

How accurately can we estimate energetic costs in a marine top predator, the king penguin?

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Abstract

King penguins (*Aptenodytes patagonicus*) are one of the greatest consumers of marine resources. However, while their influence on the marine ecosystem is likely to be significant, only an accurate knowledge of their energy demands will indicate their true food requirements. Energy consumption has been estimated for many marine species using the heart rate–rate of oxygen consumption ($f_H - \dot{V}_{O_2}$) technique, and the technique has been applied successfully to answer eco-physiological questions. However, previous studies on the energetics of king penguins, based on developing or applying this technique, have raised a number of issues about the degree of validity of the technique for this species. These include the predictive validity of the present $f_H - \dot{V}_{O_2}$ equations across different seasons and individuals and during different modes of locomotion. In many cases, these issues also apply to other species for which the $f_H - \dot{V}_{O_2}$ technique has been applied. In the present study, the accuracy of three prediction equations for king penguins was investigated based on validity studies and on estimates of \dot{V}_{O_2} from published, field f_H data. The major conclusions from the present study are: (1) in contrast to that for walking, the $f_H - \dot{V}_{O_2}$ relationship for swimming king penguins is not affected by body mass; (2) prediction equation (1), $\log(\dot{V}_{O_2}) = -0.279 + 1.24\log(f_H) + 0.0237t - 0.0157\log(f_H)t$, derived in a previous study, is the most suitable equation presently available for estimating \dot{V}_{O_2} in king penguins for all locomotory and nutritional states. A number of possible problems associated with producing an $f_H - \dot{V}_{O_2}$ relationship are discussed in the present study. Finally, a statistical method to include easy-to-measure morphometric characteristics, which may improve the accuracy of $f_H - \dot{V}_{O_2}$ prediction equations, is explained.

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Introduction

Penguins constitute up to 90% of the avian biomass in the Southern Oceans (Woehler, 1995) and consume key species in the trophic chains of these waters. Thus, in

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order to use them as monitors of change in prey availability (Le Maho et al., 1993; Ropert-Coudert et al., 2001), it is important to be able to make effective inferences about their food requirements (Croxall et al., 1999). One way to assess food requirements is to accurately measure metabolic costs. A number of studies have successfully used the heart rate–rate of oxygen consumption ($f_H - \dot{V}_{O_2}$) technique to obtain energetic data on free-ranging animals. The method is based on the relationship between \dot{V}_{O_2} and f_H as formulated in the Fick equation for convection of oxygen in the cardiovascular system (Dejours, 1981). For several species of seabird, estimates of \dot{V}_{O_2} from f_H , averaged across several individuals, are reported to be within a few per cent of the mean measured \dot{V}_{O_2} for those same individuals (Butler, 2004; and see Froget et al., 2001 for a review). For example, Fahlman et al. (2004) reported the mean percentage error of the estimate of \dot{V}_{O_2} for king penguins (*Aptenodytes patagonicus*) walking on a treadmill to be 3%.

However, while the validation experiments conducted by Fahlman et al. (2004) are typical for such calibration studies using the $f_H - \dot{V}_{O_2}$ technique, the methodology employed for these validations is not ideal. In such studies, validation experiments are often performed on the same birds used to derive the prediction equations, or at least on birds from the same field season. Given that a large degree of variation in behaviour and physiology is often recorded both between individuals (e.g. Fahlman et al., 2005) and between study seasons in the field (e.g. Charrassin et al., 1999), it seems important to validate prediction equations with a large number of individuals taken from different years and independent of the individuals used in the calibration experiments.

King penguins are estimated to be the third highest consumer by mass of all the penguin species (Brooke, 2003), thus they are a particularly important species to understand in terms of their energy requirements. Although Fahlman et al. (2004) obtained prediction equations for king penguins walking, king penguins forage at sea and spend the majority of their time away from land (Descamps et al., 2002). The $f_H - \dot{V}_{O_2}$ relationship for walking and swimming is similar in both gentoo and macaroni penguins (Bevan et al., 1995; Green et al., 2005), however, it has been shown to be different in certain other species (e.g. eider ducks; Hawkins et al., 2000), and between walking and flying (Ward et al., 2002). Furthermore, in humans, the $f_H - \dot{V}_{O_2}$ relationship is known to be moderated by the proportion of active muscle mass (Vokac et al., 1975), whether the activity is continuous or intermittent and by physical fitness (Henderson et al., 1927). In king penguins, the pectoralis and supracoracoideus muscles, which are used to move the flippers through the water during swimming, are 3.6 times larger than the leg muscles at the onset of the moult fast (Cherel et al.,

1993, 1994). Thus, it should not be assumed that the $f_H - \dot{V}_{O_2}$ relationship remains the same during different locomotor activities in king penguins given their differences from other penguin species in terms of phylogeny, body mass (M_b), fasting and diving behaviour.

Froget et al. (2001) and Fahlman et al. (2004) found that the $f_H - \dot{V}_{O_2}$ relationship in walking king penguins changes with duration of fasting, where over a 28 d period, M_b can decrease by up to 30% (Fahlman et al., 2004). The pectoral muscles are only 2.5 times the size of the leg muscles by the end of the fast (Cherel et al., 1994) and thus it may be the case that the $f_H - \dot{V}_{O_2}$ relationship during swimming changes with nutritional state as well. Potentially, the situation is complicated further because as king penguins forage at sea, their physiological state changes from fasted to fed once they start to feed. However, the rate of this reversal is not known, which may produce considerable errors in estimating \dot{V}_{O_2} from f_H during the initial portion of the foraging trip (Fahlman et al., 2004). Thus, there are a number of factors that may well make the present prediction equations for king penguins, derived from walking data (Fahlman et al., 2004), inaccurate when applied to f_H data recorded at sea.

It is clear that in order to be sure of obtaining a valid estimation of \dot{V}_{O_2} from f_H in king penguins during swimming as well as while walking, certain aspects of the relationship need to be studied further. The need to use single or multiple equations to predict \dot{V}_{O_2} from f_H during different types of locomotion and nutritional state must be investigated. Also, the accuracies of the prediction equations for estimating broad-scale \dot{V}_{O_2} needs to be assessed robustly, both across different groups of birds and seasons. These are therefore the objectives of the present study. Further studies are also proposed that could serve to provide data to refine the accuracy of the prediction equations presently available for king penguins and, indeed, for other species.

Materials and methods

Ethical approval for all procedures was granted by the ethics committee of the French Polar Research Institute (IPEV) and of the Ministère de l'Environnement. The requirements of the United Kingdom (Scientific Procedures) Act 1986 were also followed. Our procedures also conformed to the Code of Ethics of Animal Experimentation in the Antarctic.

Animals

The experiments were carried out on Possession Island (Crozet Archipelago 46° 25'S, 51° 45'E) over the austral summers of 2003–2004 and 2004–2005.

A total of 16 courting male king penguins was used for the calibration/validation experiments. The experimental protocols in terms of the timing and frequency of treadmill and water channel experiments differed between the two seasons as explained below. All birds were caught on the beach, near the breeding site. They were caught in November or December, at the beginning of courtship when mate choice for that season had not yet been made. The birds were caught in the afternoon and immediately weighed. A picric acid mark was painted on the chest of each animal for identification purposes. They were then placed in open top pens (3 × 3 m). During the periods that the birds were captured, they were kept in these pens (other than when they were in respirometry experiments) and were not provided with any food i.e. they were left to fast.

Experimental timetable

Mean mass at capture was 14.2 ± 0.1 kg (mean \pm 1 SE). One to ten days (5.1 ± 0.8 d) after capture, each bird underwent surgery for implantation of a heart rate data logger (see Froget et al., 2004; Fahlman et al., 2005). Each bird was then allowed to rest for around 2 weeks in the pen without human intervention except for when it was weighed, every other day. Each bird was then released in the vicinity of where it had been caught. Mean mass at this time was 10.2 ± 0.1 kg. All birds from both seasons were subsequently recaptured between 6 and 25 d later (14.1 ± 1.6 d), in the same area of the colony, after they had returned from a foraging trip. The birds had gained, on average, a mass of 3.2 ± 0.2 kg (range 1.9–4.96 kg) and were at a mean mass of 13.4 ± 0.2 kg. They were caught on average 12.7 ± 3.0 h (range 0.5–42.0 h) after their last dive during daylight hours to more than 50 m (ascertained from the data loggers after retrieval), which was assumed to be their last feeding dive (Pütz et al., 1998). At this point, each bird commenced a series of walking and swimming experiments spread out over several weeks. The series of respirometry experiments differed for the birds in the two seasons and a summary is shown in Table 1, which includes M_b during each set of experiments. While treadmill experiments were undertaken in both seasons, water channel experiments were only undertaken in the 2003–2004 season.

Firstly, and within the 2 d after recapture, each bird from both seasons undertook a treadmill experiment and the 2003–2004 birds also undertook a water channel experiment. Since the birds had recently fed and thus had been fasting for only a short period of time, their condition during these experiments was termed ‘fed’. Subsequently, the birds were placed back in the pen and fasted. The 2003–2004 season birds then underwent another treadmill and water channel experiment, on

average 7.1 ± 0.4 d (range 6–9 d) after their last feeding dives (termed the ‘short fasted’ condition), and then a final treadmill and water channel experiment, on average 13.3 ± 1.3 d (range 9–19 d) after their last feeding dive (termed ‘long fasted’). The 2004–2005 season birds underwent a treadmill experiment on average 18.3 ± 1.3 d (range 12–22 d) after their last feeding dives (also termed ‘long fasted’). For each season, this completed the experimental protocol. For all birds, the second surgery to remove the logger was undertaken before each bird had fasted to within 10% of its critical M_b (Gauthier-Clerc et al., 2001). The birds were then maintained in the pen for several days while being monitored daily and were then released close to where they had been first caught.

Treadmill experiments

Treadmill experiments were conducted as previously detailed in Fahlman et al. (2004). Air temperature ranged between 12 and 21 °C, which is similar to that for the treadmill experiments of Fahlman et al. (2004), and is within the thermal neutral zone for king penguins (Froget et al., 2002). In brief, each bird was placed in the respirometer chamber (80 × 46 × 86 cm high) and allowed to rest for at least 1 h. The final 5 min of consistent readings during this rest period was considered to be the resting \dot{V}_{O_2} in air. Next, each bird was made to walk at five different speeds (0.3, 0.7, 1.0, 1.5 and 1.8 km h^{-1}) interspersed by a resting periods. The sequence of walking speeds was assigned at random for each bird. The bird walked at each speed until steady values of \dot{V}_{O_2} and \dot{V}_{CO_2} were obtained for at least 3 min, which was considered to indicate that both the respirometry system had reached equilibrium and that the bird was in a steady state. Stable values were observed after 12–17 min of walking, while the time required for the system to reach a 95% fractional transformation to a new steady state was around 9.5 min.

Water channel experiments

Experiments were conducted in a static water channel (30.0 × 1.4 × 1.2 m deep), as described by Fahlman et al. (2005). Water temperature ranged between 7.5 and 9.5 °C across experiments and seasons. In brief, at each end of the water channel, a respirometer chamber in the form of an acrylic box was submerged approximately 5 cm into the water through an opening in the wooden cover. The air space of each respirometer chamber measured approximately 89 × 39 × 16 cm and the time required for a 95% fractional transformation to a new steady state was around 3.5 min, irrespective of the chamber. Each chamber had two internal fans attached to the top to ensure rapid mixing of the internal gases.

Table 1. Experiments undertaken with king penguins during the 2003–2004 and 2004–2005 seasons, and the body masses and duration of fasting of the penguins at the times of the experiments

Condition and season	Body mass during experiment (kg)	Change in body mass at recapture ^a (kg)	Number of days fasted	<i>N</i> ^b
Fed (12.7 ± 3.0 h)				
Season 2003–2004				
Treadmill	13.63 ± 0.35	0.40 ± 0.10	1.33 ± 0.17	9
Water channel	13.39 ± 0.38	0.52 ± 0.10	1.11 ± 0.20	9
Season 2004–2005				
Treadmill	13.06 ± 0.28	0.49 ± 0.06	1.57 ± 0.20	7
Short fasted (7.1 ± 0.4 d)				
Season 2003–2004				
Treadmill	12.24 ± 0.39	1.94 ± 0.2	7.14 ± 0.40	7
Water channel	12.24 ± 0.39	1.94 ± 0.2	7.14 ± 0.40	7
Long fasted				
Season 2003–2004 (13.3 ± 1.3 d)				
Treadmill	11.25 ± 5.63	2.88 ± 0.47	15.25 ± 1.44	4
Water channel	11.20 ± 0.13	3.07 ± 0.61	13.30 ± 1.32	5
Season 2004–2005 (18.3 ± 1.3 d)				
Treadmill	10.61 ± 0.13	2.95 ± 0.15	18.14 ± 1.32	7

Values in brackets refer to the mean time \pm SE since the last foraging dive at sea.

^aRefers to the time that the birds were re-caught having been released and undertaken a foraging trip at sea.

^bOn occasions, a bird that underwent experiments in a particular condition was not included in the subsequent analyses (Table 2).

The experiment began by placing the bird in one of the openings to the water channel and then fitting the respirometer over the top (with the other respirometer already in place at the other end of the water channel). Data collection started approximately 1 min after the animal had been placed in the respirometer chamber until the end of the experiment. Throughout the experiment, the animal was allowed to behave freely and experiments continued for between 2 and 12 h. Generally, birds spent at least some of the time swimming along the water channel from one respirometer box to the other and the rest of the time present in one of the boxes. Swimming and resting behaviours typically lasted many minutes at a time once the birds were used to the water channel. Observations were made continuously without intervention except for times when the respirometer chambers were covered to tempt an active bird to rest.

Respirometry

\dot{V}_{O_2} and \dot{V}_{CO_2} for the treadmill and water channel experiments were measured by a common respirometry system, which could be switched to sample gas from either the respirometry chamber in air or the two respirometry chambers on water. The exact system used is detailed in Fahlman et al. (2005). In brief, \dot{V}_{O_2} and \dot{V}_{CO_2} were

calculated using standard equations (Depocas and Hart, 1957; Withers, 1977) as described in Froget et al. (2001). All flows were corrected to standard temperature (273 K) and pressure (101.3 kPa) dry. The measurement error of the respirometry system was within $\pm 3\%$, determined by dilution tests (Fedak et al., 1981).

Data assessment and statistical analysis

Treadmill experiments conducted in the present study were used to validate treadmill prediction equations derived in Fahlman et al. (2004). Water channel experiments were used to derive a new prediction equation based on swimming data.

All values are reported as means \pm 1SEM except for estimates of \dot{V}_{O_2} (\dot{V}_{O_2} est), which are reported with standard errors of the estimates (\pm 1SEE). Analysis of the residuals calculated from validations of the prediction equations (i.e. \dot{V}_{O_2} measured – \dot{V}_{O_2} estimated for each individual) was used to test the assumptions of normality and equal variance (Cottingham et al., 2005; Neter et al., 1996) by checking that they were not correlated with f_H . This was done by visual inspection of correlations (Townend, 2002).

For the water channel experiments, \dot{V}_{O_2} and \dot{V}_{CO_2} were averaged over 5 min periods for the whole experimental period. Data were selected to give a wide

range of f_H values for use in constructing relationships between f_H and \dot{V}_{O_2} (Green et al., 2005). Values of f_H and \dot{V}_{O_2} were coupled having accounted for the lag time and time constants of the system. Because the water channel experiments varied in duration, they also varied in the number of data points. Furthermore, the number of data points for the water channel experiments also differed from the number of data points for the treadmill experiments ($n = 6$, therefore six \dot{V}_{O_2} bins; one for each treadmill speed and one for rest). Thus, the range of \dot{V}_{O_2} values over a single water channel experiment was divided into six bins of equal width. Data for the water channel experiments were binned by \dot{V}_{O_2} rather than f_H . This was because values of very high f_H were often associated with lower values of \dot{V}_{O_2} , which often occurred towards the start of a water channel experiment and were thus likely to be indications of stress causing an uncoupling of the f_H – \dot{V}_{O_2} relationship. Because the calibrations are primarily devised for estimating \dot{V}_{O_2} over a broad time scale, which is likely to incorporate a wide range of f_H and hence \dot{V}_{O_2} values, the f_H and \dot{V}_{O_2} data in each \dot{V}_{O_2} bin were averaged and these averages were used to create regression equations using the number of samples in each bin in a weighted regression analysis (Neter et al., 1996). This avoided pseudo replication and made it possible to compare regression equations without sampling bias due to differences in the length of water channel experiments or spreads of f_H data during those experiments.

Multivariate mixed model ANOVA was used to determine the effect of t (the number of days fasted) and M_b on the relationship between \dot{V}_{O_2} and f_H in the water channel, using \dot{V}_{O_2} as the dependent variable, and f_H , t and M_b as independent fixed covariates. Backward and forward stepwise regression techniques were used to search for the best parameters as previously detailed by Fahlman et al. (2004), to derive a prediction equation for these data. Further, an r^2 value for this equation was calculated using an analysis of variance general linear model, for which the parameter estimates were not significantly different between the multivariate mixed model ANOVA and the general linear model.

There are three prediction equations assessed in the present study; two derived in Fahlman et al. (2004; Eqs. (1) and (2b), hereafter termed (1) and (2)) relating to treadmill data and the equation derived from the water channel experiments in the present study (termed Eq. (3)). Eqs. (1) and (2) are reported here for convenience:

$$\log(\dot{V}_{O_2}) = -0.279 + 1.24\log(f_H) + 0.0237t - 0.0157\log(f_H)t, \quad r^2 = 0.81, \quad (1)$$

$$\log(\dot{V}_{O_2}) = -11.1 + 11.3\log(f_H) - 2.33\log(f_H)^2, \quad r^2 = 0.91. \quad (2)$$

Prediction equation (1) includes the term t and is derived from treadmill data. Thus, Eq. (1) is designed primarily to estimate \dot{V}_{O_2} in king penguins walking. Prediction equation (2) does not include t and is designed specifically to estimate \dot{V}_{O_2} in birds that are in a fed state while walking. However, Eq. (2) may also be valid for use with swimming birds that have recently fed. Eq. (1) can also be used to predict \dot{V}_{O_2} in king penguins in a fed state, by setting the value of t to 0. Prediction equation (3) is derived from water channel data and is thus for estimating \dot{V}_{O_2} during swimming.

To assess the accuracy of the three prediction equations, validation tests are available for f_H and \dot{V}_{O_2} of king penguins on the treadmill during two seasons and in the water channel during one season. ANCOVA analysis is commonly employed in the analysis of f_H – \dot{V}_{O_2} relationships to test for differences between the regression lines derived from each set of experiments. However, in the present study, a goodness-of-fit analysis of the difference between the equations in terms of the validation data was undertaken, which has two general advantages over the ANCOVA approach. Firstly, goodness-of-fit analysis circumvents the issue of violating assumptions of ANCOVA when comparing regression models which, for example, do not contain the same number of parameters and do not follow the assumption of equal variance and independence of each data point. Secondly, goodness of fit allows a direct assessment of how the predictions of different equations vary, both between equations and seasons. Where ranges of f_H and/or \dot{V}_{O_2} are similar between groups, the ANCOVA approach may nevertheless indicate a difference between regression slopes due to different elevations or gradients despite large regions where the two equations are not significantly different (quantified by the Johnson–Neyman technique; White, 2003).

To assess the practical implications of using different prediction equations for determining broad-scale energetics in the field, f_H data are available for birds in the field during two seasons combined (Froget et al., 2004). Furthermore, all these data are distinct from the group (and the season) of birds used to derive prediction equations 1 and 2 in Fahlman et al. (2004). Thus, in the present study, assessment of the most suitable equations for use in future studies concerned with estimating \dot{V}_{O_2} from f_H data in king penguins is based upon (1) comparisons of errors of estimated \dot{V}_{O_2} associated with each prediction equation for each experimental condition of the validation tests; (2) comparisons of estimated \dot{V}_{O_2} derived from each appropriate equation using a range of f_H values obtained in the field (reported in Froget et al., 2004) to test if these \dot{V}_{O_2} estimates significantly differ between equations.

Statistical analyses were performed using NCSS 2000 (NCSS statistical software, Kaysville, UT, USA), Minitab (Version 13.32, Minitab Inc., State College,

PA, USA) and JMP (Version 5.1.2, SAS Institute Inc., USA). Acceptance of significance was set at $P < 0.05$.

Results

Derivation of an $f_H - \dot{V}_{O_2}$ prediction equation for swimming

The mean water temperature during the water channel experiments was $8.7 \pm 0.6^\circ\text{C}$ ($7.5\text{--}9.5^\circ\text{C}$). There was no significant difference in mean temperature between experiments ($P > 0.05$, paired t test). A univariate parameter search was used to decide whether M_b or t would be important covariates alongside f_H for a prediction equation derived from the water channel data. In contrast to the similar findings for the treadmill data of both Fahlman et al. (2004) and the present study, it was found that neither M_b nor t significantly affected the relationship in the water channel. This is exemplified in Fig. 1, which shows similar regression lines for fed and long-fasted birds in the water channel in contrast to a shallow regression in long-fasted compared to fed birds on the treadmill during the experiments of the present study.

The resulting model was of the following form:

$$\dot{V}_{O_2} = -81.03 + 2.938f_H - 0.005f_H^2, \quad r^2 = 0.46. \quad (3)$$

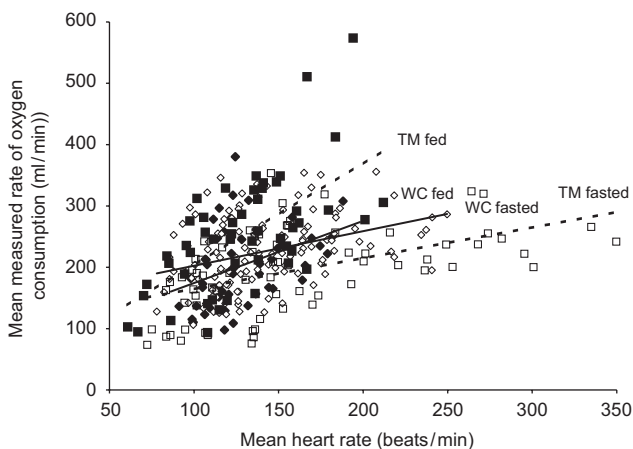


Fig. 1. Linear regression relationships of mean measured rate of oxygen consumption as a function of mean heart rate (f_H) in up to nine king penguins in the fed (12.7 ± 3.0 h since last feeding dive) and long-fasted (13.3 ± 1.3 d after last feeding dive) experimental conditions during the field season 2003–2004. These experimental conditions comprised both treadmill and water channel experiments. The treadmill experiments are represented by squares and dashed regression lines, while the water channel experiments are represented by diamonds and solid regression lines. Filled symbols denote the fed condition, while open symbols denote the fasted condition. The relationships are plotted over the range of mean f_H measured during the experiment for each individual.

Comparing the three prediction equations

The regression lines of \dot{V}_{O_2} against f_H representing each of the three prediction equations are shown in Fig. 2. To test the accuracy of Eqs. (1) and (2), values of \dot{V}_{O_2} est were calculated from the f_H data for both the treadmill and water channel experiments, using both equations, and these were compared to the measured values of \dot{V}_{O_2} . It was not possible to investigate Eq. (3) in this fashion because suitable validation data (i.e. water channel data) were only available for one season and these were from the same birds used to derive the equation. For each individual bird in each condition and season, the relative difference between the observed and estimated values of \dot{V}_{O_2} derived from Eqs. (1) and (2), i.e. the per cent errors of the residuals ($[(\text{observed} - \text{predicted}) / \text{observed}] \times 100$), were calculated for each of the six \dot{V}_{O_2} bins. From these values, the mean per cent error of the residuals was calculated for each bird. The mean algebraic error and mean absolute error for each condition and season were then calculated from the mean per cent error of the residuals. Details of the errors associated with each condition, for Eqs. (1) and (2), are given in Table 2.

The range of mean per cent error of the residuals indicates the range of error for individual birds associated with a particular equation. The mean absolute error indicates the mean absolute error in an estimate of \dot{V}_{O_2} from f_H across a number of birds. The mean algebraic error indicates the algebraic error in an estimate of \dot{V}_{O_2} from the mean f_H from a number of birds and so is particularly important when considering the application of a prediction equation for estimating field energetics. As such, the algebraic error is the value most often considered when assessing the accuracy of a prediction equation. It is important to be aware that all of these error values are affected by the number of birds used in the validation study (see also Green et al., 2001). The pertinent trends in mean algebraic error in Table 2 suggest that Eq. (1) is as accurate as Eq. (2) for estimating \dot{V}_{O_2} for fed birds walking, Eq. (1) is more accurate than Eq. (2) for fasted birds walking, Eq. (1) with $t = 0$ is more accurate than with $t =$ number of days fasted for birds swimming and Eq. (1) is equally accurate for predicting \dot{V}_{O_2} in walking and swimming birds.

For the values of \dot{V}_{O_2} est derived in the present study from the field f_H data reported in Froget et al. (2004) and the values of the lowest f_H reported at sea in Halsey et al. (unpubl. data), in each case using each of the three prediction equations (Table 3), SEEs were computed using the procedures described in Green et al. (2001) and Froget et al. (2004). To compare values of \dot{V}_{O_2} est derived from the different equations, a form of the proximate normal test for comparing the differences between two estimates was used, which employs the SEEs and is described in Green et al. (2001). Given that

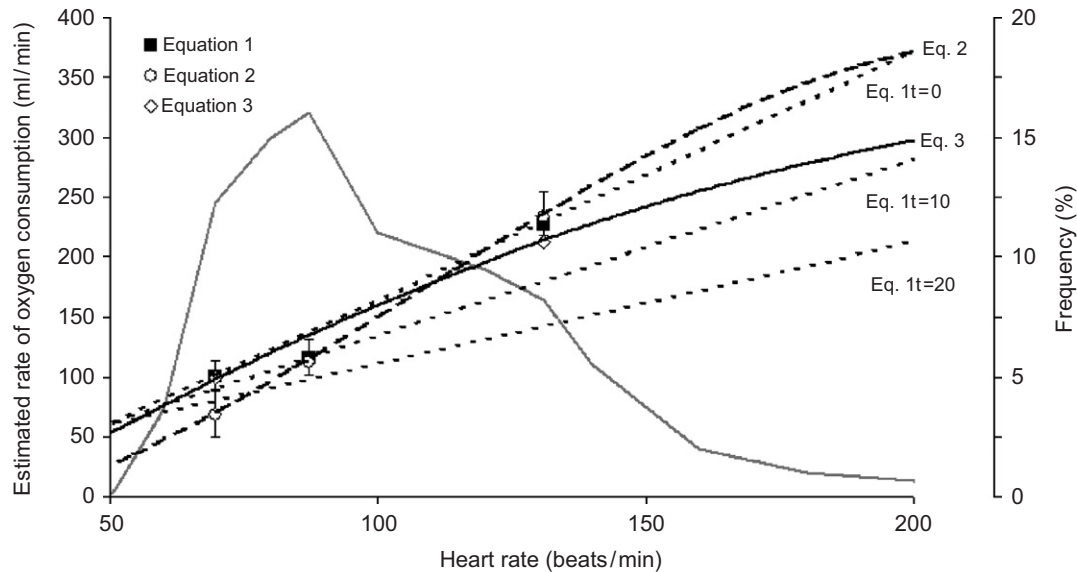


Fig. 2. Estimates of rate of oxygen consumption (\dot{V}_{O_2} est) at different heart rates (f_H) derived from different prediction equations for king penguins (see text for details). The black solid line represents Eq. (3), while the black dashed line represents Eq. (2). Each of the three black stippled lines represents Eq. (1) with different values for t , the number of days fasted: $t = 0$, upper line; $t = 10$, middle line; $t = 20$, lower line. Data points represent values of \dot{V}_{O_2} est \pm SEEs for field values of f_H reported in Froget et al. (2004) and in Halsey et al. (unpubl. data); 69.6, 87.2 and 130.8 beats/min in ascending order, see Table 3. Filled squares represent \dot{V}_{O_2} est calculated from Eq. (1), open circles represent Eq. (2) and open diamonds represent Eq. (3). Sometimes the data points and/or error bars overlap. The grey line represents an approximate frequency distribution of f_H for breeding king penguins in the field during the austral summer, at sea and on shore combined, based on Fig. 3 in Froget et al. (2004) and assuming that approximately equal time is spent at sea and on shore.

the $f_H - \dot{V}_{O_2}$ technique is robust only when averaged across a number of animals, statistical techniques that include consideration of values of measurements for individual animals are not valid for comparing values of \dot{V}_{O_2} est derived from f_H . Instead, it is preferable to employ a proximate normal test that considers only the values of \dot{V}_{O_2} est and the SEEs associated with them and, as such, these tests are used in the present study.

Furthermore, since Eqs. (1) and (2) incorporate log transformed data while Eq. (3) does not, when comparing these three equations for the at sea data, \dot{V}_{O_2} est and the associated SEEs had to be converted from logged values to real values for the first two equations. This produces asymmetrical SEEs and thus in each case the larger SEE for the converted equation was used when it was compared to Eq. (3). According to the proximate normal tests applied in the present study, values of \dot{V}_{O_2} est derived with different equations from the onshore (Eqs. (1) and (2)) and at sea (Eqs. (1), (2) and (3)) f_H data (Froget et al., 2004) were not significantly different (all Z values < 1.96). The value of \dot{V}_{O_2} est derived with Eq. (2) from f_H during periods of particularly low f_H during rest at sea (Halsey et al., unpubl. data) was significantly different from the equivalent value of \dot{V}_{O_2} est derived from Eq. (1) ($P < 0.05$). There were no differences in the estimates derived from Eqs. (1) and (3).

Discussion

Assessing validation tests

In the present study, for the treadmill experiments, different birds from those used to derive the prediction equations were used to validate those equations. Unlike the majority of previous calibration based studies (see Froget et al., 2001 for a review), the methodology employed in the present study represents a true validation test of the prediction equation being assessed. Furthermore, given that each group of birds was tested during different field campaigns, the validation tests can be used to assess the accuracy, and hence the robustness, of the prediction equations to estimate \dot{V}_{O_2} in different years.

While certain studies that have compared calibrations for exercise in a number of bird species in different years have found no difference in the $f_H - \dot{V}_{O_2}$ relationship (barnacle geese, Nolet et al., 1992; Ward et al., 2002; tufted ducks, Woakes and Butler, 1983; Bevan et al., 1992; macaroni penguins, Green et al., 2005), nevertheless it is reasonable to suppose that at least some species of bird can vary behaviourally and/or physiologically in different years. Thus it may be the case that prediction equations derived from one field season will sometimes be more or less accurate at estimating \dot{V}_{O_2} from f_H data from another season.

Table 2. Validation tests of prediction Eqs. (1) and (2) from Fahlman et al. (2004) in the fasted conditions using king penguins from both the 2003–2004 and 2004–2005 seasons

Condition and season	Prediction equation	Mean algebraic error (%)	Mean absolute error (%)	Range of errors for individuals (%)	<i>N</i>
Fed					
Season 2003–2004					
Treadmill	Eq. (1) ^a (1–2)	10.0	20.8	–33.6 to 35.9	9
Treadmill	Eq. (2) ^b	6.6	23.4	–42.8 to 47.2	9
Water channel	Eq. (1) ^a	–11.7	24.0	–44.2 to 27.5	9
Water channel	Eq. (1) ^{a,c}	–14.3	24.6	–47.5 to 10.9	9
Water channel	Eq. (2) ^b	–18.5	29.6	–59.7 to 22.8	9
Season 2004–2005					
Treadmill	Eq. (1) ^a (1–2)	–2.0	19.7	–46.3 to 39.9	7
Treadmill	Eq. (2) ^b	–4.3	20.8	–53.4 to 38.2	7
Short fasted					
Season 2003–2004					
Treadmill	Eq. (1) ^a (12–20)	13.8	16.5	–9.5 to 27.4	7
Treadmill	Eq. (2) ^b	–27.6	27.6	–48.0 to –12.6	7
Water channel	Eq. (1) ^a (6–9)	13.9	25.2	–21.6 to 51.7	7
Water channel	Eq. (1) ^{a,c}	–11.0	28.6	–51.0 to 37.8	7
Water channel	Eq. (2) ^b	–15.7	33.9	–57.5 to 44.1	7
Long fasted					
Season 2003–2004					
Treadmill	Eq. (1) ^a (12–19)	12.6	12.6	1.3 to 28.8	4
Treadmill	Eq. (2) ^b	–21.1	27.9	–67.2 to 13.4	4
Water channel	Eq. (1) ^a	23.0	23.0	5.5 to 45.7	5
Water channel	Eq. (1) ^{a,c} (11–14)	–4.0	24.8	–31.9 to 35.4	5
Water channel	Eq. (2) ^b	–5.0	29.9	–39.1 to 44.8	5
Season 2004–2005					
Treadmill	Eq. (1) ^a (12–20)	21.3	31.3	–30.1 to 56.1	6
Treadmill	Eq. (2) ^b	30.2	46.1	–96.1 to 76.6	6

Values in brackets represent ranges of the number of days fasted, *t*, which may be included in Eq. (1).

^aThe equation termed Eq. (1) in Fahlman et al. (2004).

^bThe equation termed Eq. (2b) in Fahlman et al. (2004).

^cThe term *t* within Eq. (1) is set to 0.

To highlight these validation issues, a direct comparison can be made between the validation errors for the treadmill data in the present study and those reported in Fahlman et al. (2004), in both cases using Eq. (1). In the

aforementioned study, the mean algebraic error for Eq. (1) was 3.0%. However, the same birds used in the calibration experiments to derive the prediction equations were also used to validate the equations. In the

Table 3. Estimates of $\dot{V}_{O_2} \pm \text{SEE}$ from f_H data on individual birds onshore and at sea provided in Froget et al. (2004) and Halsey et al. (2007, unpubl. data)

Activity	f_H (beats/min)	Prediction equation	\dot{V}_{O_2} est (ml O_2 /min)
Ashore	87.2	Eq. (1)	116.5 ^a (+12.1, –11.0)
		Eq. (2)	113.2 (+10.6, –9.7)
At sea	130.8	Eq. (1)	222.0 ± (+19.4, –17.8)
		Eq. (2)	239.3 (+15.4, –14.4)
		Eq. (3)	212.3 ± 20.8
During 5 min period of lowest heart rate while resting at sea	69.6	Eq. (1)	100.2 (+8.9, –8.5) ^b
		Eq. (2)	64.7 (+13.4, –11.1)
		Eq. (3)	99.2 ± 19.5

^aAssuming a mean fasting state while on shore of 9 d, estimated from details provided in Froget et al. (2004).

^bSignificant difference between Eqs. (1) and (2), $P < 0.05$.

present study, for the fed, short-fasted and long-fasted studies, using Eq. (1) (and setting either $t = 0$ or $t =$ number of days fasted as appropriate), the mean of the mean algebraic error across conditions and seasons was 11.4% (Table 2). Green et al. (2005) also used birds from different years to calibrate and validate prediction equations, for macaroni penguins, and tended to calculate slightly higher algebraic errors than those obtained from previous studies, despite having a large number of subject birds.

Estimating \dot{V}_{O_2} on land

For the two fasted conditions, the algebraic errors calculated in the present study suggest that Eq. (1) is a better predictor of \dot{V}_{O_2} of king penguins walking than is Eq. (2). This confirms that it is important to consider t when estimating \dot{V}_{O_2} while walking. For the fed condition, Eq. (1) was as accurate as Eq. (2) over the two seasons, despite the particularly high r^2 value associated with Eq. (2), indicating that there is no need for a specialist equation to predict \dot{V}_{O_2} est in fed king penguins walking. Finally, when using Eqs. (1) and (2) to derive \dot{V}_{O_2} est from values of f_H on shore reported in Froget et al. (2004), the estimates are not statistically different (Table 3; for Eq. (1), t was set to 9 d, the mean fasting state for these birds while on shore, estimated from details provided in Froget et al. (2004)). This suggests that the use of one equation over the other for predicting broad-scale mean \dot{V}_{O_2} est from f_H will have a negligible effect on the results. Thus these validation studies, across several nutritional conditions and using different birds from those used to derive the equations, coupled with comparisons of \dot{V}_{O_2} est for field f_H data, suggest that presently the most accurate and simple way to derive \dot{V}_{O_2} est during a period on shore is to use Eq. (1) for all nutritional states.

Estimating \dot{V}_{O_2} at sea

Not only is it important to establish whether a separate equation is required to predict \dot{V}_{O_2} during swimming, it is also necessary to ascertain whether the f_H – \dot{V}_{O_2} relationship changes at sea at some point after the birds have started feeding. If so, this might require a prediction equation with additional terms or multiple equations. However, Eq. (3), derived from the water channel data, does not include the term t , suggesting that, in contrast to the relationship derived from the treadmill data, the f_H – \dot{V}_{O_2} relationship in king penguins on water is not affected by their nutritional state (Fig. 1). While the water channel is the best available rather than the ideal method for assessing at sea energetics, nevertheless the lack of requirement for a term to describe the nutritional state of king penguins

swimming in the water channel is a particularly important finding. This is because it suggests that there is not a need to ascertain at what point king penguins start to feed at sea, how long after this point the f_H – \dot{V}_{O_2} relationship begins to change and how quickly it changes.

As already discussed, it was not possible to test whether a separate equation is required for walking and swimming energetics through validation tests in the water channel for each equation. However, for the present study, predicting \dot{V}_{O_2} est in the water channel from Eqs. (1) and (2) represents a true validation test for these two equations and thus allows investigation of the effectiveness of these equations for predicting \dot{V}_{O_2} est from f_H data during swimming. Eqs. (1) and (2) were applied to the f_H data in each water channel condition, and in the case of Eq. (1), t was set both to 0 and to the number of days fasted. In the fed condition, Eq. (1), with $t = 0$ and $t =$ the number of days fasted, produced similar values of \dot{V}_{O_2} est and both were more accurate than Eq. (2). In the two fasted conditions, \dot{V}_{O_2} was more accurately estimated when using Eq. (1) with $t = 0$ than with $t =$ number of days fasted or than when using Eq. (2). This again supports the finding that nutritional state is not important for estimating \dot{V}_{O_2} in king penguins swimming.

Importantly, across the three conditions, Eq. (1) was as accurate at estimating \dot{V}_{O_2} in the water channel (with $t = 0$) as it was at estimating \dot{V}_{O_2} on the treadmill (with $t =$ the number of days fasted). This indicates that the f_H – \dot{V}_{O_2} relationship is similar between fed king penguins while walking and king penguins in any nutritional state while swimming. Fig. 3 provides an

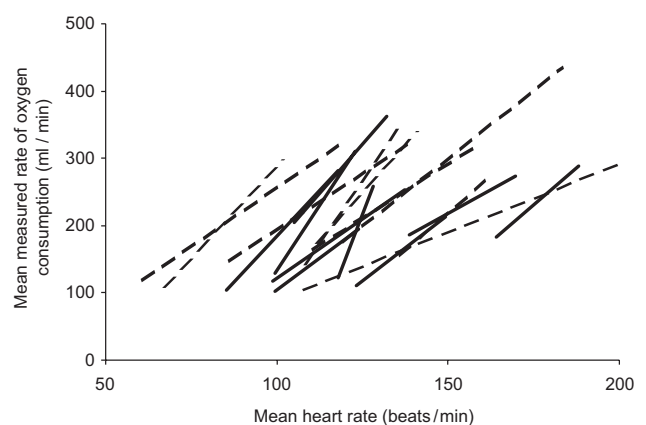


Fig. 3. Linear regression relationships of mean measured rate of oxygen consumption (\dot{V}_{O_2} est) as a function of mean heart rate (f_H) in nine king penguins in the fed condition, walking on a treadmill (dashed lines) and swimming in a water channel (solid lines). Experiments were undertaken on these birds during the 2003–2004 season. The relationships are plotted over the range of mean f_H measured during the experiment for each individual.

example of this similarity in the $f_H - \dot{V}_{O_2}$ relationship on the treadmill and in the water channel. This similarity is in agreement with the findings of Bevan et al. (1995) and Green et al. (2005) who found that there was no difference in the $f_H - \dot{V}_{O_2}$ relationship in gentoo and macaroni penguins, respectively, walking and swimming.

A comparison can be made between Eqs. (1) and (2) and also (3), in terms of \dot{V}_{O_2} est derived for field f_H data at sea reported in Froget et al. (2004). For Eq. (1), t is set at 0 since it is assumed that for the majority of the time at sea, king penguins are in a fed nutritional state. The estimates of \dot{V}_{O_2} were not significantly different between the three equations (Table 3). This suggests that, at least in terms of the practical implications of estimating \dot{V}_{O_2} from f_H data recorded across broad temporal scales in the field, equations derived from treadmill data and water channel data are similar, at least once nutritional state is accounted for. While Froget et al. (2004) used Eq. (2) for estimating \dot{V}_{O_2} est at sea, the application of Eq. (1) instead would not affect any of the conclusions in their study.

Therefore, the conclusion of the various validation tests using both the treadmill and the water channel, along with comparisons of \dot{V}_{O_2} est from field f_H data, is that Eq. (1) from Fahlman et al. (2004) is the most suitable equation available for estimating \dot{V}_{O_2} from f_H in king penguins, both walking and swimming. However, while the estimates of \dot{V}_{O_2} based on the onshore and at sea data of Froget et al. (2004) suggest that there is little practical difference between using the different equations, Fig. 2 indicates that this may not be the case at some point beyond the modal values of f_H reported for king penguins in the field. It can be seen in Fig. 2 that around the mean overall f_H values for onshore and at sea reported in Froget et al. (2004); 87.2 and 130.8 beats/min, respectively, and accounting for t with regards to Eq. (1), there is only a small difference between the estimates of \dot{V}_{O_2} for each relevant equation. However, at more extreme f_H values, differences between the equations become more apparent. Though extreme values occur relatively infrequently and short periods of very high f_H in the field may indicate an uncoupling of the $f_H - \dot{V}_{O_2}$ relationship, for instance due to diving or stress (Blix et al., 1974; Bevan and Butler, 1992), particularly low values can, for example, be recorded during certain periods of rest at sea (Halsey et al., unpubl. data).

Thus, when working with f_H data averaged across relatively short periods of time and when those data represent energetic extremes, the choice of equation makes a more substantial difference to the values of estimated \dot{V}_{O_2} . For instance, \dot{V}_{O_2} estimated by Eq. (2) for periods of particularly low f_H during rest at sea based on the f_H data recorded by Halsey et al. (unpubl. data: 69.6 ± 6.8 beats/min) is significantly lower than

\dot{V}_{O_2} estimated both by Eqs. (3) and (1) (Fig. 2). Furthermore, such f_H values, though rarely exhibited at sea, are more common on land (refer to Fig. 3 of Froget et al., 2004), with mean f_H less than 70 beats/min approximately 20% of the time. Therefore, it is important to consider the limits to temporal resolution when predicting \dot{V}_{O_2} from f_H and to employ the most suitable prediction equation when estimating energetic costs from field f_H data.

Refinement of Eq. (1)

Consideration of the algebraic errors of Eq. (1) in Table 2 shows that although the predictive error is often less than 10%, on occasions it can be higher than 20%. While these values are often higher than those reported by other $f_H - \dot{V}_{O_2}$ studies on seabirds, the individual errors are still lower than those obtained for diving species using the doubly labelled water method (see Froget et al., 2001 for a review). Indeed, at present, the $f_H - \dot{V}_{O_2}$ technique is perhaps the only method for obtaining multiple, valid estimates of energy expenditure for the duration of an experiment. Furthermore, for the reasons discussed above, the error values of the present prediction equations are more representative of the errors likely to be obtained by future king penguin studies employing Eq. (1) to estimate \dot{V}_{O_2} from recorded f_H data.

The potential may exist to reduce the errors in estimates of \dot{V}_{O_2} from f_H in king penguins by developing prediction equations to include further relevant independent variables over and above f_H and t . The inclusion of morphometric measures, specifically measures of the length of the bill, flipper and foot, have been shown to enhance the accuracy of predictions of \dot{V}_{O_2} from f_H during periods of rest in king penguins (Fahlman et al., 2006). Such metrics are relatively easy to obtain, and, unlike for example mass, do not change in adult birds over time. It is this lack of variation, however, that makes the inclusion of morphometric measures into prediction equations problematic, thus at least in part explaining their absence from such equations. The difficulty arises because if a general linear model is run on a data set where each bird is included multiple times, for example with different masses, the model is unable to calculate the variation in \dot{V}_{O_2} associated with morphometric measures since the morphometric measures themselves do not vary at the level of each individual bird. In this situation, the model will run if individual bird is not included as a random factor in the model, thus assuming that each pairing of \dot{V}_{O_2} and f_H is for a different bird. Yet this violates the assumption of the analysis that each data point is independent, thus increasing the chances of a type I error.

However, we suggest an alternative method that would enable inclusion of morphometric measures as well as M_b while allowing recognition of the fact that repeated measures of individual birds are present in the data. It also enables the increase in the variation in \dot{V}_{O_2} accounted for by including morphometric measures to be calculated. By firstly running a general linear model without the inclusion of morphometric measures but with the inclusion of the individual bird as a random factor, the analysis output will provide an individual estimate for each bird, which represents the difference between each individual bird and the mean of all individuals. These estimates can then be regressed against the morphometric measures to test for a relationship, i.e. to test whether morphometric aspects of the birds can explain some of their individual variation. As the sum of the individual estimates is zero, the relationship between morphometrics and individual difference from the mean (e.g. (i) individual difference = $d \times$ morphometrics + e) can be added to, for example, a predictive equation based on f_H and t (e.g. (ii) $\dot{V}_{O_2} = af_H + bt + c$) to refine the estimate of \dot{V}_{O_2} . Thus, in this example (iii) $\dot{V}_{O_2} = [af_H + bt + c] + [d \text{ morphometrics} + e]$. The coefficients of determination for Eqs. (ii) and (iii) can be calculated by regressing measured \dot{V}_{O_2} against the relevant model prediction of \dot{V}_{O_2} .

The use of morphometrics in such prediction equations is always worthy of investigation since they can provide considerable information about the structural size of the bird and, together with M_b , give an indication of nutritional state, both of which are important influences on \dot{V}_{O_2} as shown by Fahlman et al. (2006). Irrespective of whether or not the inclusion of morphometrics would refine current prediction equations to estimate \dot{V}_{O_2} from f_H in king penguins, the present analyses indicate that Eq. (1) is a valid, and the most suitable, equation presently available for estimating \dot{V}_{O_2} during all periods in king penguins in the field.

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