The heart rate/oxygen consumption relationship during cold exposure of the king penguin: a comparison with that during exercise

G. Froget[1,2], Y. Handrich[2], Y. Le Maho[2], J.-L. Rouanet[3], A. J. Woakes[1] and P. J. Butler[1,*]

[1]*School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*, [2]*Centre d'Ecologie et Physiologie Energétiques, CNRS, 23 rue Becquerel, 67087 Strasbourg Cedex 02, France* and [3]*Laboratoire de Thermorégulation et Energétique de l'Exercice, CNRS, Faculté de Médecine Lyon-Nord, 69373 Lyon Cedex 08, France*

*Author for correspondence (e-mail: p.j.butler@bham.ac.uk)

Summary

This study investigated whether exposure to low ambient temperature could be used as an alternative to exercise for calibrating heart rate (f_H) against rate of oxygen consumption ($\dot{V}_{O_2}$) for subsequent use of f_H to estimate $\dot{V}_{O_2}$ in free-ranging animals. Using the relationship between the oxygen pulse (OP, the amount of oxygen used per heart beat) and an index of body condition (or nutritional index, NI), a relationship between f_H and $\dot{V}_{O_2}$ was established for resting king penguins exposed to a variety of environmental temperatures. Although there was a small but significant increase in the OP above and below the lower critical temperature ($-4.9\,^{\circ}\text{C}$), there was no difference in the relationship obtained between the OP and body condition (NI) obtained above or below the lower critical temperature. These results were then compared with those obtained in a previous study in which the relationship between f_H and $\dot{V}_{O_2}$ had been established for king penguins during steady-state exercise. The relationship between OP and NI in the present study was not significantly different from the relationship between resting OP and NI in the previous study. However, the relationship was different from that between active OP and NI. We conclude that, at least for king penguins, although thermoregulation does not affect the relationship between resting OP and NI, temperature cannot be used as an alternative to exercise for calibrating f_H against $\dot{V}_{O_2}$ for subsequent use of f_H to estimate $\dot{V}_{O_2}$ in free-ranging animals.

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

Introduction

Heart rate (f_H), doubly labelled water and time/energy budgets are the three most commonly used measures for estimating the rate of oxygen consumption ($\dot{V}_{O_2}$) and, hence, field metabolic rate in free-ranging animals. The f_H method is based on the Fick equation (equation 1) and, if cardiac stroke volume (V_S) and the rate of tissue oxygen extraction ($Ca_{O_2}-\bar{C}v_{O_2}$) remain constant or vary systematically, there is a linear relationship between f_H and $\dot{V}_{O_2}$ (Owen, 1969; Butler, 1993):

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

where $\dot{V}_{O_2}$ is the rate of oxygen consumption, f_H is heart rate, V_S is cardiac stroke volume, Ca_{O_2} is the oxygen content of arterial blood and $\bar{C}v_{O_2}$ is the oxygen content of mixed venous blood. $V_S(Ca_{O_2}-\bar{C}v_{O_2})$ is also referred to as the oxygen pulse (OP) and is expressed in $\text{ml O}_2\,\text{heart beat}^{-1}$.

An increasing number of studies have investigated the relationship between f_H and $\dot{V}_{O_2}$ (Bevan et al., 1994, 1995; Nolet et al., 1992; Boyd et al., 1995; Butler et al., 1995; Hawkins et al., 2000; Froget et al., 2001; Green et al., 2001).

In most of these studies, exercise (running or swimming) was used to increase both metabolic rate and f_H. However, several factors have been found to influence the relationship between f_H and $\dot{V}_{O_2}$, such as the type of activity (Nolet et al., 1992; Butler et al., 2000), variation in body condition (Froget et al., 2001) or even season (Holter et al., 1976).

Antarctic penguins are regularly faced with two thermal challenges (exposure to cold wind on land and diving in cold sea water). Indeed, at Possession Island, Crozet Archipelago, our research site, the climate is cold ($5\,^{\circ}\text{C}$ annual average, $-3\,^{\circ}\text{C}$ in winter and $+7\,^{\circ}\text{C}$ in summer), wet (mean rainfall $247\,\text{cm year}^{-1}$) and windy (mean wind speed $45\,\text{km h}^{-1}$ with blasts attaining $180\,\text{km h}^{-1}$). Thus, the apparent temperature, using the equation from Siple and Passel (1945) for wind-chill effect on an animal, is on average $-18\,^{\circ}\text{C}$ in winter and $+4\,^{\circ}\text{C}$ in summer. This environmental variation is likely to influence metabolic rate.

In a previous study, Froget et al. (2001) found that the relationship between heart rate and the rate of oxygen consumption obtained for king penguins walking on a

treadmill was affected by the body condition of the animal. They concluded that the best estimate of the rate of oxygen consumption was obtained by relating the OP to the body condition of the bird and multiplying this by f_H. Thus, in the present study, we compared the relationship between f_H and $\dot{V}_{O_2}$ obtained by exposing king penguins to environmental temperatures that exceeded the average range routinely experienced in the field with that obtained in the previous study of king penguins walking on a treadmill.

The aims of the present study were therefore (i) to investigate whether exposure to low ambient temperature could be used as an alternative to exercise for calibrating f_H against $\dot{V}_{O_2}$ for subsequent use in free-ranging animals and (ii) to establish the relationship between $\dot{V}_{O_2}$, body temperature and ambient temperature and to determine the lower critical temperature (LCT) of adult king penguins.

Materials and methods
Animals

The experiments were carried out on Possession Island (Crozet Archipelago) over the two austral summers of 1997–1998 and 1999–2000.

In 1997–1998, 22 breeding king penguins were captured. As king penguins are less likely to desert their nest while brooding a small chick, males were captured either at the beginning or the end of their third foraging trip and females at the beginning or the end of their second foraging shift (see Fig. 1 in Froget et al., 2001). Sex was determined either by the song (Jouventin, 1982) or by the behaviour (such as mating or egg-laying). All birds were weighed, and measurements of their flipper size, bill length and foot length to +1 mm were taken according to standard techniques (Stonehouse, 1960). At the end of the experiment, each bird was weighed to ±20 g, and the stomach contents of the bird were retrieved using the 'water off-loading technique' (Wilson, 1984).

A nutritional index (NI) was then calculated using equations 3 and 4 from Froget et al. (2001):

$$NI = M_b - (0.102L_b - 3.43), \qquad (2)$$

where M_b is the body mass in kg and L_b is the length of the bill in mm. The bird was then re-fed with its own stomach contents prior to its release.

In 1999–2000, nine king penguins were captured using the protocol described above. The only difference from the 1997–1998 experiment was that the stomach contents of the bird were retrieved before the experiment to obtain a better estimate of the body mass and the NI (Froget et al., 2001). The bird was rested overnight prior to being placed in the respirometer. The bird was then re-fed before its release.

Equipment

Each bird was equipped with an externally mounted pulse-interval-modulated heart rate radio transmitter (Woakes and Butler, 1975) or heart rate data logger in 1999–2000 (Woakes
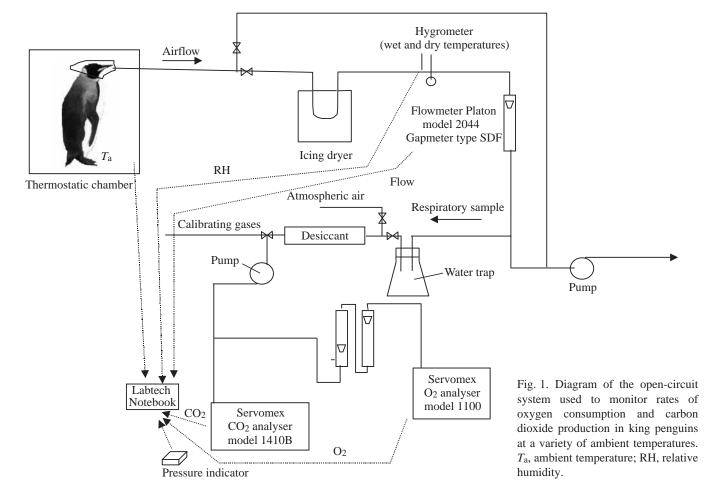
et al., 1995). Both were the same size and mass (4.5 cm×2.5 cm×0.6 cm and 15 g). Each transmitter or logger had electrode leads made of stainless-steel wire which terminated with hypodermic needles. *In situ*, the maximum distance between the two electrodes was 37 cm. The body of the transmitter or logger was wrapped in insulating foam and covered with Tesa tape (Beierdorsf AG, Germany) for protection from attacks by the bird. The electrodes were placed subcutaneously in a dorsal, midline position. One electrode was placed level with the heart and the other in a more caudal location. This arrangement provided a good electrocardiogram (ECG) signal. The body of the transmitter or logger was attached to the back feathers using Tesa tape (Bannasch et al., 1994). The transmitter or logger was externally mounted rather than implanted to avoid any post-operative recovery time.

Rate of oxygen consumption was measured in an open-circuit system (Fig. 1) similar to that described by Barré and Roussel (1986). The penguin was placed in a thermostatic chamber with its head enclosed in an opaque respiratory hood connected to the open-circuit flow for measurement of the rates of O_2 consumption and CO_2 production. The hood was ventilated with a constant airflow of approximately $24 \, l \, min^{-1}$, measured using a digital flowmeter (Platon, model 2044). A sub-sample of the outlet airflow was passed, *via* a drying agent (Silica gel), to a paramagnetic oxygen analyser (Servomex 1100) and then to an infrared carbon dioxide analyser (Servomex 1410B). Data were recorded on a PC using the Labtech-Notebook software. The O_2 and CO_2 analysers were calibrated before each experiment using oxygen-free nitrogen, atmospheric air and a calibrating gas of 5 % CO_2 in N_2. The signal from the externally mounted transmitter was detected by a receiver (International 877R) and converted to an ECG by a decoder (Woakes and Butler, 1975). The ECG was directed to a chart recorder (Graphtec). Heart rate was calculated by counting the number of QRS waves of the ECG over 3 min.

In 1999–2000, the same system was used but, to determine whether the flow rate had not been too low in 1997–1998, the airflow circulating through the hood was higher, at approximately $45 \, l \, min^{-1}$. There were no differences in measured $\dot{V}_{O_2}$ between the two years. Heart rate was recorded in the data logger every 2 s and later downloaded to a computer for analysis.

Experimental protocol

After being equipped with a radio transmitter or data logger, the penguin was placed in a container in the thermostatic chamber at 10 °C and left resting for at least an hour. Ambient temperature (T_a) was then randomly varied between –30 and +10 °C (with an increment of approximately 5 °C). The penguin was left at the chosen temperature for at least 30 min or until steady-state conditions had been achieved (i.e. stabilisation of the gas concentrations in the respirometer). Heart rate was then recorded on a chart recorder over a 3 min period (for the birds of the 1998–1999 experiment). Each bird

Fig. 1. Diagram of the open-circuit system used to monitor rates of oxygen consumption and carbon dioxide production in king penguins at a variety of ambient temperatures. T_a, ambient temperature; RH, relative humidity.

was exposed to at least nine different temperatures. During all the experiments, the bird was resting while standing in the container. In 1999–2000, body temperature was measured using a thermistor (accuracy $\pm 0.2\,°C$) that was 'ingested' by the bird, and the connecting lead was fixed with Tesa tape at the opening of the bill. The thermistor probe was located approximately 30 cm into the digestive tract.

Data analysis
Calculation of rate of oxygen consumption

The rate of oxygen consumption was calculated from the gas concentration using the equation derived from Depocas and Hart (1957) as modified by Withers (1977):

$$\dot{V}_{O_2} = \dot{V}_{STPD} \left\{ \frac{F_{IO_2} - F_{EO_2}}{1 - [1 - (F_{ECO_2} - F_{ICO_2})/(F_{IO_2} - F_{EO_2})]F_{IO_2}} \right\},$$
(3)

where $\dot{V}_{STPD}$ is the calculated dry air outflow at standard temperature and pressure, and F_{IO_2}, F_{EO_2}, F_{ICO_2} and F_{ECO_2} are the fractional concentrations of O_2 and CO_2 in the inlet and outlet air respectively. Mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$) was calculated from the body mass without the stomach contents.

Statistical analyses

All statistical tests were performed using the statistical package MINITAB 12.22 for Windows (Minitab Inc.). All values are presented as mean $\pm$ S.E.M. The relationship between heart rate and the rate of oxygen consumption was determined using least-squares regression. Regression equations were compared using an analysis of variance general linear model (GLM, as reviewed in Zar, 1999). Student's *t*-tests were used to compare the significance of any difference between the means of two populations. One-way analysis of variance (ANOVA) with Tukey's HSD *post-hoc* testing was used when more than two populations were compared. Results were considered significant at $P<0.05$.

Results

No significant changes in body temperature (T_b) occurred within the range of the ambient temperatures studied, the mean T_b remaining at $37.5\pm0.06\,°C$ throughout the experiment (Fig. 2).

There were no significant differences in f_H and mass-specific $\dot{V}_{O_2}$ ($s\dot{V}_{O_2}$) between the two years (Fig. 3, multiple comparisons with an unbalanced nested design: $F_{1,17}=1.91$, $P=0.17$; $F_{1,17}=1$, $P=0.32$; $F_{1,17}=0.05$, $P=0.82$, respectively). It was

therefore possible to pool all the data from the two years.

Metabolic response to varying ambient temperature

According to the classic model for heat loss (Scholander et al., 1950), the relationship between $s\dot{V}_{O_2}$ and T_a is expressed by two linear regression lines that intersect at the lower critical temperature (LCT). The LCT is defined as the lowest temperature in the thermoneutral zone and was determined from the pooled data of the 31 king penguins by using the least-squares method (Zar, 1999). The mean $s\dot{V}_{O_2}$ at $10\,°C$ was $10.5\pm0.46\,ml\,min^{-1}\,kg^{-1}$; between $10\,°C$ and $-5\,°C$, $s\dot{V}_{O_2}$ remained relatively constant (at $10.6\pm1.52\,ml\,min^{-1}\,kg^{-1}$). Between -5 and $-31\,°C$, $s\dot{V}_{O_2}$ increased significantly to $18.5\pm0.57\,ml\,min^{-1}\,kg^{-1}$ (Fig. 4). The linear regression equations (equations 4 and 5), for the relationship between $s\dot{V}_{O_2}$ and ambient temperature are as follows.

Between 10 and $-5\,°C$:

$$s\dot{V}_{O_2} = -0.057T_a + 10.32, \qquad (4)$$

($r^2=0.021$, $P>0.06$); between -5 and $-30\,°C$:

$$s\dot{V}_{O_2} = -0.343T_a + 8.93, \qquad (5)$$

($r^2=0.43$, $P<0.0001$).

The lines for these two equations intersect at $-4.9\,°C$, which is taken to be the lower critical temperature.

Exercise versus temperature to calibrate heart rate against $\dot{V}_{O_2}$

Although the range of f_H (from 66 to $204\,beats\,min^{-1}$) for birds exposed to varying ambient temperatures was similar to that obtained for birds resting and walking on a treadmill (57–$189\,beats\,min^{-1}$; Froget et al., 2001), the range of $\dot{V}_{O_2}$ during cold exposure (82.8–$314.6\,ml\,min^{-1}$) was closer to that obtained for birds resting within their thermoneutral zone (62.6–$225.2\,ml\,min^{-1}$) than to that for birds exercising on the treadmill (127.1–$563.0\,ml\,min^{-1}$; Froget et al., 2001). There was a significant positive relationship between f_H and $\dot{V}_{O_2}$, but this was significantly different from that obtained from birds walking on a treadmill (Fig. 5).

The oxygen pulse was calculated above and below the LCT. There was a small but significant increase in the oxygen pulse between that measured at thermoneutrality and that measured for temperatures lower than the LCT (from 1.23 ± 0.06 to $1.47\pm0.07\,ml\,O_2\,beat^{-1}$; paired t-test, $t=9.72$, $N=31$, $P<0.001$).

There was a significant correlation between the nutritional index (NI) and the oxygen pulse above or below the LCT.
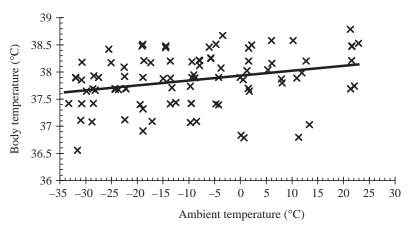

Fig. 2. Relationship between body temperature (T_b) and ambient temperature (T_a) in nine adult king penguins. The regression line is $T_b=0.007T_a+37.5$ ($r^2=0.045$, $P>0.05$, $N=92$).
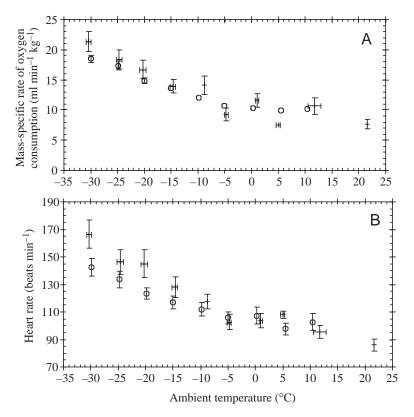

Fig. 3. Comparison of data obtained in two different seasons (open circles, 1997–1998; plus signs, 1999–2000). (A) Mass-specific rate of oxygen consumption; (B) heart rate plotted against ambient temperature. Values are means ± S.E.M., $N=203$. Some error bars are within the size of the symbol.

However, an analysis of covariance (Zar, 1999) showed that there was no significant difference in the slopes and the intercepts between the equation obtained using the OP above the LCT and that obtained using the OP below the LCT. It is then possible to use a common regression ($r^2=0.32$, $P=0.015$, Fig. 6):

$$OP = 1.09 + 0.096NI. \qquad (6)$$

Discussion

Metabolic response to variation of ambient temperatures

Our values of $s\dot{V}_{O_2}$ for animals resting at thermoneutrality ($10.5\pm0.46\,\mathrm{ml\,min^{-1}\,kg^{-1}}$) are not significantly different from those obtained in previous studies ($7.93\pm0.40\,\mathrm{ml\,min^{-1}\,kg^{-1}}$, $N=3$; $t=1.68$, $P<0.05$; Le Maho and Despin, 1976; $8.81\pm0.98\,\mathrm{ml\,min^{-1}\,kg^{-1}}$, $N=73$ measurements from 12 king penguins; $t=1.09$, $P<0.05$; Barré, 1980, 1984). The mean value for the lower critical temperature determined in the present study ($-4.9\,^{\circ}\mathrm{C}$) is consistent with what would be expected from the studies mentioned above. Le Maho and Despin (1976) did not observe an increase in metabolic rate in adult king penguins between +15 and $-5\,^{\circ}\mathrm{C}$, suggesting that between these two temperatures king penguins were in their thermoneutral zone; Le Maho et al. (1979) even mentioned that the LCT of king penguins was $-5\,^{\circ}\mathrm{C}$. Furthermore, Barré (1984) reported the LCT of king penguin chicks to be between -10 and $5\,^{\circ}\mathrm{C}$.

The $f_H/\dot{V}_{O_2}$ relationship and cold exposure

Although most previous relationships between f_H and $\dot{V}_{O_2}$ have been obtained by exposing animals to different levels of activity (Nolet et al., 1992; Bevan et al., 1994, 1995; Hawkins et al., 2000; Froget et al., 2001; Green et al., 2001), in some studies (e.g. Morhardt and Morhardt, 1971), a large range of f_H and $\dot{V}_{O_2}$ values were obtained by exposing the animals to a variety of temperatures. Froget et al. (2001) established the relationship between OP and NI for king penguins walking on a treadmill. Two equations were derived depending upon whether the animal was active or resting. Using an analysis of covariance (Zar, 1999) to compare the relationship between OP and NI obtained in the present study (equation 6) and that between resting oxygen pulse (ROP) and NI from penguins resting on a treadmill ($T_a=15\,^{\circ}\mathrm{C}$, range 7 to $20\,^{\circ}\mathrm{C}$; equation 8 in Froget et al., 2001), we established that there was no significant difference between the slopes of the two equations ($F_{1,39}=2.96$; $P=0.0904$):

$$\mathrm{ROP} = 1.033 + 0.18\mathrm{NI} \qquad (10)$$

($r^2=0.34$, $P=0.004$). The heart rate of king penguins resting on sea water is approximately $115\,\mathrm{beats\,min^{-1}}$ (measured in free-ranging birds at night; G. Froget, unpublished data). It is therefore possible to estimate OP, and then $\dot{V}_{O_2}$, obtained for a hypothetical penguin resting on sea water using the different equations available (equations 6 and 10; Fig. 7). $s\dot{V}_{O_2}$ was thus estimated to lie between 13.6 and $15.7\,\mathrm{ml\,O_2\,min^{-1}\,kg^{-1}}$, which is consistent with direct estimates of the rate of energy expenditure of king penguins resting on sea water given in the literature (between 8.03 and $15.45\,\mathrm{ml\,O_2\,min^{-1}\,kg^{-1}}$, Kooyman et al., 1992;
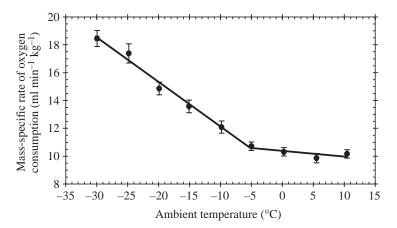
Fig. 4. Relationship between mass-specific rate of oxygen consumption ($s\dot{V}_{O_2}$, $\mathrm{ml\,min^{-1}\,kg^{-1}}$) and ambient temperature (T_a, $^{\circ}\mathrm{C}$) in 31 adult king penguins. The regression lines for mass-specific rate of oxygen consumption *versus* ambient temperature are as follows: above $-5\,^{\circ}\mathrm{C}$, $s\dot{V}_{O_2}=-0.057T_a+10.32$, $r^2=0.021$, $P>0.06$; below $-5\,^{\circ}\mathrm{C}$, $s\dot{V}_{O_2}=-0.343T_a+8.93$, $r^2=0.43$, $P<0.0001$. The two regression lines intersect at $-4.9\,^{\circ}\mathrm{C}$, the lower critical temperature (LCT). Values are means $\pm$ S.E.M., $N=203$.

$13.9\,\mathrm{ml\,O_2\,min^{-1}\,kg^{-1}}$, Culik et al., 1996). However, using the equation for active penguins in air (AOP=1.565+0.36NI, Froget et al., 2001), energy expenditure would be approximately $26.5\,\mathrm{ml\,O_2\,min^{-1}\,kg^{-1}}$.

This suggests that, in king penguins, the use of reduced environmental temperature to calibrate f_H against $\dot{V}_{O_2}$ is inappropriate if the relationship is to be used for different levels of activity, but should still be employed to estimate the metabolic rate of penguins resting on sea water and on land.
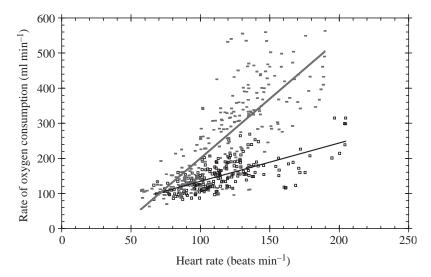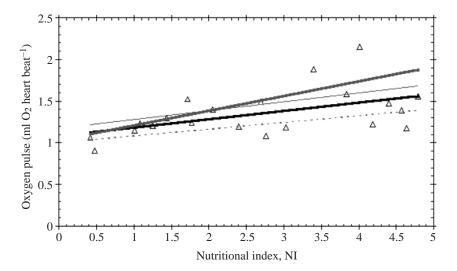
Fig. 5. Rate of oxygen consumption ($\dot{V}_{O_2}$) as a function of heart rate (f_H) for 24 adult king penguins walking on a treadmill (grey symbols, the grey line is the regression equation: $\dot{V}_{O_2}=3.39f_H-136.86$, $r^2=0.63$, $P<0.001$; data are from Froget et al., 2001) and for 26 resting adults exposed to different temperatures (open squares, the black line is the regression equation: $\dot{V}_{O_2}=1.09f_H+26.10$, $r^2=0.46$, $P<0.001$).

Fig. 6. Resting oxygen pulse (ROP) of 26 king penguins at a variety of temperatures plotted against the nutritional index (NI, for details, see text). The solid black line is the regression line between ROP and NI for resting penguins at a variety of temperatures (ROP=1.09+0.096NI, r^2=0.32, P=0.015), and the thin grey line and the dotted line are the regression lines between NI and the resting oxygen pulse (ROP) above and below the lower critical temperature, respectively. The thick grey line is the ROP obtained in the previous study (Froget et al., 2001).

In other words, cold exposure simply extends the range of resting f_H and $\dot{V}_{O_2}$ values. This study confirms the influence of body composition (NI) on the $f_H/\dot{V}_{O_2}$ relationship. Rates of energy consumption estimated from f_H for king penguins resting on sea water or at varying ambient temperature are in agreement with values taken from the literature.

A major difference between walking on a treadmill and swimming in penguins is that the birds do not use the same musculature for both activities: they use their pectoral muscles during swimming and their leg muscles during walking. Previous studies, on non-diving birds, showed that the OP could differ depending on the muscle mass engaged in the activity (an increase in OP from walking to flying in barnacle geese; Nolet et al., 1992; Butler et al., 2000). However, in gentoo penguins *Pygoscelis papua*, Bevan et al. (1995) found

no significant difference in the relationship between f_H and $\dot{V}_{O_2}$ during swimming and walking.

Finally, while walking on a treadmill, king penguins are exposed to an unnatural situation, that is performing sustained intense exercise in their thermoneutral zone (or maybe sometimes above it). Thus, they have to face an extra challenge, the elimination of exercise-generated heat. While at sea, the elimination of exercise-generated heat is eased because the thermal conductance of water is 25 times that of air.

Thus, to use f_H as an indicator of $\dot{V}_{O_2}$ with more confidence in free-ranging king penguins, the relationship between f_H and $\dot{V}_{O_2}$ should be calculated for animals resting at different temperatures (this study), while walking (Froget et al., 2001) and perhaps also while swimming both at the surface and when submerged. It would also be useful to perform experiments that associate thermoregulation and activity; i.e. walking or swimming at different temperatures, although it is possible that endotherms could use the 'wasted' heat produced during exercise, at least partly, to offset the costs of thermoregulation if the animals are below their LCT (Butler and Jones, 1982; Handrich et al., 1997).
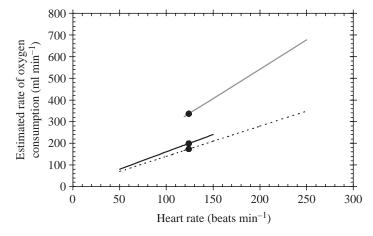
Fig. 7. Graphic representation of the different equations available to estimate rate of oxygen consumption from heart rate using the equations from Froget et al. (2001) when the animal is resting (solid line) or active (walking on a treadmill, grey line) or using the equation from the present study (broken line). The regression equations are derived from a hypothetical bird with a bill length of 123.8 mm and a body mass of 12.2 kg. The black circles represent the estimated rate of oxygen consumption when the animal is resting on sea water.

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