

**THE USE OF HEART RATE TO ESTIMATE OXYGEN
CONSUMPTION OF FREE-RANGING BLACK-BROWED
ALBATROSSES *DIOMEDEA MELANOPHRYS***

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Summary

Heart rates (f_H) and rates of oxygen consumption (\dot{V}_{O_2}) were measured in eight black-browed albatrosses (*Diomedea melanophrys*) when walking on a treadmill, with the aim of using f_H to predict \dot{V}_{O_2} in free-ranging albatrosses. The resulting relationship between the variables was: \dot{V}_{O_2} (ml min^{-1}) = $[0.0157f_H$ (beats min^{-1})]^{1.60}, $r^2=0.80$, $P<0.001$. In addition to the calibration procedure, six of the albatrosses were injected with doubly labelled water (DLW), and f_H and \dot{V}_{O_2} were monitored continuously over a 3 day period while the birds were held in a respirometer. During the 3 day period, the birds were walked for up to 3–4 h day⁻¹ in bouts lasting approximately 0.5 h. The heart rate data were used to estimate the metabolic rates of these birds using the above regression. Estimates of metabolic rate derived from f_H , DLW and respirometry did not differ (ANOVA; $P=0.94$), primarily because of the variance between individual birds. There was also no significant difference between the different estimates obtained from the different equations used to calculate energy expenditure from the DLW technique (ANOVA; $P=0.95$). Mean estimates of \dot{V}_{O_2} from f_H under active and inactive conditions differed from measured values of \dot{V}_{O_2} by –5.9% and –1.7% respectively. In addition, the estimates of \dot{V}_{O_2} from f_H at different walking speeds did not differ significantly from the measured values. It appears that, in the black-browed albatross, f_H is as good a predictor of the mean metabolic rate of free-ranging birds as DLW or time–energy budgets combined with either respirometry or DLW. However, the method should be applied to as many individuals and as many instances of a particular behaviour as possible. The heart rate technique offers potential for much more detailed analyses of the daily energy budgets of these birds, and over much longer periods, than has previously been possible.

Key words: heart rate, oxygen consumption, albatross, *Diomedea melanophrys*, metabolic rate, energy expenditure, doubly labelled water, walking.

Introduction

Estimating the energy expenditures of free-ranging animals is an essential component in studies of foraging ecology, but such estimates are difficult to obtain for flying birds, especially albatrosses. At present, there are two methods in general use: time–energy budgets (TEB; Weathers and Nagy, 1980; Walsberg, 1983; Weathers *et al.* 1984) and the doubly labelled water method (DLW; Lifson *et al.* 1955). Both methods have their drawbacks. The TEB method can be very laborious and demands that the individual animal being studied is observed constantly (or that its behaviour is remotely recorded) and that the meteorological conditions, e.g. solar radiation, windspeed etc., are carefully monitored (Weather and Nagy, 1980; Weathers *et al.* 1984; Nagy, 1989a; Bakken *et al.* 1991). Activities are then assigned energy values determined from laboratory studies, and the daily energy expenditure is estimated from the proportion of time that is spent at each activity (Calder and King, 1976).

The DLW technique estimates the rate of carbon dioxide production from the difference in the rate of loss of labelled hydrogen (^2H or ^3H) and oxygen (^{18}O) from the body. This entails capturing the animal and taking a fluid sample, e.g. blood or urine, administering a mixture of H_2^{18}O and $^2\text{H}_2\text{O}$ or $^3\text{H}_2\text{O}$, allowing the labelled water to equilibrate with the body water, then taking another fluid sample before releasing the animal. The animal must then be recaptured for a final body fluid sample to be taken. The experimental period depends on the biological half-life of the H_2^{18}O in the species being studied; 1–2 half-lives being the optimum (Nagy, 1980; Tatner and Bryant, 1989). Validation studies which have simultaneously measured the energetics of the animals by respirometry or food intake have shown that the technique can be very accurate, with an error of only -0.04% in one study (Buttemer *et al.* 1986), but that the range of individual estimates can differ substantially (in birds -29.0% to $+48.5\%$; see Speakman and Racey, 1988; Nagy, 1989b). In addition, most validation studies have been performed on sedentary animals, whereas free-living animals will, in general, be active some of the time. Another drawback with the technique is that it produces only a mean estimate of energy expenditure over the experimental period. It is not inherently possible to estimate the energy costs of specific types of activity, although some studies have attempted to do this by also determining time budgets (Nagy *et al.* 1984; Costa *et al.* 1989). A further drawback of the technique is that the energy consumption of the animal has to be estimated from the rate of CO_2 production, even though the respiratory quotient (RQ) is not necessarily known; this problem can lead to the introduction of large errors.

Heart rate has been proposed as an indicator of metabolic rate (Owen, 1969; Lund and Folk, 1976; Pauls, 1980; Ceesay *et al.* 1989; Bevan *et al.* 1992) because of the relationship between the two variables as illustrated by the Fick equation: oxygen consumption (\dot{V}_{O_2}) = heart rate (f_{H}) \times cardiac stroke volume \times tissue oxygen extraction. As long as cardiac stroke volume and tissue oxygen extraction remain constant or change systematically, f_{H} should be a good estimate of oxygen consumption (Butler, 1993). In birds, tissue oxygen extraction increases with exercise (Butler *et al.* 1977; Grubb, 1982; Woakes and Butler, 1986; Faraci, 1986) but stroke volume only varies slightly (Butler *et al.* 1977; Bech and Nomoto, 1982; Grubb, 1982; Kiley *et al.* 1985). Therefore, variations

in f_H are the main contributor to changes in cardiac output. Studies have shown that f_H in birds varies either linearly (Bamford and Maloiy, 1980; Woakes and Butler, 1983; Barnas *et al.* 1985) or sometimes curvilinearly (Grubb, 1982; Grubb *et al.* 1983) with \dot{V}_{O_2} .

In a comprehensive study on the barnacle goose (*Branta leucopsis*), Nolet *et al.* (1992) showed that f_H could be used in that species to determine f_H to an accuracy that was as good as that of the DLW technique. However, the f_H method must be limited to the range of f_H over which the f_H/\dot{V}_{O_2} relationship has been determined. It must also be applied to a number of individuals in the field, i.e. it cannot be used to estimate the energy costs of a single animal.

Use of f_H in determining metabolic rate is, therefore, highly attractive for several reasons: (1) the mean estimate is as accurate as any of the other available methods; (2) monitoring periods can be divided into smaller units to determine the energetic costs of specific activities; (3) new technology (Woakes *et al.* 1994) allows the monitoring of f_H for periods up to several months; (4) it also provides physiological information.

In this study, the relationship between f_H and \dot{V}_{O_2} was determined in the black-browed albatross (*Diomedea melanophrys* Temminck). The use of f_H in estimating energy expenditure was then validated by continuously monitoring f_H and \dot{V}_{O_2} for 3 days and comparing the estimates derived from f_H with the measured energy expenditures and those estimated from the DLW technique.

Materials and methods

The study was divided into two separate protocols. Protocol 1 was used to determine the relationship between \dot{V}_{O_2} and heart rate in the black-browed albatross (calibrations). Protocol 2 used this relationship to estimate the \dot{V}_{O_2} from f_H monitored over a 3 day period. This derived estimate was then compared with the \dot{V}_{O_2} that had been continuously measured directly by respirometry over the recording period and with the \dot{V}_{O_2} estimated using the doubly labelled water technique (DLW) (validations; see also Nolet *et al.* 1992).

Animals

The experiments were performed at the British Antarctic Survey base on Bird Island, South Georgia, during the austral summers of 1990–1991 and 1991–1992. Eight black-browed albatrosses were caught at a nearby colony during the incubation period (the egg being placed under a neighbouring, incubating bird) and were taken to the base. A pulse interval modulated transmitter (Woakes and Butler, 1975) was then implanted into the abdomen under halothane anaesthesia (for the implantation procedure, see Stephenson *et al.* 1986). The initial incision was made in the brood patch and did not necessitate the removal of any feathers. After the implantation, the birds were given an intramuscular injection of long-acting penicillin (LA Terramycin) and returned to the nest. All birds were monitored after the implantation procedure and were deemed to be behaving normally because incubation and foraging shifts were of normal duration. They were recaptured when required for the actual experiments.

Experimental apparatus

A wooden frame was attached to a variable-gradient, variable-speed treadmill (model EG10, Powerjog, Sports Engineering Ltd) onto which a Perspex respirometer could be placed. The respirometer measured 79 cm×64 cm×45 cm and was equipped with two fan units to ensure an even mixing of air. The whole system had an internal volume of 246 l. Brush-style draught excluders ensured a good seal between the frame and the treadmill belt, and rubber seals ensured an air-tight fit between the respirometer and the frame. Air was drawn through the box at approximately 60 l min⁻¹, measured with two variable-area flowmeters (series 1100, KDG Flowmeters) linked in parallel. A subsample of the outlet air flow was passed, *via* a container of drying agent (silica gel), to an infrared carbon dioxide analyser (Servomex 1410) and then to a paramagnetic oxygen analyser (Servomex 570A) *via* a container of soda lime to remove CO₂. A solenoid valve (RS Components Ltd) switched sampling between the inlet (ambient) and outlet air. The humidity and temperature of both the inlet and outlet air flows were continuously monitored (HMP 35A, Vaisala Sensor Systems). The O₂ and CO₂ analysers were calibrated with N₂ and a gas mixture 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 from the O₂ and CO₂ analysers were passed to a purpose-built interface/display unit which amplified the signals so that a 1 % change in gas concentration was equivalent to a 10 V output voltage. The amplified signals were sampled by a laptop computer (Dell LT316), fitted with an A/D converter (PCL-711 PC-MultiLab Card, Advantech Co., Ltd). Temperature and humidity were also recorded *via* the interface unit.

The signal from the implanted transmitter was detected by a Sony 3090 receiver and converted to an electrocardiogram (ECG) by a decoder (Woakes, 1980). The ECG was directed to the interface unit, then to the computer. *f*_H was calculated by counting the number of times the QRS wave of the electrocardiogram passed a threshold voltage. A BASIC program sampled the other inputs every 5 s, derived a mean value for each minute and stored the data for later analysis.

Protocol 1: calibrations

The albatrosses walked on a treadmill at speeds of 0.2–1.1 km h⁻¹ (depending on the maximum sustainable speed that each bird could attain) and at inclines of 0–15 %. The sequence of work loads was randomly assigned. When not walking, the albatrosses always rested on their bellies. They only stood up if disturbed or in order to preen. As the birds could not walk for long durations, they were rested for at least 30 min between the different speed trials. They were walked for up to 25 min at each speed until steady-state conditions had been attained, i.e. the gas concentrations within the respirometer had stabilised. The rate of oxygen uptake, rate of carbon dioxide production (\dot{V}_{CO_2}) and *f*_H were then recorded over the subsequent 5 min of the exercise period. Two calibration runs were carried out whenever possible, the second run coming after the validation experiment (see below).

Heart rate, \dot{V}_{O_2} and \dot{V}_{CO_2} were also measured in four black-browed albatrosses resting on water (water temperature 6.1 ± 0.4 °C). The birds were held in a respirometer placed on a water channel (13 m \times 1.1 m \times 1.1 m). The same measuring system was used as in the calibration procedure.

Protocol 2: validations

After a calibration run had been completed, the birds were allowed to rest for 24 h in an outdoor enclosure. They were then weighed and a blood sample was obtained from the brachial vein to measure background levels of $H_2^{18}O$ and 3H_2O . They were injected intramuscularly with approximately 1.0 ml of $H_2^{18}O$ (50.2 %) and approximately 1.0 ml of 3H_2O (7.4 MBq ml⁻¹) and left for 2 h to allow the isotopes to equilibrate with the body water (Costa and Prince, 1983). The syringes were weighed before and after injection to determine accurately the amounts injected. A second blood sample was obtained and the bird was put into the respirometer. \dot{V}_{O_2} , \dot{V}_{CO_2} and f_H were then continuously monitored for the next 3 days. The birds were walked at submaximal speeds for periods of up to 30 min, after which they were allowed to rest for at least 30 min. The total time spent walking was 3–4 h day⁻¹. All exercise periods were performed during daylight hours; during the hours of darkness, the birds were left undisturbed. After 3 days, the birds were removed from the respirometer and a final blood sample was taken. All blood samples were flame-sealed in micro-haematocrit tubes for later analysis. During the complete experiment, the gas analysers were calibrated at least twice a day. The birds were not fed, nor did they have access to water, during the course of the experiment but, as the mean incubation shift of the black-browed albatross is 11.1 days (Tickell and Pinder, 1975), it was felt that the time spent without access to food and water was not abnormal for this time of year.

Plasma samples were analysed for ^{18}O abundance at SURRC, East Kilbride, Scotland, and Centrum voor Isotopen Onderzoek, Gröningen, Netherlands. Tritium analyses were performed on water distilled from separate plasma samples (Ortiz *et al.* 1978). Approximately 100 μ l of water was distilled from the plasma and accurately weighed in glass scintillation vials. 5 ml of scintillation fluid (Ultima Gold MV, Packard) was added to the vial, which was then placed in a scintillation counter (Beckman LS1701). All samples were analysed in duplicate.

Calculations

Respirometry

The \dot{V}_{O_2} and \dot{V}_{CO_2} were calculated from the measured gas concentrations using the equations of Woakes and Butler (1983) as modified by Culik *et al.* (1990):

$$\dot{V}_{O_2}, \dot{V}_{CO_2} = (C_2 - C_1)V + (C_1 + C_2 - 2C_a) \times \Delta t \times \dot{Q}/2, \quad (1)$$

where C_1 and C_2 , are the gas concentrations (%) at times t_1 and t_2 , respectively, C_a is the ambient gas concentration of O_2 or CO_2 (%), Δt is the time between t_1 and t_2 , V is the volume of the respirometer, \dot{Q} is the rate of air flow through the respirometer, \dot{V}_{O_2} is the rate of O_2 consumption over the period Δt and \dot{V}_{CO_2} is the rate of CO_2 production over the period Δt .

The flow was corrected for RQ using the following equations:

$$\dot{Q} = \dot{V}_{\text{out}}/[1 - (1 - \text{RQ})F_{\text{IO}_2}] \quad (\text{for } \dot{V}_{\text{O}_2}) \quad (2)$$

and

$$\dot{Q} = \dot{V}_{\text{out}}/[1 - (1 - \text{RQ})F_{\text{ICO}_2}] \quad (\text{for } \dot{V}_{\text{CO}_2}), \quad (3)$$

where \dot{V} is the measured air flow out of the respirometer, RQ is the respiratory quotient estimated from the ratio of the O₂ and CO₂ concentrations in the respirometer and F_{IO_2} and F_{ICO_2} are the fractional concentrations of O₂ and CO₂ entering the respirometer. All gas measurements were converted to STPD.

Doubly labelled water technique

The rate of CO₂ production was calculated from the isotopic enrichment of the blood samples using the following equations (equations 4–10).

$$r\text{CO}_2 = \frac{N}{2.08} (k_o - k_h) - 0.015k_h N, \quad (4)$$

equation L+M, from Lifson and McClintock (1966), where $r\text{CO}_2$ is the rate of CO₂ production (mol h⁻¹), N is the total body water (mol), and k_o and k_h are the fractional turnover rates of the oxygen and hydrogen pools, respectively, and are calculated thus:

$$k_{o/h} = [\log_e(I_{o/h} - B_{o/h}) - \log_e(F_{o/h} - B_{o/h})]/t, \quad (5)$$

where I_o and I_h , F_o and F_h , and B_o and B_h are the initial, final and background enrichments of ¹⁸O and ³H, and t is the period between the initial and final samples.

$$r\text{CO}_2 = 0.481(N_o k_o - N_h k_h) - 0.015k_h N_h, \quad (6)$$

equation C3, from Coward *et al.* (1985), where N_o and N_h are the dilution spaces of ¹⁸O and ³H, respectively.

$$r\text{CO}_2 = \frac{N}{2.08} (1.01k_o - 1.04k_h) - 0.0246r_{\text{gf}}, \quad (7)$$

equation S(A6), from Schoeller *et al.* (1986), where $r_{\text{gf}} = 1.05N_o(k_o - k_h)$.

$$r\text{CO}_2 = \frac{N}{2.196} (1.01k_o - 1.053k_h), \quad (8)$$

equation SNG3, from Speakman *et al.* (1993), where $N = [N_o/1.01 + (N_h/1.053)]/2$.

$$r\text{CO}_2 = \frac{N}{2.196} (k_o - 1.0427k_h), \quad (9)$$

equation SNG5, from Speakman *et al.* (1993), where $N = [N_o + (N_h/1.0427)]/2$.

$$r\text{CO}_2 = \frac{N_o}{2.08} (k_o - R_{\text{dilspace}}k_h) - r_{\text{gf}}, \quad (10)$$

equation S4, from Speakman (1993), where $R_{\text{dilspace}} = N_h/N_o$. \dot{V}_{O_2} was estimated from the

rates of CO₂ production using the value of RQ derived from measured gas exchange measurements.

Heart rate

Heart was used to estimate mean \dot{V}_{O_2} for all birds using a number of different methods: (1) using the individual linear regression equations obtained from each bird and applying the relationship to the heart rate data obtained over the 3 day period for that bird; (2) using the mean equation of all the individual linear regression equations and applying the relationship to the 3 day data of each bird; (3) using the mean of the individual regression equations derived from \log_e -transformed f_H and \dot{V}_{O_2} to estimate \dot{V}_{O_2} over the 3 day period for each bird. For all three methods, a mean estimate of \dot{V}_{O_2} from all birds and the percentage deviation of that estimate from the measured \dot{V}_{O_2} was obtained.

Periods of activity and inactivity, which corresponded to day and night, respectively, were extracted from the 3 day trials. The mean estimates of \dot{V}_{O_2} obtained from the f_H data were then compared with the mean measured \dot{V}_{O_2} . In addition, the mean estimates of \dot{V}_{O_2} at different specific activity levels (corresponding to resting and different walking speeds) were compared with the mean measured values.

Statistics

All statistical tests were performed using the statistical package SYSTAT (SYSTAT Inc.). All mean values are presented as \pm S.E.M. The relationship between f_H and \dot{V}_{O_2} during the calibration runs was determined using least-squares regression. As f_H was to be used to estimate \dot{V}_{O_2} , it was plotted on the abscissa. The linear regression equations obtained from each individual were compared using analysis of covariance (ANCOVA) (Zar, 1984). Analysis of variance (ANOVA; Zar, 1984) was used to detect differences between the different methods of determining the energy expenditure of the birds during the validation experiments. Probability levels of $P < 0.05$ were regarded as significant.

Results

Calibrations

The minimum values of f_H and \dot{V}_{O_2} for the albatrosses were measured while they were sitting and resting. The mean value (\pm S.E.M.) for f_H was 110.4 ± 9.9 beats min^{-1} ($N=8$, range 66.4–166.8 beats min^{-1}) and that for \dot{V}_{O_2} was 8.42 ± 0.69 ml $\text{min}^{-1} \text{kg}^{-1}$ (range 6.38–12.22 ml $\text{min}^{-1} \text{kg}^{-1}$; Fig. 1). All birds showed a considerable increase in both f_H and \dot{V}_{O_2} when walking (Fig. 1). The mean maximum f_H of 349.4 ± 17.7 beats min^{-1} (range 246.6–409.1 beats min^{-1}) was 3.1 times the lowest f_H recorded. \dot{V}_{O_2} showed an even more dramatic increase of nearly sevenfold, rising to a mean maximum of 57.39 ± 3.56 ml $\text{min}^{-1} \text{kg}^{-1}$ (range 44.62–73.00 ml $\text{min}^{-1} \text{kg}^{-1}$; Fig. 1).

Although the albatrosses would walk on the treadmill, they could not do so for long periods nor could they walk very fast. The maximum speed attained by any of the birds was 1.1 km h^{-1} , with most only reaching 0.8 km h^{-1} . As a result, the birds had to rest after each period of walking.

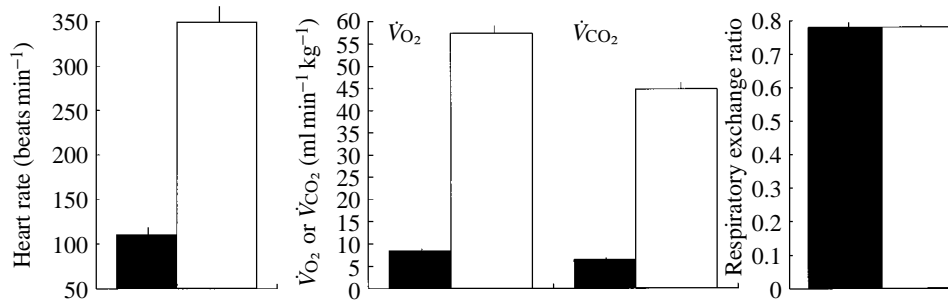


Fig. 1. Mean minimum (resting, filled columns) and maximum (walking, open columns) values of heart rate (f_H , beats min^{-1}), rates of oxygen consumption and carbon dioxide production (\dot{V}_{O_2} , \dot{V}_{CO_2} ; $\text{ml min}^{-1} \text{kg}^{-1}$) and respiratory exchange ratio ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) obtained from eight black-browed albatrosses during the calibration protocol. The vertical lines above each column represent $+1$ S.E.M.

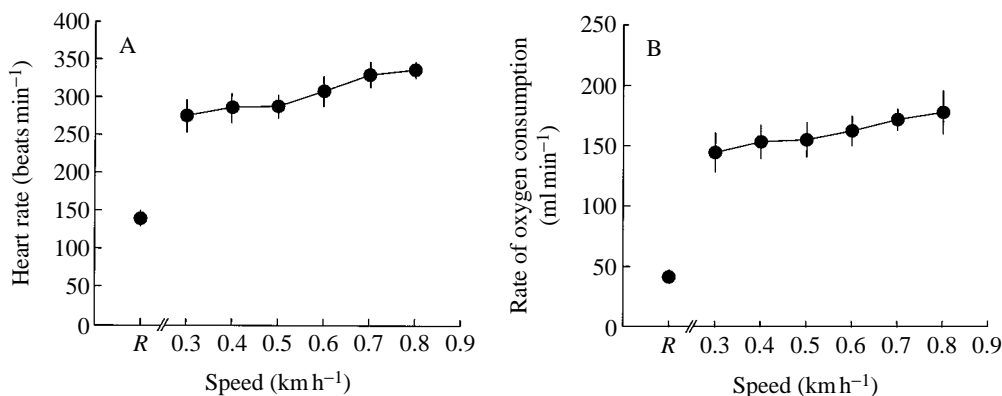


Fig. 2. (A) Mean heart rate (beats min^{-1}) and (B) rate of mean oxygen consumption (ml min^{-1}) of eight black-browed albatrosses walking at different speeds (km h^{-1}) on a treadmill. The vertical lines at each point represent ± 1 S.E.M. *R*, rest.

After an initial large increase between rest and the first walking speed, both f_H and \dot{V}_{O_2} increased linearly with walking speed in all eight albatrosses (Fig. 2). There was no statistical difference between the first and second calibrations for an individual, so the data from the two runs were pooled. The two variables were found to be well correlated, especially when \log_e -transformed, with coefficients of determination ranging between 0.71 and 0.97 (Table 1 and Fig. 3). Both the slopes and the intercepts of the individual linear regression equations were found to be significantly different ($P < 0.001$), whereas only the intercepts of the \log_e -transformed data of the individual birds were significantly different. In this latter situation, it is statistically acceptable to use the slope from the pooled data and the mean intercept to predict \dot{V}_{O_2} from f_H (P. Davies, personal communication). From the pooled data, the equation relating f_H to \dot{V}_{O_2} was therefore:

$$\dot{V}_{O_2} = 0.0157f_H^{1.60}, \quad (11)$$

Table 1. Regression equations of fH (beats min^{-1}) versus \dot{V}_{O_2} (ml min^{-1}) obtained from eight black-browed albatrosses walking on a treadmill

Bird number	N	a	b	r^2_{adj}	P
1	9	-4.050	1.635	0.97	<0.001
2	21	-3.825	1.586	0.84	<0.001
3	30	-4.215	1.583	0.71	<0.001
5	15	-4.263	1.606	0.90	<0.001
6	12	-3.756	1.475	0.89	<0.001
8	23	-5.423	1.820	0.95	<0.001
6-91	16	-3.829	1.603	0.91	<0.001
V1	14	-3.863	1.504	0.84	<0.001
Group	140	-4.153	1.602	0.80	

The form of the equation is $\ln \dot{V}_{O_2} = a + b \log_e fH$.

r^2 values were adjusted (r^2_{adj}) to a level expected when using the derived equation on a new sample from the same population.

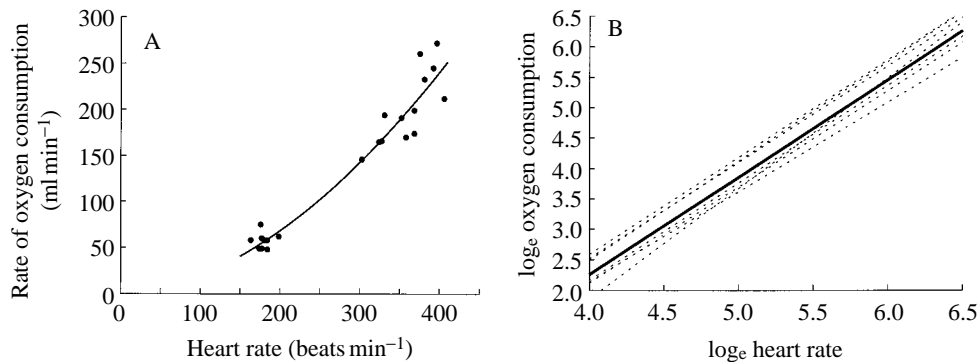


Fig. 3. (A) Oxygen consumption (ml min^{-1}) as a function of heart rate (beats min^{-1}) in a single black-browed albatross while resting and walking on a variable-speed treadmill. (B) Oxygen consumption (ml min^{-1}) as a function of heart rate (beats min^{-1}) in eight black-browed albatrosses. Both variables have been \log_e -transformed. The dashed lines are regression equations obtained from individual birds, and the solid line is the mean regression. The mean regression equation is: $\dot{V}_{O_2} = 0.0157fH^{1.60}$ ($r^2=0.80$, $P<0.001$).

($r^2=0.80$, $P<0.001$), where \dot{V}_{O_2} is in ml min^{-1} and fH in beats min^{-1} (Fig. 3B).

Validations

Doubly labelled water: a comparison between the methods of calculation

Apart from the equation of Lifson and McClintock (1966) (equation 4 above), all the equations gave estimates of the mean oxygen consumption of the albatrosses that were within 4% of those measured by respirometry. Even the equation of Lifson and

Table 2. *Estimates of oxygen consumption from the doubly labelled water technique for six albatrosses using the different equations available*

Bird number	Mass (kg)	N_o (mol)	N_h (mol)	N_h/N_o	$\dot{V}O_2$ (ml min ⁻¹ kg ⁻¹)	Estimated $\dot{V}O_2$ (ml min ⁻¹ kg ⁻¹)					
						L+M	C3	S(A6)	SNG3	SNG5	S4
2	3.62	100.17	113.23	1.13	15.87	19.75	17.98	18.35	18.25	18.25	17.36
3	3.30	83.74	85.10	1.02	14.41	11.33	11.17	10.54	10.46	10.46	10.43
5	2.98	83.00	93.31	1.12	11.90	16.26	14.63	15.11	15.01	15.01	14.25
6	3.17	79.34	88.44	1.12	15.00	21.09	18.44	19.61	19.41	19.40	18.31
8	3.82	102.87	104.56	1.02	18.98	15.91	15.60	14.81	14.64	14.64	14.45
6-91	2.98	89.24	92.95	1.04	16.80	15.01	14.46	13.96	13.86	13.85	13.65
Mean	3.31	89.73	96.26	1.08	15.49	16.55	15.38	15.40	15.27	15.26	14.74
S.E.M.	0.14	3.96	4.33	0.02	0.97	1.43	1.08	1.32	1.31	1.31	1.15
Algebraic mean Δ						8.9	0.8	1.3	0.5	0.5	-3.2
Absolute mean Δ						25.0	18.9	23.2	23.0	23.0	20.2

ANOVA

Source	SS	d.f.	MS	F	P
Method	10.815	5	2.163	0.222	0.950
Error	291.633	30	9.721		

N_o is the oxygen dilution space; N_h is the hydrogen dilution space.

Δ is the difference between the estimated and the measured oxygen consumption as a percentage.

L+M, equation from Lifson and McClintock (1966); C3, equation from Coward *et al.* (1985); S(A6), equation from Schoeller *et al.* (1986); SNG3 and SNG5 from Speakman *et al.* (1993) and S4 from Speakman (1993). rCO_2 was converted to $\dot{V}O_2$ using the measured respiratory exchange ratios; rCO_2 is the rate of CO_2 production.

In the ANOVA, method refers to the equation used to estimate oxygen consumption.

SS, sum of squares; d.f., degrees of freedom; MS, mean squares.

McClintock (1966) was within 10%. If, however, we look at the range of errors involved, it can be seen that these can be quite substantial [-21.4% to +40.6% in the case of the equation of Lifson and McClintock (1966)]. It is, therefore, more appropriate to look at the absolute errors involved, i.e. to ignore the sign of the error (Table 2). From this analysis, it can be seen that the mean deviation is much greater than that shown by the mean error alone. This phenomenon is illustrated by the equation proposed by Coward *et al.* (1985) which, although giving the best mean estimate with a mean error of only 0.8%, has a mean deviation of 18.9%. The equation used to calculate energy expenditure was therefore not a significant factor (Table 2).

Heart rate as a predictor of oxygen consumption

The estimates of $\dot{V}O_2$ from fH were not significantly different from those measured by respirometry (t -test; $t=-0.781$, $P=0.466$) (Table 3). The mean algebraic difference was -2.8%, but this again hides the individual errors involved, which ranged from -17.0% to +12.1% (mean absolute error 8.9%). There was no significant difference between the

Table 3. Mean rate of oxygen consumption (\dot{V}_{O_2}), rate of carbon dioxide production (\dot{V}_{CO_2}) and heart rate (f_H) obtained from six black-browed albatrosses over 3 days and the estimates of the rate of oxygen consumption derived from the heart rate data using equation 11

Bird number	Mass	\dot{V}_{O_2} (ml min ⁻¹ kg ⁻¹)	\dot{V}_{CO_2} (ml min ⁻¹ kg ⁻¹)	f_H (beats min ⁻¹)	$\dot{V}_{O_{2est}}$ (ml min ⁻¹ kg ⁻¹)	Δ
2	3.62	15.87	10.38	149.1	13.17	-17.0
3	3.30	14.41	8.88	159.9	16.16	12.1
5	2.98	11.90	8.32	128.6	12.62	6.1
6	3.17	15.00	7.77	148.6	14.96	-0.3
8	3.82	18.98	13.48	175.2	16.15	-14.9
6-91	2.98	16.80	13.37	151.1	16.34	-2.7
Mean	3.31	15.49	10.37	152.1	14.90	-2.8
S.E.M.	0.14	0.97	1.03	6.2	0.67	4.7
Absolute Δ						8.9

Paired *t*-test between the estimated and the measured oxygen consumptions: $t=-0.789$, $P=0.466$.

Δ is the difference between the estimated and the measured rates of oxygen consumption expressed as a percentage.

estimates of energy expenditure over the 3 day period obtained by respirometry, DLW and f_H (ANOVA; $P=0.94$).

If f_H is to be used as a predictor of aerobic metabolic rate in animals in the field, then it has to be able to predict the energy costs of different activities and over different time periods. To test this, f_H and \dot{V}_{O_2} were monitored at different activity levels (walking speed) during the validation procedure. Values were averaged over the last 5 min at any given speed, when steady-state conditions had been attained. The equation describing the relationship between the two variables from the validation experiments (Fig. 4) is:

$$\dot{V}_{O_2} = 0.0158f_H^{1.60}, \quad (12)$$

($r^2=0.85$, $P<0.001$), which is not significantly different from the line obtained from the calibration data (equation 11). The estimates of \dot{V}_{O_2} from f_H using equation 11 were plotted against those measured directly. Fig. 5 shows all the values obtained from six birds, while Fig. 6 shows the mean value for these birds at each particular speed, e.g. at rest, 0.2 km h⁻¹ etc. The linear regression between the estimated \dot{V}_{O_2} and the measured \dot{V}_{O_2} is not significantly different from the line of equality. However, it should be noted that resting heart rates tended to produce an overestimate of \dot{V}_{O_2} (Figs 5 and 6).

To test whether f_H could be used to predict \dot{V}_{O_2} over different periods that encompassed both different levels of exercise and periods of transition from one level of activity to another, the f_H and \dot{V}_{O_2} data during the 3 day validation period were divided into periods of inactivity and activity. Inactive periods were during the night when the birds were not disturbed and were not made to walk, whereas activity periods were those periods during the day while the birds were walked. Activity periods generally lasted for 8–12 h each day and included the quiescent periods between each exercise session. There were 3–4 active periods and three inactive periods per bird. The results are summarised in Table 4. The

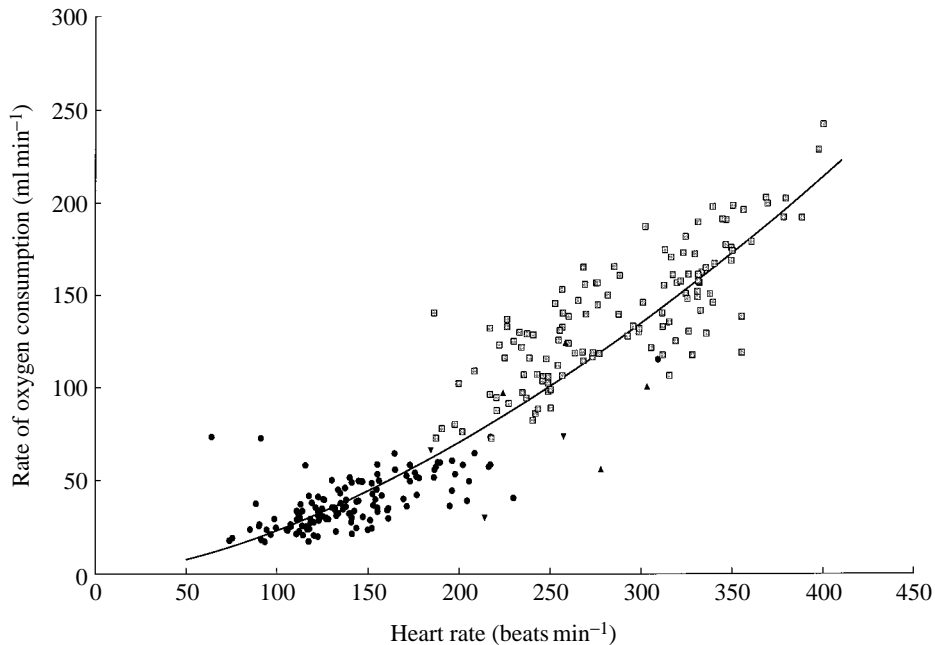


Fig. 4. Heart rate (f_H , beats min^{-1}) and rate of oxygen consumption (\dot{V}_{O_2} , ml min^{-1}) of six black-browed albatrosses whilst resting (filled circles) and walking (filled squares) on a treadmill. Data were obtained during the 3 day validation period. Each point represents the mean value taken over a 5 min period when steady-state conditions had been attained. The regression equation shown is: $\dot{V}_{O_2} = 0.0158f_H^{1.60}$ ($r^2=0.85$, $P<0.001$). Also represented are the f_H and \dot{V}_{O_2} measurements of four albatrosses while sitting on the treadmill (\blacktriangledown) and whilst resting on water (\blacktriangle) (see also Fig. 7).

mean estimate of \dot{V}_{O_2} from f_H over the inactive periods was $11.90 \pm 1.09 \text{ ml min}^{-1} \text{ kg}^{-1}$ and over the active periods it was $16.29 \pm 0.92 \text{ ml min}^{-1} \text{ kg}^{-1}$, underestimates of 1.7% and 5.9% respectively. Mean deviation of errors over the same periods were 11.7% and 10.9%.

Resting on water caused both f_H and \dot{V}_{O_2} to increase (Fig. 7); f_H rose, on average, by 36% and \dot{V}_{O_2} nearly doubled. In two of the birds, the estimates of \dot{V}_{O_2} from f_H were on the line of equality, but in the two other birds \dot{V}_{O_2} was overestimated, both while the birds were in the air (sitting on the treadmill) and whilst they floated on the water (Fig. 7). From Fig. 4 it can be seen that these measured values of f_H and \dot{V}_{O_2} lie within the range of resting or active values obtained from the treadmill during the validation experiment.

Discussion

Calibrations

Once the birds had started walking, f_H and \dot{V}_{O_2} increased linearly with work load, as they do in other birds, e.g. barnacle geese (Nolet *et al.* 1992) and maribou stork (Bamford and Maloiy, 1980). Although the two variables showed a high mean linear correlation, a better

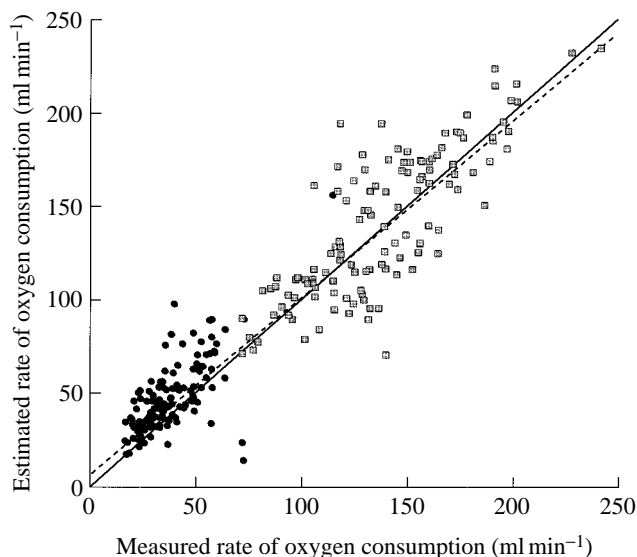


Fig. 5. Oxygen consumption (ml min^{-1}) estimated from heart rate as a function of measured rate of oxygen consumption (ml min^{-1}) at different exercise levels (walking speed) in six black-browed albatross. Oxygen consumption was estimated from heart rate using the mean regression equation in Fig. 3 for eight birds. Data were obtained during the 3 day validation period and are pooled from all six birds. Each point is the mean value obtained over a 5 min period when steady-state conditions had been attained at that exercise level. Resting measurements are represented by a filled circle, active ones by a filled square. The solid line is the line of equality and the dashed line is the regression equation between the two variables described by the equation: estimated $\dot{V}_{\text{O}_2} = 9.56 + 0.94(\text{measured } \dot{V}_{\text{O}_2})$ ($r^2=0.88$, $P<0.001$).

fit was found when the data were \log_e -transformed (Table 1). The logarithmic equation is superior because it better describes the f_H/\dot{V}_{O_2} relationship at low levels of energy expenditure, where relatively large changes in heart rate do not necessarily indicate any major change in \dot{V}_{O_2} (Blix *et al.* 1974; cf. appropriate oxygen pulse technique used in Nolet *et al.* 1992). Walking the birds on the treadmill immediately caused a large increase in both f_H and \dot{V}_{O_2} . It was not possible, therefore, to measure the intermediate values of the variables for an individual bird. However, if the data from several birds are pooled, then the variation between individuals gives a much wider spread of data points. These data (see Fig. 4) show that a curvilinear relationship exists between the two variables and that it is not significantly different from that obtained from the calibrations.

Unlike the situation in other studies (Flynn and Gessaman, 1979; Pauls, 1980), there was no difference in the relationship between f_H and \dot{V}_{O_2} between the two calibration runs performed on each bird. These were performed 4–5 days apart, suggesting that, in the black-browed albatross, the relationship between the variables is relatively constant within an individual, at least over the short period of a week. It is not known, however, whether this relationship will change over the breeding season.

An interesting observation was that leg exercise in the albatross caused a substantial increase in f_H and \dot{V}_{O_2} (Fig. 2). The only times that the birds were seen to stand when not

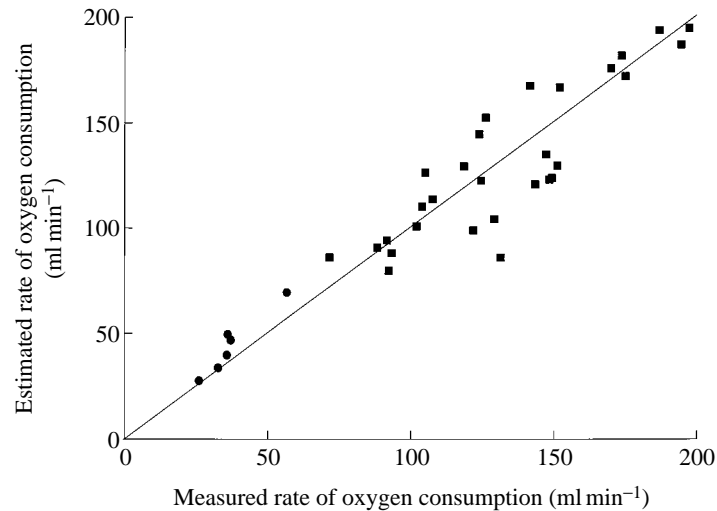


Fig. 6. Mean oxygen consumption (ml min^{-1}) estimated from heart rate as a function of mean measured rate of oxygen consumption (ml min^{-1}) at different exercise levels (walking speed) in six black-browed albatrosses. Data were obtained during the 3 day validation period and each point is the mean value for the bird at each walking speed that it attained. Resting measurements are represented by a filled circle, active ones by a filled square. The solid line is the line of equality. The equation describing the relationship between the two variables is: $\dot{V}_{\text{O}_2} = 9.63 + 0.92(\text{measured } \dot{V}_{\text{O}_2})$ ($r^2 = 0.88$, $P < 0.001$).

walking was when they were disturbed or preening. So, in going from resting to walking, the birds must first stand up and it is this action that is almost certainly the cause of the initial increase in f_{H} and \dot{V}_{O_2} . This capacity for increasing \dot{V}_{O_2} through leg exercise is surprising because the birds appear to spend very little time walking during the breeding season (R. M. Bevan, personal observation). The capacity is, therefore, more likely to be related to their feeding behaviour: the birds have been observed participating in 'feeding frenzies' (P. Prince, personal communication). In these, the birds will swim vigorously towards krill swarms that have come to the sea surface and, when feeding, they may also perform foot-propelled dives, an energetically costly behaviour (Woakes and Butler, 1983). Indeed, preliminary observations indicate that the leg muscles of the black-browed albatross form 13% of its body mass, whereas only 8% of body mass is flight muscles (pectoralis and supracoracoideus) (R. M. Bevan and P. J. Butler, unpublished data). This is low compared with other birds, where the flight muscles constitute, on average, 17% of the body mass (Greenwalt, 1962).

Validation study

There are now many equations that can be used to calculate the energy expenditure of an animal from the doubly labelled water technique, from the simplest one-compartment model, which assumes that the oxygen and hydrogen dilution spaces are equal (Lifson and McClintock, 1966), to the more complex two-compartment models, which assume that the oxygen and hydrogen dilution spaces are different (Coward *et al.* 1985; Schoeller *et al.* 1986; Speakman *et al.* 1993; Speakman, 1993). However, the present study found

Table 4. Comparison between measured oxygen consumption ($\dot{V}O_2$) and that estimated from heart rate ($\dot{V}O_{2est}$) using equation 11 over periods of activity and inactivity in six black-browed albatrosses (see text for further details)

	Bird number	f_H (beats min^{-1})	$\dot{V}O_2$	$\dot{V}O_{2est}$	Δ
Inactive					
	2	109.9	9.20	8.47	-8.0
	3	156.2	13.23	16.02	21.1
	5	108.0	9.33	9.66	3.5
	6	133.2	16.41	12.61	-23.2
	8	129.4	12.32	12.97	5.3
	6-91	141.3	12.86	11.66	-9.4
	Mean	129.7	12.23	11.90	-1.7
	S.E.M.	7.5	1.10	1.09	
	Absolute error				11.7
Active					
	2	152.0	18.15	13.81	-23.9
	3	165.4	15.43	17.44	13.0
	5	129.3	13.33	13.10	-1.7
	6	162.2	17.03	17.28	1.5
	8	161.3	18.52	18.6	0.4
	6-91	183.8	23.29	17.55	-24.6
	Mean	159.0	17.62	16.29	-5.9
	S.E.M.	7.3	1.38	0.92	
	Absolute error				10.9

Periods of activity included walking trials and the quiescent periods between walking trials; periods of inactivity were when the birds were undisturbed.

no significant differences between the estimates of energy expenditure derived from different equations (cf. Speakman and Racey, 1988). This was due to the large individual errors. Thus, although the mean estimate is accurate when using the two-compartment models (Table 2), the mean deviation of the errors showed substantial variation between the individual estimates and the measured values. This is a phenomenon that is often seen with the DLW technique (see Speakman and Racey, 1988).

Using f_H to predict $\dot{V}O_2$ showed the same phenomenon. Even though the mean estimate was accurate to -2.8% (Table 3), the range of individual errors was quite large (-17.0% to +12.1%). This was also observed by Bevan *et al.* (1992) and Nolet *et al.* (1992) and again demonstrates that, although f_H can be used to determine the energetics of the albatrosses, it cannot be used to determine accurately the energy costs of an individual; it can only be applied to a group of animals (Bevan *et al.* 1992; Nolet *et al.* 1992). This was borne out by the data obtained from the birds resting on water, where the small number of animals used probably contributed to the mean values of $\dot{V}O_2$ in air and on water being overestimated by 44% and 19% respectively. There was no significant difference between the estimates of energy expenditure over the 3 day period obtained by

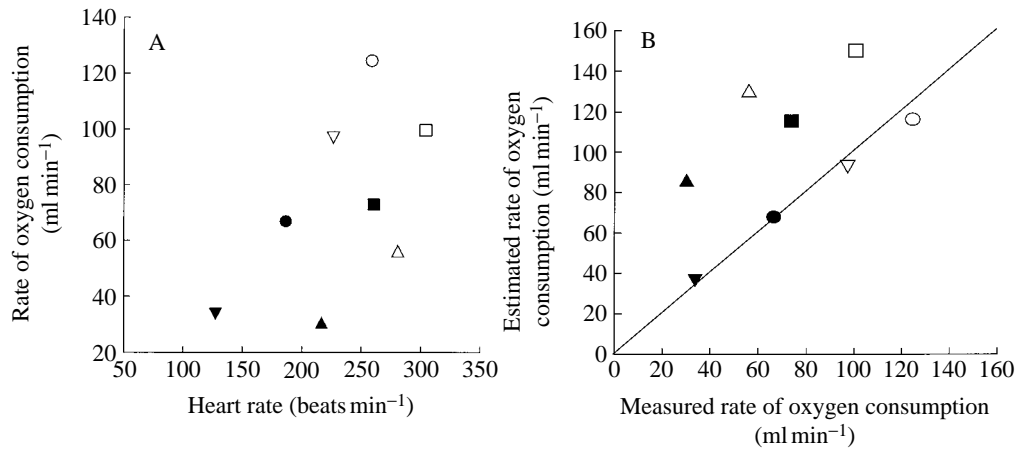


Fig. 7. (A) Heart rates (beats min^{-1}) and rates of oxygen consumption (ml min^{-1}) of four black-browed albatrosses (represented by the square, the circle, the triangle and the inverted triangle) while resting in air (filled symbols) and while resting on water (open symbols). (B) Rate of oxygen consumption (ml min^{-1}) estimated from heart rate as a function of measured oxygen consumption (ml min^{-1}) in four black-browed albatrosses (see A for details of symbols). The solid line is the line of equality.

respirometry, DLW and f_H , showing that, in the black-browed albatross, f_H is at least as good as the DLW technique for estimating energy consumption.

The real benefit of using f_H to estimate energy expenditure in these birds is that it can be used to determine the energy costs of specific activities and over different time periods (Figs 5, 6; Table 4). There was very close agreement between the estimates of \dot{V}_{O_2} derived from the f_H data and those measured directly when the data were analysed in terms of levels of activity (walking speed) over the 3 day period (Figs 5, 6). However, the resting values were nearly all overestimated (Fig. 5), although mean values for the individual birds were only slightly overestimated (Fig. 6). This error again highlights the need to use mean data from a group of animals when applying this technique. By applying the technique in this way to heart rates obtained from free-ranging birds, the energetics of particular behaviours, e.g. gliding, incubating or feeding, can be estimated.

95 % confidence limits can also be added to the estimates using the equation:

$$(S\hat{Y}_i)_m = \sqrt{S_{X,Y}^2 \left[\frac{1}{m} + \frac{1}{n} + \frac{(X_i - \bar{X})^2}{\sum x^2} \right]} \quad (13)$$

Zar (1984), where $(S\hat{Y}_i)_m$ is the standard error of the predicted value of Y_i estimated from m values of X_i , $S_{X,Y}$ is the standard error of the estimate, n is the number of data points, \bar{X} is mean heart rate, $\sum x^2$ is the sum of squares (all obtained from the original regression analysis), X_i is the mean heart rate from which oxygen consumption is to be estimated and m is the number of heart rate measurements that X_i is derived from. The 95 % confidence limits for the estimated oxygen consumption, \hat{Y} , equals $\pm(t_{0.05(2n)})(S\hat{Y}_i)_m$. It should be noted that, for a large number of samples, the term $1/m$ can be ignored, and that the smallest confidence intervals will occur when $X_i = \bar{X}$. For the regression equation 11, the

95% confidence intervals of the mean, assuming that $1/m$ is negligible, are $198.54 + 11.48 \text{ ml min}^{-1}$ and $198.54 - 10.81 \text{ ml min}^{-1}$.

Nolet *et al.* (1992) concluded that, in using f_H to predict daily energy expenditure, its application has to be restricted to 'the range of exercise levels in which f_H has been calibrated against \dot{V}_{O_2} '. The authors point out that the derived equation would not be applicable to flapping flight and that the general applicability of the equation would also depend on how long the bird spends in flapping flight (Nolet *et al.* 1992). In the present study, the range of f_H used in the calibrations covered the entire range of f_H found in free-ranging albatrosses, including flight, (R. M. Bevan, P. J. Butler, A. J. Woakes and P. A. Prince, in preparation). Consequently, the equations will be applicable for estimating energy expenditure of birds in the field, although this makes the assumption that the flight muscles have the same f_H/\dot{V}_{O_2} relationship as the leg muscles. The probable reason for the heart rates of black-browed albatrosses not attaining the same level as those found during flapping flight in other birds, e.g. the barnacle goose (Butler and Woakes, 1980), is that gliding (the main method of locomotion in the albatrosses) is, energetically, relatively inexpensive (Baudinette and Schmidt-Nielsen, 1974; Costa and Prince, 1983). Hence, cardiac output, and therefore f_H , should not need to be as high during gliding as during flapping flight. This is further borne out by the relatively small flight muscles of the black-browed albatross (R. M. Bevan and P. J. Butler, unpublished data).

In conclusion, the present study has demonstrated that the use of f_H in predicting the mean metabolic rate of black-browed albatrosses is as accurate as any of the available techniques for determining the energy expenditure in free-ranging birds. f_H , though, has a unique advantage in that it enables a much more detailed analysis of the energetics of the birds when performing specific behaviours. There is also the potential, when using this method, for monitoring the energetics of the black-browed albatross over much longer periods, e.g. the entire breeding season or even the whole year.

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