumption and carbon dioxide production in king penguins walking at different speeds.

subcutaneously in a midline dorsal position. One electrode was placed level with the heart and the other in a more caudal position. This arrangement provided a good electrocardiogram signal. The body of the logger was attached to the back feathers using Tesa tape (Beierdorsf AG, Germany; Bannasch et al., 1994).

Apparatus

Rates of oxygen consumption were measured using an open-circuit system (Fig. 2) similar to that described previously (Bevan et al., 1994; Bevan et al., 1995). A wooden frame was attached to a variable-speed treadmill (Powerjog, Sport Engineering Ltd) onto which a Perspex respirometer was placed. The respirometer measured 64.5 cm×45 cm×79 cm and was equipped with two fan units to ensure thorough and even mixing of its gaseous content. The whole system had a total volume of 366.6 l. Brushes between the frame and the treadmill ensured a good seal, and rubber seals ensured an airtight fit between the frame and the respirometer. Air was drawn through the box by two air pumps linked in parallel (Charles Austen B105), at approximately 90 l min⁻¹, measured by an electronic flowmeter (McMillan 100 Flo-Sen) calibrated using a rotameter (Fisher Control Limited). A sub-sample of the outlet airflow (approximately 3 l min⁻¹) was passed, using a pump (Charles Austen L2C), via a drying agent (silica gel), to a paramagnetic oxygen and infrared carbon dioxide analyser

(Servomex 1440). Temperature, humidity and atmospheric pressure were also monitored, using appropriate sensors (Farnell Instrument Services), both inside and outside the respirometry chamber. The O₂ and CO₂ analysers were calibrated before each experiment using O₂-free N₂, atmospheric air and a mixture of 1% CO₂ in N₂ prepared by a precision gas-mixing pump (2M301/1-f, Wöstoff Pumps, Bochum, Germany). N₂ dilution tests (Fedak et al., 1981) showed that the accuracy of the system was within 1%.

The output signals of the gas analysers, relative humidity sensors, temperature probes and barometer were passed into a purpose-built interface unit that amplified the signals and transferred them to a laptop computer (Compaq) fitted with an A/D converter (DAQ500 card, National Instruments). Data were displayed and sampled every 10 s using LabVIEW software (National Instruments). Heart rate was recorded in the data logger every 2 s and later downloaded to a computer for analysis.

Experimental protocol

After being placed on the treadmill, the bird was left to settle for at least 1.5 h before the start of a walking session, until \dot{V}_{O_2} reached steady values. The penguin then walked on the treadmill at eight different speeds between 0.3 and 2.5 km h⁻¹ (with an increment of approximately 0.3 km h⁻¹), depending on the maximum sustainable speed that each bird could attain. The

sequence of walking speeds was assigned at random. Birds walked for at least 25 min at each speed until steady-state conditions were obtained (i.e. stabilisation of the gas concentrations in the respirometer). After each hour of walking, the bird was left to rest for 0.5 h before the next walking session commenced. The bird was then allowed to rest for at least 2 h after the fourth walking period.

Protocol 2: validation

The same measuring system was used as in protocol 1. The birds, equipped with an externally mounted data logger, were placed on the treadmill and left to rest for at least 3 h. They were then walked at a selected speed, assigned at random, for an hour. After a run, they were left to rest for at least another hour. The total time spent walking was approximately 4 h per day. All exercise periods were during the day; at night, the bird was left undisturbed, resting in the dark on the treadmill. During the entire duration of the experiment, heart rate was sampled every 2 s by the data logger. After 56 h, the bird was removed from the respirometer and weighed before it was released with its partner in the colony. The birds were not fed and had no access to water over the entire period of the experiments because at this stage of their breeding cycle they would normally be fasting ashore.

Data analysis

Calculation of rates of oxygen consumption

Rates of oxygen consumption and carbon dioxide production were calculated from the gas concentrations using equation 2 (derived from Depocas and Hart, 1957, as modified by Withers, 1977):

$$\dot{V}_{O_2} = \dot{V}_{STPD} \times \frac{F_{IO_2} - F_{EO_2}}{1 - [1 - (F_{ECO_2} - F_{ICO_2}) / (F_{IO_2} - F_{EO_2})] F_{IO_2}}, \quad (2)$$

where \dot{V}_{STPD} is the calculated dry air outflow at standard temperature and pressure, and F_{IO_2} , F_{EO_2} , F_{ICO_2} and F_{ECO_2} are fractional concentrations of inlet and outlet air for O_2 and CO_2 respectively.

Determination of body condition

Body condition, or the amount of body fat stores, was determined for each bird using a nutritional index (*NI*). This index was used to take into account differences in size (height) and the structural mass of each individual.

NI is the amount of body mass that could be considered as energy stores and was determined as the difference between the measured body mass *BM* (in kg) and the critical body mass *cBM* (in kg) (equation 3):

$$NI = BM - cBM. \quad (3)$$

Gauthier-Clerc et al. (Gauthier-Clerc et al., 2001) defined the critical body mass as the mass at which a bird spontaneously deserts its nest, and they found that *cBM* could be estimated using equation 4:

$$cBM = 0.102L_b - 3.43, \quad (4)$$

where L_b is the length of the bill (in mm), an indicator of the height of the bird.

Statistical analyses

All statistical tests were performed using the statistical package MINITAB 12.22 for windows (Minitab Inc.). All values are presented as means \pm S.E.M. The relationships between f_H and \dot{V}_{O_2} during calibration experiments were determined using least-squares regression. Regression equations were compared using an analysis of variance general linear model (GLM, as reviewed in Zar, 1999). After testing the data for normality using a Kolmogorov–Smirnov normality test, a Student's *t*-test was used to compare the significance of any difference between the means of two populations. One-way analysis of variance (ANOVA) with Tukey's HSD *post-hoc* testing was used when more than two populations were compared. Results were considered significant at $P < 0.05$.

Results

Heart rate and \dot{V}_{O_2} during resting and exercise

Individual body masses, the duration of the fast or foraging trip and group means are summarised in Table 1. There were significant differences in body mass between the four groups ($F = 0.339$, $P = 0.041$), with arriving birds being significantly heavier than departing birds (mean body mass of arriving birds 11.89 ± 0.29 kg, mean body mass of departing birds 10.73 ± 0.24 kg, $t = 2.99$, $P = 0.007$, $N = 22$). On the basis of bill length, there was no difference in height of arriving and departing males ($t = -0.16$, $P = 0.088$, $N = 11$) or females ($t = -0.64$, $P = 0.054$, $N = 11$), but females had significantly shorter bills than males ($t = -2.1$, $P = 0.048$, $N = 22$). Departing males fasted on average for 5 days longer than departing females ($t = -2.9$, $P < 0.05$, $N = 8$).

Values for resting and maximum \dot{V}_{O_2} and heart rate (f_H) are given in Table 2. Of particular interest is the fact that the resting and maximum f_H of departing males were significantly greater than those of the three other groups. Also, resting and maximum \dot{V}_{O_2} of arriving males were significantly greater than those of the three other groups.

Calibration

The maximum sustainable speed obtained was 2.4 km h^{-1} . During walking, \dot{V}_{O_2} increased linearly with speed, and there was no significant difference in slope between the four groups (ANCOVA, $F_{3,154} = 0.21$, $P = 0.89$, Fig. 3B). Similarly, mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) increased linearly with speed, and again there was no significant difference in slope between the four groups (ANCOVA, $F_{3,154} = 1.98$, $P = 0.85$, Fig. 3C). When the four groups are pooled, the relationship between speed v (in km h^{-1}) and $s\dot{V}_{O_2}$ (in $\text{ml min}^{-1} \text{ kg}^{-1}$) can be described by equation 5:

$$s\dot{V}_{O_2} = 16.8 + 9.8v. \quad (5)$$

Table 1. Details of the birds used in the calibration experiments

Bird	Sex	BM (kg)	Fast duration (days)	L_{bill} (mm)	L_{flipper} (mm)	L_{foot} (mm)	CS (g)
F1A	F	11.556	-20	112	300	162	826
F2A	F	12.078	-13	123	323	168	2060
F4A	F	11.122	-12	113	299	162	1003
F5A	F	12.31	-12	125	N/A	N/A	1106
F6A	F	12.026	N/A	130	313	175	0
F7A	F	10.492	N/A	116	316	170	1760
Mean FA group		11.60	-14.25	119.83	310.2	167.4	1125.8
F2D	F	10.186	8	123	316	166	0
F3D	F	10.66	9	124	317	170	0
F4D	F	10.176	15	122	309	165	536
F5D	F	9.806	7	116	316	178	0
F6D	F	12.46	N/A	117	329	171	0
Mean FD group		10.66	9.75	120.40	317.40	170.00	107.2
M1A	M	12.918	-14	121	315	172	522
M2A	M	12.52	-18	122	302	165	45
M4A	M	11.18	-15	119	312	165	1232
M6A	M	10.186	-14	127	318	172	2116
M7A	M	13.016	-13	125	324	168	765
M9A	M	13.38	-10	129	330	174	1810
Mean MA group		12.2	-14	123.83	316.83	169.33	1081.7
M3D	M	10.608	14	119	315	170	452
M5D	M	11.264	N/A	125	329	170	80
M6D	M	11.106	17	134	338	175	0
M7D	M	10.778	14	125	320	168	0
M8D	M	10.24	17	125	325	175	189
Mean MD group		10.7992	15.5	125.6	325.4	171.6	144.2

Data shown are sex (F, female; M, male), body mass after the stomach contents had been retrieved (BM), shift duration (negative values are days spent at sea before capture, and positive values are days spent ashore fasting and incubating before capture), length of the bill (L_{bill}), length of the flipper (L_{flipper}), length of the foot (L_{foot}) and stomach content (CS) of the 22 king penguins used in the calibration experiment.

N/A indicates non-available data.

A or D in the bird identification means measurements taken from birds arriving or departing from the colony, respectively.

Table 2. Mean resting and maximum heart rate and rate of oxygen consumption in the four different groups of king penguins

Group	Number of birds	Resting f_H (beats min^{-1})	Resting \dot{V}_{O_2} (ml min^{-1})	Max f_H (beats min^{-1})	Max \dot{V}_{O_2} (ml min^{-1})
Arriving males	6	90.51±1.3 ^a (27)	152.06±5.3 (27)	148.8±13.9 ^{c,d} (13)	472.7±53.8 (13)
Departing males	5	106.95±4.7 (20)	112.52±5.5 ^b (20)	196.9±24.4 (9)	363.8±52.0 ^e (9)
Arriving females	6	86.75±2.0 ^a (28)	125.59±3.5 ^b (28)	156.5±23.1 ^c (15)	405.1±48.9 ^e (15)
Departing females	5	76.95±2.8 ^a (26)	119.30±6.1 ^b (26)	133.8±8.4 ^d (11)	396.2±84.6 ^e (11)

The maximum values were calculated for walking speeds greater than 1.7 km h^{-1} .

Numbers in parentheses indicate the total number of data points used for each group.

Both resting rate of oxygen consumption (\dot{V}_{O_2}) and heart rate (f_H) were significantly different between the four groups ($F=11.34$, $P<0.0001$ for \dot{V}_{O_2} and $F=19.29$, $P<0.0001$ for f_H).

Similarly, maximum \dot{V}_{O_2} and maximum f_H were significantly different between the four groups ($F=6.55$, $P<0.001$ for \dot{V}_{O_2} and $F=20.44$, $P<0.0001$ for f_H). Values with the same superscript letters are not significantly different (*post-hoc* Tukey test, $P<0.05$).

($N=156$, $r^2=0.66$, $P<0.001$). In all groups, \dot{V}_{O_2} showed an initial increase during the transition between resting and walking (mean \dot{V}_{O_2} increase was 72 % for all groups, range 50–106 %). At maximum speed, f_H and \dot{V}_{O_2} were 1.8 and 3.2 times the

resting values, respectively. At any given speed, departing males had a significantly higher heart rate (on average 22 % higher over the whole speed range) than those of the other three groups (Fig. 3A). The regression equations for departing males

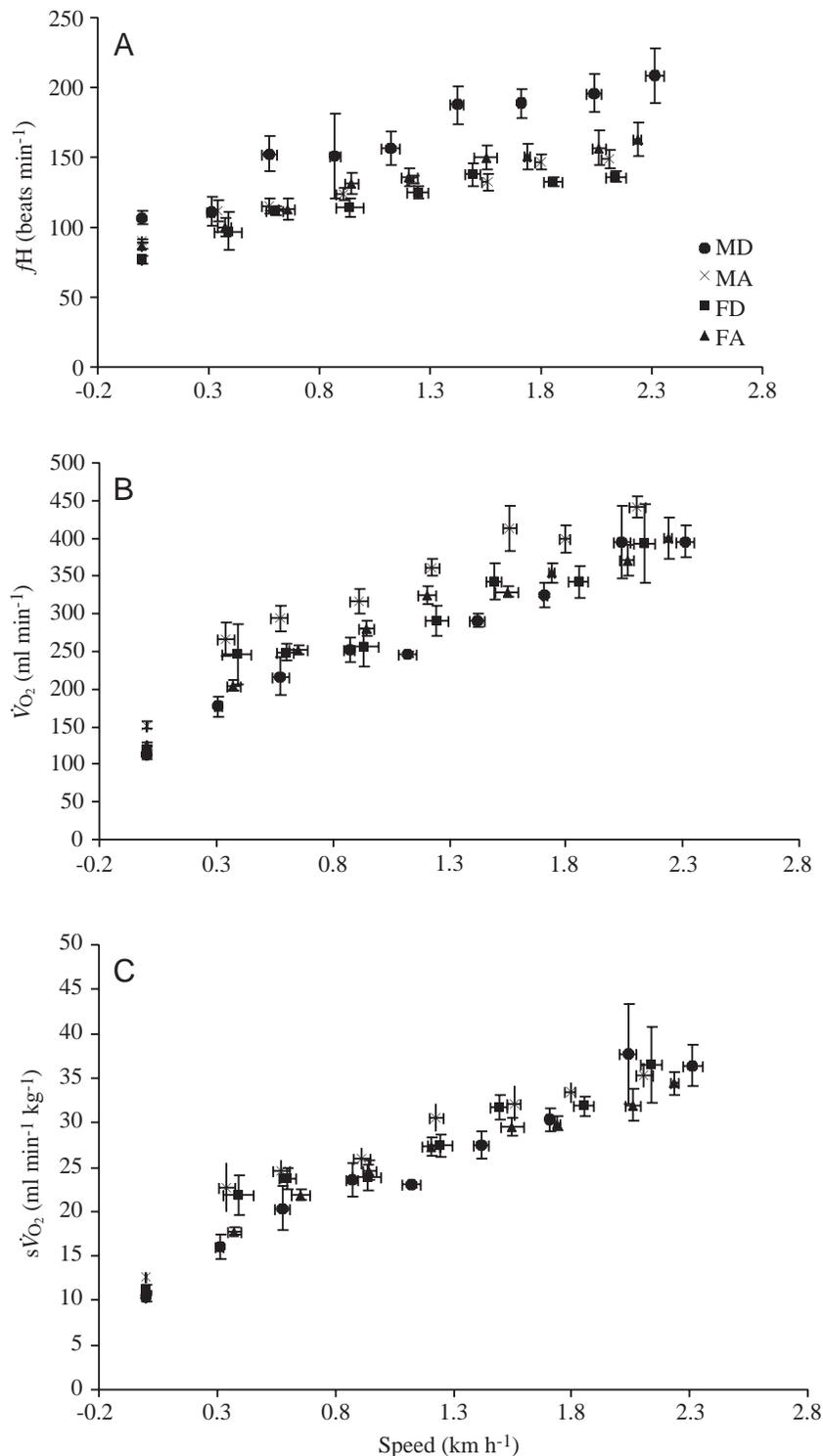


Fig. 3. Mean heart rate (f_H , A), rate of oxygen of consumption ($\dot{V}O_2$, B) and mass-specific $\dot{V}O_2$ ($s\dot{V}O_2$, C) of 22 king penguins walking at different speeds on a treadmill. The birds are in four groups, females (squares, $N=5$, FD) and males (circles, $N=5$, MD) departing from the colony and females (triangles, $N=6$, FA) and males (crosses, $N=6$, MA) arriving at the colony after a foraging shift. The vertical and horizontal lines at each point represent \pm S.E.M.

(equation 6) and the other three groups combined (equation 7) were:

$$f_H = 44.5v + 110.9 \quad (6)$$

($N=31$, $r^2=0.93$, $P<0.001$) and

$$f_H = 29.6v + 91.8 \quad (7)$$

($N=31$, $r^2=0.86$, $P<0.001$), where speed v is expressed in km h^{-1} .

There was a good correlation between f_H and $\dot{V}O_2$ in individual birds. However, when all data points from each bird were considered, there were significant within-group differences both in the slope and in the intercept of the regression equations ($F=2.6$, $P=0.049$ for departing males; $F=2.70$, $P=0.029$ for arriving males; $F=2.76$, $P=0.026$ for departing females; $F=9.70$, $P<0.0001$ for arriving females). Thus, each group was best described using the pooled regression (in which all birds in each group are pooled; Green et al., 2001; see Table 3).

However, when resting and exercise data were separated, there was no significant difference in the slopes of the regression equations during exercise for each penguin within the same group ($F=0.41$, $P=0.80$ for departing males; $F=1.07$, $P=0.40$ for arriving males; $F=0.91$, $P=0.47$ for departing females; $F=1.81$, $P=0.14$ for arriving females). It was thus possible to determine a general regression (see Green et al., 2001) for each group using an analysis of covariance.

Note that each equation (see Table 4) was obtained using data points obtained only when the bird was exercising. The slopes of the four equations (Table 4) were significantly different from each other ($F=9.67$, $P<0.0001$). The O_2 pulses of birds at rest (resting oxygen pulses, ROP s), were significantly different between the four groups ($F=19.71$, $P<0.0001$), with departing males having the lowest ROP (Table 5).

Variation in oxygen pulse with the nutritional index (NI)

When the mean ROP s and the mean O_2 pulses of birds during exercise (active oxygen pulses, AOP s) of each group were plotted against the mean NI of each group, there was a significant positive correlation in each case ($ROP=0.2548NI+0.8867$, $r^2=0.54$; $AOP=0.6609NI+0.854$, $r^2=0.87$).

There was also a significant positive correlation ($r^2=0.36$, $P=0.004$) between individual ROP and NI and between individual AOP and NI ($r^2=0.46$, $P=0.0007$, Fig. 4). It is therefore possible to predict oxygen pulse using equations 8 and 9:

Table 3. Intercept (a) and slope (b) of linear regressions of oxygen consumption rate on heart rate in the four different groups of king penguins during resting and walking at incremental speeds on a treadmill

Group	Number of birds	N	a	b	r ²	P
Arriving males	6	72	-248.44±25.89	4.723±0.21	0.73	<0.05
Departing males	5	50	-78.097±22.38	2.017±0.15	0.76	<0.05
Arriving females	6	70	-120.56±17.51	3.176±0.14	0.79	<0.05
Departing females	5	61	-164.48±19.06	3.877±0.18	0.79	<0.05

\dot{V}_{O_2} , rate of oxygen consumption (ml min⁻¹); fH , heart rate (beats min⁻¹).

N is the number of data points in each group.

Values are means ± S.E.M., where S.E.M. is the standard error of the intercept of the slope, and r² is the average coefficient of determination in each group.

Table 4. Intercept (a) and slope (b) of linear regressions of oxygen consumption rate on heart rate in the four different groups of king penguins during walking at incremental speeds on a treadmill

Group	Number of birds	N	a	b	r ²	P
Arriving males	6	45	-267.5±55.76	4.9699±0.42	0.76	<0.05
Departing males	5	31	-89.63±47.56	2.1852±0.28	0.75	<0.05
Arriving females	6	43	-99.88±37.27	3.1378±0.27	0.79	<0.05
Departing females	5	36	-212.37±4.34	4.3363±0.44	0.82	<0.05

\dot{V}_{O_2} , rate of oxygen consumption (ml min⁻¹); fH , heart rate (beats min⁻¹).

N is the number of data points in each group.

Values are means ± S.E.M., where S.E.M. is the standard error of the intercept of the slope, and r² is the average coefficient of determination in each group.

$$ROP = 1.033 + 0.18[BM - (0.102L_b - 3.43)] \quad (8)$$

$$AOP = 1.56 + 0.36[BM - (0.102L_b - 3.43)], \quad (9)$$

where ROP and AOP are in ml O₂ min⁻¹, BM is in kg and L_b is in mm. BM-(0.102L_b-3.43) is the nutritional index NI (see equations 3 and 4).

\dot{V}_{O_2} was then estimated using the equations $\dot{V}_{O_2}=ROP \times fH$ when the animal was resting and $\dot{V}_{O_2}=AOP \times fH$ when the animal was active (i.e. exercising on the treadmill).

Heart rate as a predictor of \dot{V}_{O_2}

The data obtained during the calibration experiments were used to derive an equation for estimating \dot{V}_{O_2} from fH in six penguins caught during their courting period, resting and exercising on the treadmill, over a 56 h period. During the validation experiments, the appropriate equation (see below) was used to derive predicted values of \dot{V}_{O_2} over two time scales. First, \dot{V}_{O_2} was estimated for 30 min periods throughout the whole experimental period (up to 56 h) to determine whether short-term changes in metabolic rate could be accurately predicted. Second, \dot{V}_{O_2} was estimated over the whole experimental period to determine whether longer-term estimates of metabolic rate were accurate.

The aim of this validation experiment was to determine which of the three models (Fig. 5) for estimating \dot{V}_{O_2} gives the best prediction. Consequently, \dot{V}_{O_2} was predicted using (i) the

appropriate general linear regression (GLR, equations in Table 3); (ii) a combination between ROPs and the appropriate linear regression equations (Table 4, depending on sex and nutritional status) between fH and \dot{V}_{O_2} during exercise or (iii) AOPs and ROPs as a function of NI (equations 8 and 9).

The difference between measured and estimated \dot{V}_{O_2} was calculated as a percentage of measured \dot{V}_{O_2} to give the error of the estimate. A positive value of percentage error indicates an over-estimation of \dot{V}_{O_2} and a negative value indicates an under-estimation of \dot{V}_{O_2} .

The mean measured \dot{V}_{O_2} over the experimental period for the six birds was 148.8 ml O₂ min⁻¹ (Table 6). The mean calculated \dot{V}_{O_2} values were 153.1 ml O₂ min⁻¹ using model i (the general linear regression model), 148.7 ml O₂ min⁻¹ using model ii (linear regression and ROP) and 150.0 ml O₂ min⁻¹ using model iii (oxygen pulse as a function of NI). This gave estimated errors, for the six experimental birds, of 2.9%, -0.1% and 0.8% for models i, ii and iii respectively. Individual error estimation of the mean rate of oxygen consumption ranged from -12.7% to +26.5% when using the appropriate linear regression, from -14.4% to +22.6% when using a combination of linear regression and ROP and from -12.4% to 8.5% using ROP and AOP as a function of NI (Table 6).

The error of 30 min estimate ranged from -62% to +182% when using model i, from -41% to 106% when using model

Table 5. The mean oxygen pulse of king penguins while resting (ROP) or while walking on the treadmill (active O₂ pulse: AOP) in the four groups of birds

Group	Number of birds	ROP (ml O ₂ beat ⁻¹)	AOP (ml O ₂ beat ⁻¹)
Arriving males	6	1.693±0.352	2.963±0.572
Departing males	5	1.071±0.215	1.654±0.294
Arriving females	6	1.455±0.199	2.447±0.356
Departing females	5	1.568±0.327	2.613±0.476

Values are means ± S.E.M.

ii and from -52% to +89% when using model iii (Table 7). Although the mean of 30 min error was lowest when using model ii (2.5% compared with 8.3% for model i and 5.5% for model iii), the absolute error was lowest when $\dot{V}O_2$ was estimated using model iii (15.8% compared with 17.7% for model ii and 23.8% for model iii) (Table 7; Fig. 6).

Fig. 4. Resting individual oxygen pulse (ROP, circles) and active oxygen pulse (AOP, squares) for 22 king penguins plotted against the nutritional index *NI* (see text for details). The continuous line is the regression line between ROP and *NI* ($ROP=1.0326+0.1761NI$, $r^2=0.36$, $P=0.004$), and the broken line is the regression line between AOP and *NI* ($AOP=1.5637+0.36NI$, $r^2=0.46$, $P=0.0007$).

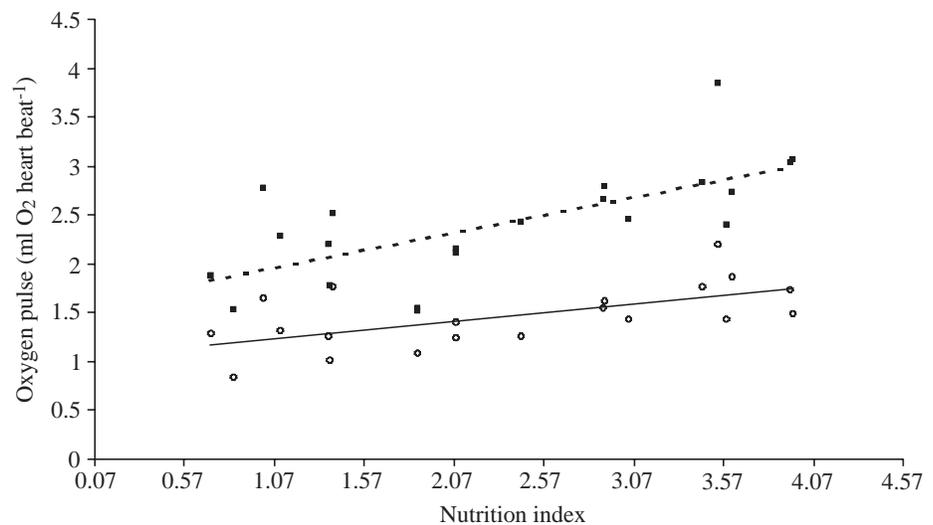
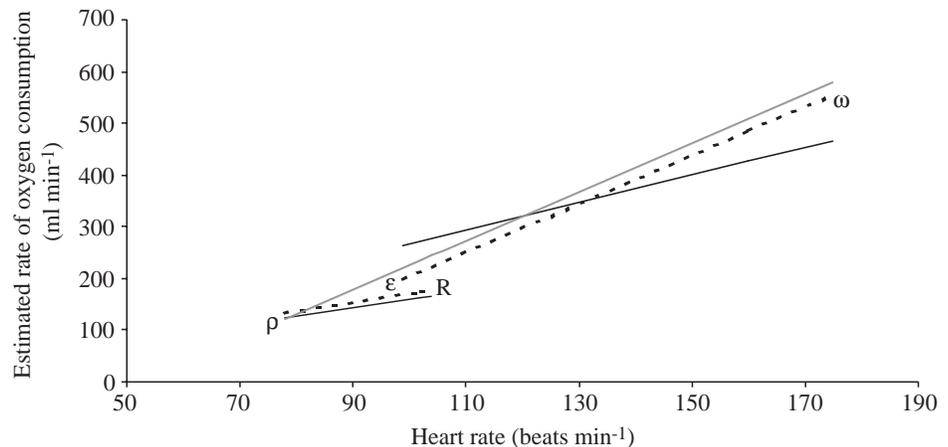


Fig. 5. Graphical representation of the three different models used to estimate the rate of oxygen consumption $\dot{V}O_2$ from heart rate *fH* in a hypothetical arriving male with a bill length of 123.8 mm and a body mass of 12.2 kg (nutritional index *NI*=3.2). The grey line represents the general linear regression model (GLR) for departing males, the broken lines use a combination of resting oxygen pulse (ROP) (from minimum resting *fH*, ρ , to maximum resting *fH*, *R*) and the linear regression for the birds walking on the treadmill (from minimum exercising *fH*, ϵ , to maximum walking *fH*, ω). The solid black lines represent the model using ROPs and active oxygen pulses (AOPs) as function of the nutritional index (*NI*).



Discussion

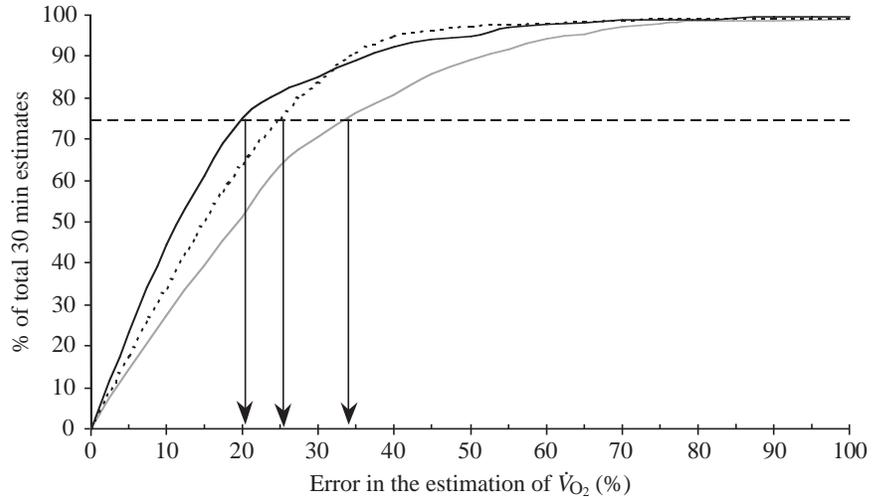
Cost of transport

The resting $\dot{V}O_2$ values obtained (112.5–152.1 ml O₂ min⁻¹) fell within the range 74.9–169.8 ml O₂ min⁻¹ obtained by Le Maho and Despin (Le Maho and Despin, 1976) for king penguins in their thermoneutral zone at the beginning and at the end of 30 days of fasting.

There was a distinct increase in $\dot{V}O_2$ when the penguins started to walk. Similar increases have been observed in other species: black-browed albatross *Diomedea melanophrys* (Bevan et al., 1994), gentoo penguin *Pygoscelis papua* (Bevan et al., 1995) barnacle goose *Branta leucopsis* (Nolet et al., 1992) and eider duck *Somateria mollissima* (Hawkins et al., 2000). It has been suggested that this initial increase may be associated with the change in attitude of the bird from a lying or sitting position to a standing and walking position (Bevan et al., 1994).

To compare the relationship between mass-specific $\dot{V}O_2$ ($s\dot{V}O_2$) and walking speed (*v*) derived here with those found in

Fig. 6. Distribution of the absolute error between 30 min estimates of oxygen consumption rate $\dot{V}O_2$ and measured $\dot{V}O_2$ for the six calibrations obtained using the general linear regression model (model i, grey line) when using a combination of resting oxygen pulse (ROP) and the linear regression for the birds walking on the treadmill (model ii, broken line) or when ROPs and active oxygen pulses (AOPs) are expressed as function of the nutritional index (model iii, black line). The horizontal dashed line is the line of 75% of the total errors of 30 min estimates, and the arrows indicate the respective error for each model (75% of errors are below 34% when using model i, below 26% when using model ii and below 20% when using model iii).



other penguin species, we used E (Pinshow et al., 1976), where E is $ds\dot{V}O_2/dv$, defined as the slope of the line relating $s\dot{V}O_2$ to speed excluding resting values. The value of E for the king penguin falls on the regression line of $\log E$ against $\log BM$ determined for six other penguin species ($r^2=0.96$, $P<0.003$, Fig. 7). The higher values of E for penguins than for quadruped mammals is thought to be because penguins must compromise between aquatic and terrestrial locomotion. However, it may not be due to the waddling gait of penguins, as previously thought (Pinshow et al., 1976; Baudinette and Gill, 1985), but instead to the short legs of penguins that require them to generate muscular force more rapidly (Griffin and Kram, 2000).

Calibration experiment

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

et al., 1994; Bevan et al., 1995; Boyd et al., 1995; Hawkins et al., 2000). Following on from this work, and because of the peculiarities of the breeding cycle of the king penguin, the main aims of the present study were to determine whether fH could be used as a technique for measuring the rate of energy expenditure of these animals in the field and whether this relationship was valid for different sexes and stages of the penguins' yearly physiological cycle for an animal that can exhibit extreme variation in nutritional state.

Our main hypothesis was that the relationship between fH and $\dot{V}O_2$ would vary according to the physiological status of the bird. This was despite the fact that there are no changes in haematocrit associated with fasting (Cherel et al., 1988). Considering that haematocrit is an indirect estimate of C_{aO_2} , it might therefore be assumed that there would be no change in the fractional volume of the oxygen content of arterial blood associated with fasting. However, Butler and Turner (Butler and Turner, 1988), showed that muscular training caused an increase in muscle capillarity (blood capillary/muscle fibre

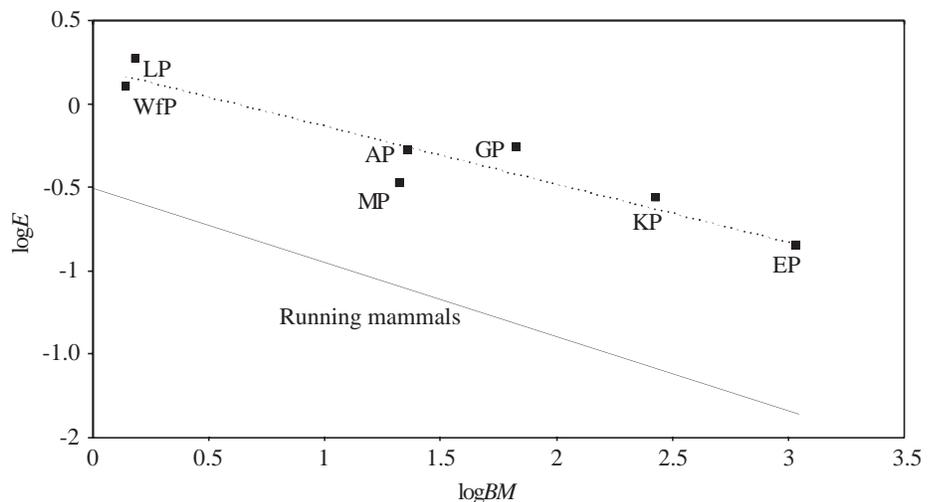


Fig. 7. Double logarithmic plot of E against body mass (BM , kg), where E is the energy cost of walking (in $lO_2 kg^{-1} km^{-1}$). Data for the emperor penguin (EP), the Adélie penguin (AP) and the white-flipped penguin (WfP) are derived from Pinshow et al. (Pinshow et al., 1976), data for the little penguin (LP) are derived from Baudinette and Gill (Baudinette and Gill, 1985), data for the gentoo penguin (GP) are derived from Bevan et al. (Bevan et al., 1995), data for the macaroni penguin (MP) are from Green et al. (Green et al., 2001) and data for king penguin (KP) data are from the present study. The dotted line is the regression line for all penguins other than the king penguin: $\log E = -0.3822 \log BM + 0.3477$, $r^2=0.9637$, $P<0.003$, $N=6$. The solid line is the line calculated for running mammals: $\log E = -0.4434 \log BM + 0.5057$ (Taylor et al., 1970).

Table 7. Summary of distribution of 30 min errors of \dot{V}_{O_2} estimates using the three different models: the appropriate general linear regression line (GLR), the appropriate combination of resting oxygen pulse (ROP) and linear regression (LR) or using resting (ROP) or active (AOP) oxygen pulse as a function f of the nutritional index (NI)

	GLR (model i)	LR and ROP (model ii)	ROPs and AOPs $f(NI)$ (model iii)
Arithmetic mean (%)	8.3	2.5	5.5
Absolute mean (%)	23.8	17.7	15.8
Range	-62.3 to +182.0	-41.6 to +106.3	-51.7 to +89.2
Arithmetic median	3.9	-0.2	3.8
Absolute median	19.5	14.9	11.3
Arithmetic mode	-20	0	10
Absolute mode	5	5	5

The arithmetic mean is the mean of the percentage differences across all six individuals.

The absolute mean is the average of the percentage differences excluding the signs of the differences.

Means were obtained using arcsine-transformed data.

ratio) of 20%. It is thus possible that inactivity results in a decrease in the capacity of the tissues to extract oxygen (i.e. in $CaO_2 - C\bar{v}O_2$). Cherel et al. (Cherel et al., 1994) also indicated that, during phase II of fasting (before the transition from the use of lipids to the use of protein as the main energy fuel; Cherel et al., 1988), the body mass of king penguins decreased by 32%, but that the majority of this was the result of the loss of body fat (reduced by 78%). There was, therefore, only an 11% reduction in total body protein levels over this period, although heart protein content was reduced by 20%. These data indicate that, at the end of phase II, heart protein as a proportion of total body protein was reduced by 10% (from 1.7% to 1.5% of the total body protein).

Cardiac stroke volume has been shown to be directly proportional to heart size in birds (Grubb, 1982), so the cardiac stroke volume (V_s) of the king penguin probably decreases as the fast progresses. Therefore, $V_s(CaO_2 - C\bar{v}O_2)$, the oxygen pulse, would be expected to decrease as a function of fast duration as body stores are reduced. This means that, at the same level of \dot{V}_{O_2} , f_H should be higher in a fasting king penguin than in a non-fasting one. Indeed, using equations 10 and 11 describing the relationship between f_H and \dot{V}_{O_2} for arriving males and departing males, at a resting \dot{V}_{O_2} of 120 ml min^{-1} , the corresponding f_H was $88 \pm 7.1 \text{ beats min}^{-1}$ for an arriving male and $109 \pm 9.2 \text{ beats min}^{-1}$ for a departing male, although these values are not significantly different ($Z = -1.81$, $P = 0.07$). However, at a maximum \dot{V}_{O_2} of 350 ml min^{-1} , the corresponding f_H was $124 \pm 6.3 \text{ beats min}^{-1}$ for an arriving male, which is significantly lower than the corresponding value of $196 \pm 10.0 \text{ beats min}^{-1}$ for a departing male ($Z = -6.1$, $P < 0.0001$).

For departing males:

$$f_H = 0.15\dot{V}_{O_2} + 69.98 \quad (10)$$

($N = 5$, $r^2 = 0.76$, $P < 0.001$). For arriving males:

$$f_H = 0.38\dot{V}_{O_2} + 63.66 \quad (11)$$

($N = 6$, $r^2 = 0.73$, $P < 0.001$).

In the present study, we have demonstrated not only that the

relationship between f_H and \dot{V}_{O_2} is dependent upon the physiological status of the animal but also that it is dependent on whether the animal is active. As in a previous study (Nolet et al., 1992), a combination of ROP and the linear regression between f_H and \dot{V}_{O_2} during exercise (model ii) gave better estimates of \dot{V}_{O_2} than the general linear regression (model i). However, although the \dot{V}_{O_2}/f_H relationship differed among the four groups described in the present study, most of this variation can be explained by differences in the physiological state of the individuals. As observed in the present study, the four groups differed not only in gender but also in fast duration, with departing males having a significantly longer fasting period ashore (on average 50% longer than that of the females).

Accuracy of heart rate as a predictor of \dot{V}_{O_2}

In the present study, we compared three different models (Fig. 5) to estimate \dot{V}_{O_2} from f_H . The model equations were then applied to six courting birds. The main problem with using courting animals was that it was not possible to determine accurately for how long the bird had been fasting: the fasting period before egg laying can last for up to an average of 20 days for males and 14 days for females (Barrat, 1976). When using either the GLR model (model i) or the ROP and linear regression model (model ii), it was therefore difficult to decide which equation should be applied (i.e. that for a departing or for an arriving animal) to estimate \dot{V}_{O_2} . In most cases, \dot{V}_{O_2} was overestimated when using the relationship for departing individuals and underestimated when using the relationship for arriving individuals. However, irrespective of the model used, the mean \dot{V}_{O_2} for the six birds over the entire experimental period could be estimated with an error of less than 3% (Table 6), with individual errors ranging between -14.4 and +26.5%. This range of errors in estimation is much lower than those obtained from birds and mammals using doubly labelled water (Nolet et al., 1992; Bevan et al., 1994; Bevan et al., 1995; Boyd et al., 1995; Hawkins et al., 2000; for a review, see Table 8).

The use of ROP and AOP as a function of nutritional index (model iii) not only gave similarly satisfactory estimates

Table 6. Comparison of mean rate of oxygen consumption measured by respirometry and predicted from heart rate in six exercising king penguins

Bird	Sex	NI	Measured \dot{V}_{O_2} (ml min ⁻¹)	Heart rate (beats min ⁻¹)	General linear regression (model i)			Linear regression and ROP (model ii)			ROP and AOP with NI (model iii)	
					\dot{V}_{O_2} (ml min ⁻¹)	(% error)	Best equation	\dot{V}_{O_2} (ml min ⁻¹)	(% error)	Best equation	\dot{V}_{O_2} (ml min ⁻¹)	(% error)
21-Dec-98	Male	3.72	151.8	84.4	150.9	-0.5	MA	157.8	3.9	MA	162.5	7.1
23-Dec-98	Female	2.53	121.8	72.1	115.1	-5.5	FD	119.4	-2.0	FA	124.4	2.1
26-Dec-98	Female	0.82	118.7	85.2	150.1	26.5	FA	145.4	22.6	FA	118.7	0.1
28-Dec-98	Male	1.48	163.5	108.3	142.7	-12.7	MD	139.9	-14.4	MD	168.1	2.8
2-Jan-99	Male	1.01	149.4	113.7	151.2	1.2	MD	145.8	-2.4	MD	162.1	8.5
5-Jan-99	Female	1.89	187.5	103.6	208.5	11.2	FA	183.9	-2.0	FD	164.3	-12.4
Mean		3.72	148.8	94.6	153.1	3.4		148.7	0.96		150.0	1.4
			Population error			2.89			-0.07			0.81

The rate of oxygen consumption \dot{V}_{O_2} was calculated using three different methods: the appropriate general linear regression (model i), an appropriate combination of linear regression and resting oxygen pulse (ROP) (model ii) and resting (ROP) or active (AOP) oxygen pulse as a function of the nutritional index (NI) (model iii).

The population error is calculated as the error between the mean measured \dot{V}_{O_2} for the six individuals and the mean estimated \dot{V}_{O_2} .

The appropriate equation is determined depending on the gender and the equation (departing or arriving) that gives the smallest percentage error; the equation used is described as the best equation. MA, males arriving; MD, males departing; FA, females arriving; FD, females departing.

Table 8. Comparison of validation studies of the heart-rate technique $\dot{V}_{O_2}=f(\text{HR})$ compared with the doubly labelled water (DLW) technique

Species	$s\dot{V}_{O_2}$ (ml min ⁻¹ kg ⁻¹)	$s\dot{V}_{O_2}=f(\text{HR})$			DLW			N	Reference
		(ml min ⁻¹ kg ⁻¹)	% Difference	Range (%)	(ml min ⁻¹ kg ⁻¹)	% Difference	Range (%)		
Barnacle geese* (<i>Branta Leucopsis</i>)	32.1±1.76*	32.3±3.1*	0.02±6.4	-13 to +18	32.3±2.9*	1.43±7.6	-21 to +23	5	Nolet et al., 1992
Black-browed albatross (<i>Diomedea melanophrys</i>)	15.49±0.97	14.9±0.67	-2.8±4.7	-17 to +12	15.27±1.31	0.46±10	-27 to +29	6	Bevan et al., 1994
Gentoo Penguin (<i>Pygoscelis papua</i>)	13.3±0.75	13.17±0.91	-0.95±4.3	-17 to +11	13.44±2.1	1.6±20.8	-45.5 to +73.1	6	Bevan et al., 1995
California sea lion (<i>Zalophus californianus</i>)	12.0±0.9	11.95±0.89	2.7±8.0	-28 to +24	14.66±1.35	38.04±13.1	-10 to +86	7	Boyd et al., 1995
Eider duck (<i>Somateria mollissima</i>)	31.3±2.5	32.3±2.6	3.7±4.2	-14 to +17	30.1±5.7	0.42±21.8	-74 to +64	6	Hawkins et al., 2000
King penguin* (<i>Aptenodytes patagonicus</i>)	148.8±11*	150.0 ±9.1*	1.4±3	-12 to +8				6	Present study

% Difference is the algebraic mean of differences between measured and estimated mass-specific \dot{V}_{O_2} ($s\dot{V}_{O_2}$) expressed as a percentage, N refers to the number of individuals used and an asterisk indicates that the calculation of \dot{V}_{O_2} consumption is not mass-specific (units are ml min⁻¹ in these cases).

(0.81% error in the estimation of the mean energetic expenditure for the six birds, with individual error ranging from -12% to +9%) but could also be more easily applied in field conditions, where foraging trip and fasting duration vary as the breeding cycle progresses.

The relatively low coefficients of determination (r^2) obtained for the relationship between the oxygen pulse and the nutritional index could be explained at least partly by the fact that we use bill length rather than body height when determining nutritional index.

In conclusion, the present study further supports the use of heart rate as a predictor of the rate of energy expenditure of free-ranging animals. However, it raises new questions about the physiological changes associated with long-term fasting. Indeed, in king penguins, long-term fasting is also associated with inactivity, and there is evidence in barnacle geese (Bishop et al., 1998) that the relative mass of the heart decreases with inactivity. It would therefore be interesting to differentiate, in the king penguin, the effects of fasting from those of inactivity to determine how the decrease in oxygen pulse in birds captured as they departed from the colony is influenced by these factors.

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