Heart rate and energetics of free-ranging king penguins
(*Aptenodytes patagonicus*)

G. Froget[1,2], P. J. Butler[1,*], A. J. Woakes[1], A. Fahlman[1], G. Kuntz[2], Y. Le Maho[2] and Y. Handrich[2]

[1]*School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK* and [2]*Centre d'Écologie et Physiologie Énergétiques, Centre National de la Recherche Scientifique, 23 rue Becquerel, 67087 Strasbourg cedex, France*

*Author for correspondence (e-mail: P.J.Butler@bham.ac.uk)

Summary

The main objective of this study was to determine heart rate (f_H) and the energetic costs of specific behaviours of king penguins while ashore and while foraging at sea during their breeding period. In particular, an estimate was made of the energetic cost of diving in order to determine the proportion of dives that may exceed the calculated aerobic dive limit (cADL; estimated usable O_2 stores/estimated rate of oxygen consumption during diving).

An implanted data logger enabled f_H and diving behaviour to be monitored from 10 free-ranging king penguins during their breeding period. Using previously determined calibration equations, it was possible to estimate rate of oxygen consumption ($\dot{V}_{O_2}$) when the birds were ashore and during various phases of their foraging trips. Diving behaviour showed a clear diurnal pattern, with a mixture of deep (>40 m), long (>3 min) and shallow (<40 m), short (<3 min) dives from dawn to dusk and shallow, short dives at night. Heart rate during dive bouts and dive cycles (dive + post-dive interval) was 42% greater than that when the birds were ashore. During diving, f_H was similar to the 'ashore' value (87 ± 4 beats min^{-1}), but it did decline to 76% of the value recorded from king penguins resting in water. During the first hour after a diving bout, f_H was significantly higher than the average value during diving (101 ± 4 beats min^{-1}) and for the remainder of the dive bout.

Rates of oxygen consumption estimated from these (and other) values of f_H indicate that when at sea, metabolic rate (MR) was 83% greater than that when the birds were ashore [3.15 W kg^{-1} (–0.71, +0.93), where the values in parentheses are the computed standard errors of the estimate], while during diving bouts and dive cycles, it was 73% greater than the 'ashore' value. Although estimated MR during the total period between dive bouts was not significantly different from that during dive bouts [5.44 W kg^{-1} (–0.30, +0.32)], MR during the first hour following a dive bout was 52% greater than that during a diving bout. It is suggested that this large increase following diving (foraging) activity is, at least in part, the result of rewarming the body, which occurs at the end of a diving bout. From the measured behaviour and estimated values of $\dot{V}_{O_2}$, it was evident that approximately 35% of the dives were in excess of the cADL. Even if $\dot{V}_{O_2}$ during diving was assumed to be the same as when the birds were resting on water, approximately 20% of dives would exceed the cADL. As $\dot{V}_{O_2}$ during diving is, in fact, that estimated for a complete dive cycle, it is quite feasible that $\dot{V}_{O_2}$ during diving itself is less than that measured for birds resting in water. It is suggested that the regional hypothermia that has been recorded in this species during diving bouts may be at least a contributing factor to such hypometabolism.

Key words: diving metabolism, foraging, heart rate, aerobic dive limit, king penguin, *Aptenodytes patagonicus*, seabird.

Introduction

Changes in the sizes of seabird populations are known to reflect variations in marine resources (Chastel et al., 1993; Montevecchi, 1993; Boyd, 1996). Thus, there has been an increasing effort to use seabirds as indicators of variation in ocean resources and of global climatic changes (Le Maho et al., 1993; Le Maho, 1994). However, in order to give a predictive model that is as accurate as possible, it is necessary to estimate accurately the energy requirement of seabirds.

Recent technologies have not only enabled a better understanding of the behaviour of free-ranging animals, e.g. *via* radio or satellite tracking (Bost et al., 1997; Butler et al., 1998) and time–depth recorders (Kooyman et al., 1980; Bevan et al., 1995), but have also made possible the acquisition of physiological data from heart rate and temperature data loggers (Bevan et al., 1997, 2002; Green et al., 2003).

King penguins breed and forage in the Antarctic Ocean,

where primary and secondary productions are high (Huntley et al., 1991). Information on their energy expenditure and thus food consumption can be used in models of energy flux within the ecosystem (Croxall et al., 1999) or for inclusion within fisheries models (Croxall, 1984). To date, estimates of the field metabolic rate (FMR) have been made using data obtained either from captive birds in a metabolic chamber or in a water channel (Culik et al., 1996), or from free-ranging birds using the time–energy budget (TEB) and the doubly labelled water (DLW) methods (Kooyman et al., 1992a).

However, although DLW is still widely used to measure FMR (Speakman, 1998), it does not enable the determination of energetic costs associated with specific activities, without a detailed time budget (Butler et al., 2004). Heart rate (f_H) has been proposed as an alternative indicator of the rate of oxygen consumption ($\dot{V}_{O_2}$; Butler, 1993). This technique has already been successfully used to monitor continuously the rate of energy expenditure in several species (Bevan et al., 1995, 2002; Boyd et al., 1999; Green et al., 2003). The advantages of this technique include a monitoring period of up to several months (Woakes et al., 1995), and the recorded f_H can be divided into small time units to allow the investigation of energetic costs of integrated behaviour categories, such as foraging at sea and incubating ashore. In the present study, miniature implantable data loggers for simultaneous recording of depth and f_H were utilised.

Froget et al. (2001) presented three models for estimating $\dot{V}_{O_2}$ of free-ranging king penguins and two of these involved the use of oxygen pulse (OP, the amount of oxygen consumed per heart beat). OP during rest (ROP) and during activity (AOP) were used according to the activity state of the birds. However, it is known that AOP is not usually constant over a range of activity levels (Butler, 1993), and it is now known that ROP is different between birds resting in air (i.e. when ashore) and resting in water (i.e. when at sea; Fahlman et al., 2004). Thus, the primary aim of the present study was to record f_H and diving behaviour of free-ranging king penguins and, using the appropriate equation from Fahlman et al. (2004), to estimate the energy expenditure of the birds during the different phases of the foraging and incubating shifts. From these, it was expected to shed new light on the 'king penguin's enigma' (Culik et al., 1996), which is that 'king penguins seem to swim too fast and dive for too long, and too often, to be in agreement with our physiological models of diving' (Kooyman et al., 1992a).

Indeed, the aerobic dive limit (ADL) or diving lactate threshold (DLT), defined as the diving duration beyond which there is an increase in post-dive plasma lactate concentration (Kooyman, 1989; Butler and Jones, 1997) has not yet been measured in free-ranging king penguins. Thus a calculated ADL (cADL, which is the dive duration during which all usable oxygen would be exhausted; Kooyman, 1989) is often used as an indicator of the DLT. Previous estimations of the cADL at 2 min for king penguins (Kooyman et al., 1992a; Culik et al., 1996) imply that 45% of all dives would exceed the cADL, which suggest that this calculation is inaccurate.

Thus, an associated aim of the present study was to estimate $\dot{V}_{O_2}$ while foraging (diving) in free-ranging king penguins, and thus cADL.

Materials and methods

Animals

The study was undertaken at the French station on Possession Island (46°25′S, 51°45′E), Crozet Archipelago during two consecutive austral summers (1998–1999 and 1999–2000). King penguins *Aptenodytes patagonicus* Miller 1778 used in the study were breeding at the colony of 'La Baie du Marin', a few hundred metres from the research station. Twenty-seven penguins were captured 3–6 days after the egg exchange (the second egg exchange for a female or the third in the case of a male; see fig. 1 in Froget et al., 2001). They were each implanted with a custom-built f_H- and depth-data logger (HRDDL: Woakes et al., 1995); 14 birds in 1998–1999 (mean mass ± S.E.M.=12.7±0.2 kg); 13 birds in 1999–2000 (mean mass ± S.E.M.=12.2±0.3 kg). Several pairs of penguins were selected for observation and/or logger implantations. Both partners from each pair were marked using temporary plastic flipper bands, when the female laid its egg. The bands were removed at the end of the season or during the implantation (i.e. studied birds were not banded; see Froget et al., 1998).

Heart rate data loggers

The data loggers (61 mm×24 mm×6 mm, 27 g, <0.3% of the body mass of king penguins) had 4 MB of memory and were able to record f_H and hydrostatic pressure (to an accuracy of 1 m depth) and were programmed to store these measurements every 2 s. After programming, the loggers were encapsulated in wax and coated with medical grade silicone rubber. Prior to implantation, the logger was bathed in a cold sterilising solution for 2 h, and rinsed thoroughly with sterile water.

Surgical procedure

The implantation was performed 3–6 days after the egg exchange, so that the bird was settled on the egg and sufficiently motivated to continue incubating despite the disturbance caused by handling and implantation. A portable enclosure was placed over the bird before capture in order to protect its territory during the surgery. The enclosure consisted of wire mesh formed into a circular fence that was approximately the area of a king penguin territory (about 1.5 m diameter and 0.6 m high). The enclosure was also used to confine the bird to the territory it previously held, while awakening and recovering from anaesthesia, and to prevent other penguins (or sheathbills and skuas) from attacking it. 30 min prior the surgery, the bird was injected with 0.5 ml of Valium (Virbac, Centravet Plancoët, France) subcutaneously on the back. The penguin was captured by placing a sack over the adult and lifting both it and its egg. The egg was immediately replaced under the bird by a plaster egg that had

previously been kept in an incubator at 38°C, to prevent breakage of the real one. This procedure generally produced little reaction from the bird. The real egg was kept in an incubator at 38°C until its replacement under the adult.

The birds were anaesthetised with halothane in a mixture of O_2:N_2O (1:1) administered *via* a hood placed over their head. Induction with 4.5% Halothane (Virbac) usually took 3–5 min. After induction, the bird was weighed on a load cell balance (accurate to ±2 g) and placed on the operating table. The bird was then intubated, and the anaesthetic gas was altered to 1.5% halothane in pure O_2. A long-acting antibiotic (Oxytetrin LA; Virbac) was injected into the pectoral muscle. Some feathers and the down surrounding the brood patch were removed. The remaining feathers around the brood patch and the incision area were deflected and held away using tape. The brood patch, tape and the surrounding feathers were then swabbed with an iodine solution (Betadine; Virbac). The electrocardiogram (ECG) and rectal temperature were continuously monitored throughout the surgical procedures. Xylocaine (lignocaine + 2% adrenaline; Virbac) was injected subcutaneously along the proposed incision lines to prevent bleeding.

The initial skin incision was made in line with the body axis, starting approximately 3 cm above the brood patch, and was about 5 cm long. The second incision, through the abdominal muscles, was made at right angles to the first, enabling an opening of the abdominal cavity of 3 cm length. The HRDDL was inserted into the body cavity, one of the ECG electrodes was placed close to the apex of the heart, its position was checked using an endoscope, and the other was placed pointing in the opposite direction, below the brood patch. The HRDDL was sutured into position with silk thread.

Each logger incorporates a transmitter that emits a click on each detected QRS wave of the ECG and a receiver was used to confirm that the logger was detecting and recording the ECG. Heart rate was calculated from its mean period, by dividing the total duration of *n* heart beat periods by *n*, over the sampling period.

After the surgery the bird, still asleep, was replaced together with a warm dummy egg within the colony in its enclosure. After full recovery from the anaesthetic (usually 4–5 h later), the enclosure was removed. 1–2 days after the surgery, the real egg was replaced under the bird. Attendance behaviour was then monitored during the following experimental period at least twice a day. 3–4 days after the equipped bird returned to the colony from its foraging trip, it was recaptured with its chick and weighed. Prior to the removal of the data logger, the bird was anaesthetised and carried to the base to be X-rayed, to check the position of the logger and of the ECG electrodes. The procedures for the removal of the logger and for the replacement of the bird in the colony after surgery were similar to those described for implantation.

Data analysis

Birds were initially divided in two groups depending on the year the data were obtained. Behavioural data were compared and, if no difference was detected, the data from the 2 years were pooled.

The data were prepared and initially analysed using a purpose-written computer program in the Labview programming package (version 5.0, National Instruments, Austin, TX, USA). Further analyses were performed with the
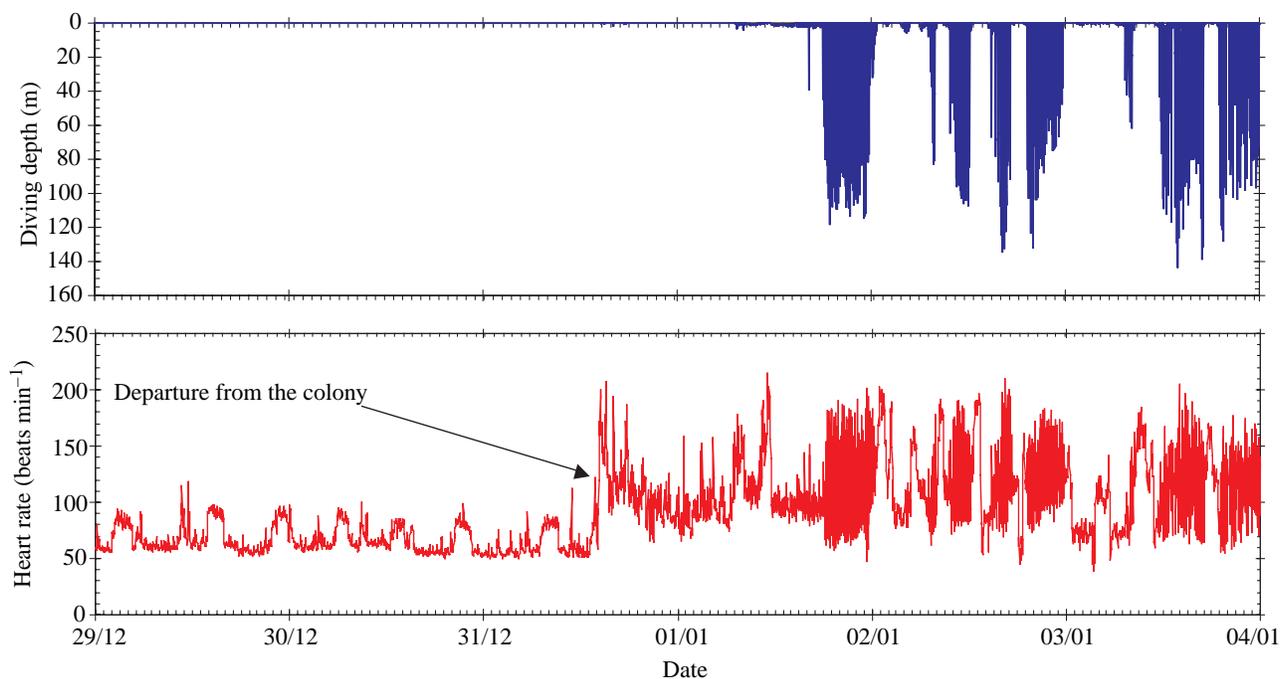

Fig. 1. Recording of depth (blue trace; top) and heart rate (red trace; bottom) of a king penguin (bird B00, mass=11.65 kg) over a 6 day period. Black arrow indicates the time when the bird went to sea.

statistical package MINITAB 12.22 for Windows (Minitab Inc.) and Excel 97 (Microsoft Corp.). Diving bouts were visually determined; a bout was deemed to have started as soon as three deep dives (below 10 m) were interspaced by a surface duration shorter than 10 min (Boyd et al., 1994) and it ended as soon as the surface duration was longer than 10 min. In king penguins, diving bouts are usually easily determined visually from the depth trace (see Fig. 1).

Estimating the rate of energy expenditure from heart rate

We used the linear regression equations 1 and 2A from Fahlman et al. (2004) to estimate $\dot{V}_{O_2}$ for animals ashore and while at sea, respectively (see Table 3). The standard error of the estimate (S.E.E.) for the estimated $\dot{V}_{O_2}$ ($\dot{V}_{O_2,est}$) was calculated from equation 11 in Green et al. (2001):

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

where d^2 is the error associated with the variation between calibration penguins (0.00165); n_1 is the number of penguins used in the calibration process (5); n_2 is the total number of data points in the regression (30); n_3 is the number of penguins from which the field value of heart rate was obtained (8; see Table 3); n_4 is the number of data points from which the field value of heart rate was obtained (>10 000); e^2 is the error associated with the scatter around the regression lines (0.00416); $\bar{X}$ is the mean value of heart rate used in the regression (2.17); X_i is the mean value of heart rate from the field from which σ_1 is to be estimated (see Table 3); and Σx^2 is the sum of all the squared values of heart rate used in the regression (0.536). The values for d^2, n_1, e^2, n_2, $\bar{X}$ and Σx^2 given above are for equation 2A from Fahlman et al. (2004). As equation 1 from Fahlman et al. (2004) is a multivariate equation, the modifications for the multivariate case (Zar, 1999) were employed: $e^2=0.00246$, $d^2=0.00881$, $n_1=5$, $n_2=150$, $n_3=8$, $n_4>10\,000$, and $\Sigma_{i=1}^{m}\Sigma_{k=1}^{m}c_{ik}x_i=1.33$ for the parameters in equation 1 of Fahlman et al. (2004), i.e. $\log(f_H),t$ and $\log(f_H)t$.

The conversion of $\dot{V}_{O_2}$ to W was achieved assuming that 1 ml O_2 s^{-1}=19.8 W when the animal was ashore (fasting and post-absorptive: respiratory quotient, RQ=0.7; Culik et al., 1996; Schmidt-Nielsen, 1997) and that 1 ml O_2 s^{-1}=18.9 W when the animal was at sea (Bevan et al., 2002, for gentoo penguins).

All values (other than $\dot{V}_{O_2est}$) are presented as means ± S.E.M. of the mean values from each animal. After verifying that the data were normally distributed and of equal variances using a Kolmogorov–Smirnov normality test and F-test, respectively, a Student's t-test was used to compare the significance of any difference between the means of two populations. One-way analysis of variance (ANOVA) with Tukey's HSD *post-hoc* test were used when more than two populations were compared. Results were considered significant at $P<0.05$. Woolf's test for differences and Z-tests were used to compare estimates of $\dot{V}_{O_2}$.

Results

Of the 27 birds implanted with data loggers, 25 were recaptured. Depth data were not recovered from four birds, due to failure of the pressure sensor. In the remaining 21 birds, ECG was not recorded accurately in six birds, due to movement of the ECG electrodes. And finally, five data loggers failed to record any data, possibly due to deficient waterproofing of the electronics. Thus, simultaneous measurements of depth and f_H were successfully recorded from ten birds. Altogether, f_H and diving data were analysed from a total of 66 days ashore and 188 days at sea.

Finally, in three of the ten birds (D99, W99 and U00) there were, due to programming problems, slight discrepancies (2–4 min over 15 days) between the recordings of f_H and depth, thus the data from these animals were excluded for the fine scale analysis of the cardiac response to diving. For this purpose a total of 10 343 dives was analysed from the remaining seven birds.

Fig. 1 illustrates f_H over a 6 day period recorded from a bird at the transition between the incubation and the foraging shift. Note that departure from the colony and diving behaviour are clearly discernible from the f_H trace.

Duration of foraging shifts

Birds left the colony on average 9±2 days after the implantation of the HRDDLs when their mate returned from the sea. The average duration of the foraging shift of the implanted birds was 16±2 days in 1998–1999 and 15±1 days in 1999–2000. In the second year, the mean duration of the foraging shifts of implanted birds was compared with that of non-implanted birds (18±5 days, N=13, P>0.05), at the same stage of reproduction and in close proximity to implanted birds. There was no significant difference between the two groups.

Diving behaviour

Due to sampling rate and the resolution of the pressure sensor, only dives for periods longer than 4 s and deeper than 6 m were analysed. The deepest dive depth recorded was
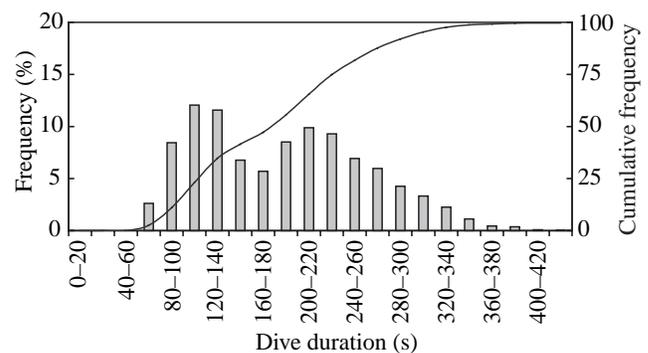

Fig. 2. Percentage frequency distribution of dive durations from 10 king penguins (only dives longer than 1 min and inter-dive interval shorter than 10 min were considered). Dive durations are divided into 20 s intervals. N=5230.

Table 1. *Summary information on the 10 implanted king penguins from which data were obtained*

Bird	Mean body mass (kg)[1]	Length of bill (mm)	Deployment duration (days)	Duration of shift (days) Incubation	Foraging	Total number of dives	Diving bouts Number	Mean duration (h:min)	Mean number of dives per bout	Interbout duration (h:min)
D99	13.1	128	30		17	1551	27	06:43	47.6	06:03
F99	12.9	124	31	14	16	2013	29	06:22	62.7	06:05
G99	13.4	128	28	14	18	2322	22	08:51	92.2	04:14
H99	12.7	126	24	12	16	696	12	06:37	58.0	07:03
W99	11.2	127	34			2886	37	08:11	65.7	05:21
X99	10.8	118	172*	6	12	2983	34	08:18	85.4	06:53
B00	11.7	118	34	14	14	302	28	04:04	39.6	05:19
S00	14.3	128	42	6+1**	7+27**	1054	8	04:13	122.6	04:10
U00	12.7	133	16	8	11	569	13	04:11	35.3	10:00
V00	15.0	127	28	10	15	1458	23	07:16	67.4	07:18
Mean	12.8±0.4	125.7±1.5					23.3±3.1	6:43±0:34	67.6±8.4	6:31±0:33
Total						15834				

[1]Calculated as the average of mass at implantation and mass at removal of data logger.

*This bird deserted the colony and was recaptured 6 months later, however, data were available only for the first 30 days.

**This bird performed two foraging shifts.

Missing data are because the beginning or end of shifts are unknown.

Table 2. *Mean heart rate of 10 king penguins when ashore and at different stages of their foraging trip*

| Bird	Heart rate, f_H (beats min^{-1}) Ashore	At sea	While travelling	During dive bout	During diving	At first hour after dive bout	Between bouts	Between dives	Increase after dive bout (%)	f_H at sea/ f_H ashore
D99	87.2	146.7								1.7
F99	86.9	146.7	127.9	133.4	106.5	183.7	176.4	192.4	38	1.7
G99	86.9	128.1	127.9	120.6	91.6	198.2	168.0	177.2	64	1.5
H99	119.2	139.8	133.4	138.8	112.8	202.4	161.8	192.5	46	1.2
W99	78.6	112.2	79.0	112.4	91.2	142.4	127.6		27	1.4
X99	88.4	156.3	93.3	118.9	91.4	182.1	123.4	182.2	53	1.8
B00	69.0	121.4	107.3	117.2	111.2	144.6	115.3	177.5	23	1.8
S00	94.3	110.9								1.2
U00	87.1	113.8	107.3	119.8	101.5	122.3	114.4	193.4	2	1.3
V00	74.6	131.6	120.9	126.9	106.5	174.1	146.2	188.1	37	1.8
Mean	87.2±4.3	130.8 ±5.1	112.1±6.7	123.5±3.1	100.9±3.6	168.7±10.2	141.6±8.8	186.2±7.1	36	1.5

Values are means ± S.E.M.
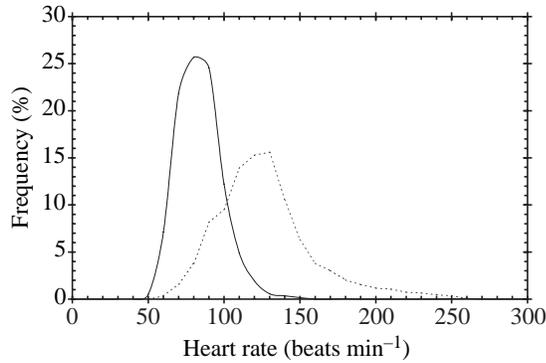
Fig. 3. Percentage frequency distribution of 30 min averaged heart rate from king penguins ashore (solid line) and while at sea (dotted line); *N*=10.
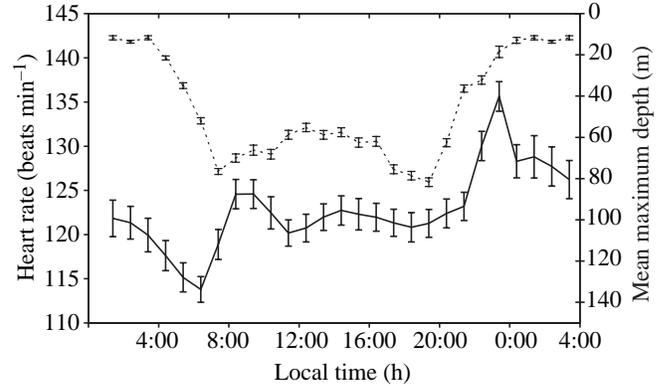

Fig. 4. Mean heart rates at half-hourly intervals (continuous line), and mean maximum diving depth (broken line) of foraging king penguins (*N*=10) during the incubation and chick-guarding period. Each point is the mean ± S.E.M. from all the birds while they were at sea.

257 m (for a duration of 7 min 42 s). General details of diving behaviour are given in Table 1. There was a significant positive correlation for the relationship of dive duration to depth (depth=0.6×duration – 56, r^2=0.82, P<0.05). There was a clear diurnal dive pattern, with a mixture of deep long and shallow short dives from dawn (between 05:00 h and 06:00 h) to dusk (around 21:00 h–22:00 h), while at night activity was limited to shallow short dives. This bimodal distribution of dive depth gave peaks at 10 m and 70 m and a related bimodal distribution of dive duration (2 and 3.5 min, Fig. 2). Thus, it was possible to establish two groups of dives: long and deep (>3 min and >40 m) and short and shallow (<3 min and <40 m). 80% of the inter-dive intervals were less than 2.5 min.

Heart rate during the monitoring period

The frequency distribution of f_H clearly differed depending upon whether the birds were ashore or at sea. When the birds were ashore, f_H ranged between 50 and 150 beats min^{-1}, whereas while they were at sea, f_H ranged between 50 and 260 beats min^{-1}, showing peaks at 80–90 beats min^{-1} when ashore and 120–130 beats min^{-1} when at sea (Fig. 3). The mean f_H of individual birds over the recording periods ranged from 69.0 to 119.2 beats min^{-1} (mean f_H=87.2±4.3 beats min^{-1}) when the animals were ashore and from 110.9 beats min^{-1} to 156.3 (mean f_H=130.8±5.1 beats min^{-1}) when they were at sea (Table 2). The overall mean f_H of the free-ranging penguins was 111.1±0.3 beats min^{-1}. All of the f_H values monitored from the birds in the wild were within the range of f_H recorded during the calibration experiments (from 47 beats min^{-1} to 308 beats min^{-1}; Fahlman et al., 2004).

When the animals were ashore, no circadian pattern of f_H was observed (see Fig. 1). On the other hand, when at sea (Fig. 4), there was a clear pattern, with a decline of f_H at dawn (from 121.8±2.0 beats min^{-1} at 00:00 h down to 113.8±1.4 beats min^{-1} at 05:30 h), when the animals start to forage actively (indicated by the increase of mean depth). During the daytime, f_H remained at a reasonably steady level (around 122 beats min^{-1}), while the birds regularly dived to greater depths (mean diving depth around 66 m). Then, around
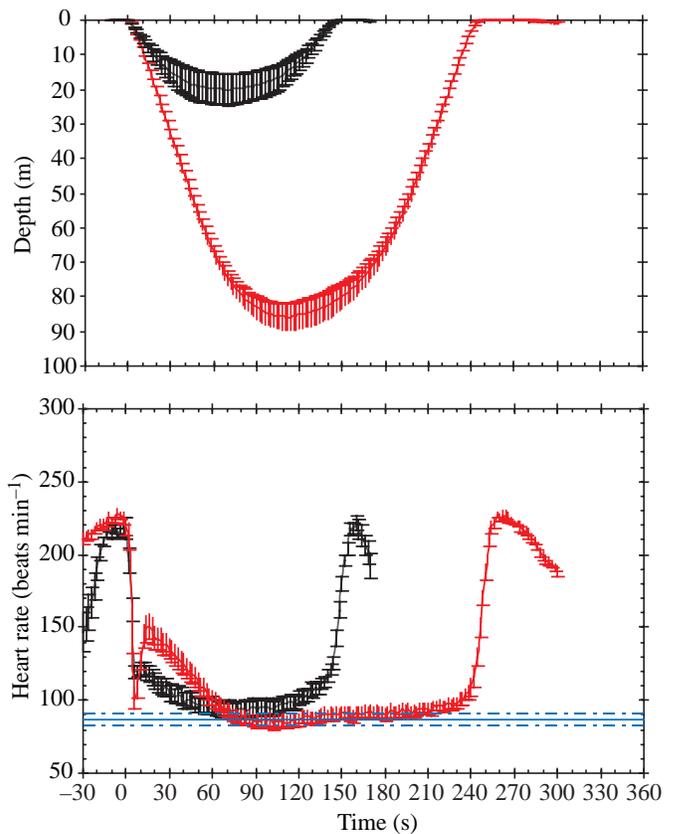

Fig. 5. Temporal changes in heart rate (beats min^{-1}; bottom), and depth (m; top) occurring during diving relative to the point of submergence and the points of surfacing, for long dives (240–250 s; red traces) and short dives (150–140 s; black traces). Each point is the mean ± S.E.M. of the mean values from 7 birds. The blue horizontal lines are mean ± S.E.M. of heart rate when ashore.

21:00 h and after diving depth had begun to decrease, f_H increased, to reach a peak of 135.6±2.2 beats min^{-1} at approximately 23:00 h, before returning to the midnight value.

Changes in heart rate associated with diving

When dives were grouped according to their duration separated by 10 s intervals (e.g. all dives between 130 to 139 s were grouped as dives of 134.5 s), a rapid decrease in f_H was observed in all categories, reaching a minimum 4 s after submergence (Fig. 5). During long dives (>3 min), f_H then showed an increase for the subsequent 6 s, before steadily decreasing to a value similar to that measured when the birds were ashore (lowest f_H, 82.1±5.4 beats min^{-1}; mean ± S.E.M.). During the shorter dives (<3 min), this secondary increase in f_H was not observed, and f_H was maintained at the low level achieved in the first 6 s of the dive, which was also similar to that measured in birds resting ashore (lowest f_H, 88.8±5.2 beats min^{-1}; mean ± S.E.M.; see Fig. 5). Mean f_H during diving, for all dives, was significantly greater than these values, but almost 30% below the value between bouts (Table 2). When f_H was plotted relative to the time of surfacing, there was no difference between short and long dives. Both showed a slight anticipatory tachycardia, with the largest increase around 4 s prior to surfacing. There was no significant difference between f_H pre- and post-dive. Similarly, in the 10 s prior to the dive, f_H did not vary significantly between short and long dives.

Heart rate values between dives, during the dive and over the dive-cycle (dive + post dive interval, 126±4.2 beats min^{-1}) were not significantly different between long and short dives. For dives longer than 3 min, however, there were significant negative correlations between dive duration and (a) mean dive f_H (r^2=0.78, P<0.001), (b) mean dive-cycle f_H (r^2=0.83, P<0.001), and (c) mean post-dive interval f_H (r^2=0.78, P<0.001).

The mean f_H during all diving bouts (123.5±3.1 beats min^{-1}; Table 2) was 40% lower than the mean f_H recorded during the hour following the diving bout (168.7±10.2 beats min^{-1}; paired t-test, t=5.3, P<0.01, N=8). The mean f_H during diving bouts was also 20% lower than the mean f_H over the entire subsequent inter-bout interval (141.6±8.8 beats min^{-1}; paired t-test, t=2.64, P<0.05, N=8). The mean f_H during the hour following a diving bout was, on average, 20% higher than mean f_H over the entire inter-bout interval (paired t-test, t=12.01, P<0.001, N=8).

Field metabolic rate

Estimated $\dot{V}_{O_2}$ of birds was calculated according to equations 1 and 2A from Fahlman et al. (2004) as appropriate, and then converted to estimated field metabolic rate FMR$_{est}$ (Table 3). When at sea, FMR$_{est}$ was 83% greater than when the birds were ashore. During diving bouts (and dive cycles), MR$_{est}$ was 73% greater than when the birds were ashore and MR$_{est}$ during the total period between bouts, although 20% greater, was not significantly different from that during diving bouts. However, during the first hour following a diving bout, MR$_{est}$ was 52% greater than that during a diving bout and 33% greater than that during the remainder of the interbout interval.

Discussion

To avoid using atypical data associated with the recovery of the birds from the surgery, the first 48 h after implantation were not used in subsequent data analysis (see Bevan et al., 1995). Furthermore, there was no significant difference in the duration of the foraging shifts in implanted and non-implanted birds. This suggests that the implantation had no long-term behavioural or physiological effects on the penguins. The foraging pattern of the king penguins used in the present study was similar to that described by other authors (Kooyman et al., 1992a; Culik et al., 1996; Charrassin et al., 1998), with deep diving activity limited to the daylight period, and diving activity at night reduced in frequency and limited to shallow depth. This daily diving pattern has been discussed extensively by the above authors, and probably reflects the photoperiodic vertical migration of their main prey (the myctophid fish species *Electrona carlsbergii, Frefftichtys andersonni* and *Protomyctophum tensoni*; Cherel and Ridoux 1992), and/or the light sensitivity of the eyes of penguins (Wilson et al., 1993).

Frequency analyses of the dive durations are consistent with those described elsewhere (Kooyman et al., 1992a; Culik et al., 1996): up to 35% of all dives (deeper than 8 m) were of longer duration than 3.5 min, and 80% of interdive intervals were shorter than 2.5 min.

The hypothesis developed by Kooyman et al. (1992a) that king penguins accumulate lactic acid during long dives and pay off the 'oxygen debt' by increasing surface times or surface frequency is not supported by the present data. Since a surface time of 2.5 min is theoretically inadequate for the metabolism of lactate after exceeding the DLT (Kooyman et al., 1992a), we reach a similar conclusion to Culik et al. (1996), that these birds must almost always dive within their DLT. This means that previous estimations of the cADL of 2 min (Kooyman et al., 1992a; Culik et al., 1996) must be inaccurate. On the other hand, it is possible, at least on some occasions, that any accumulated lactate could be metabolised by the locomotory muscles and/or heart during relatively short, shallow dives and/or when the birds are travelling to different foraging locations (Butler, 2004).

Physiology of diving

Few studies have investigated the variation of f_H during natural dives of birds or mammals. Most of the available data are either from captive animals during forced (Scholander 1940) or voluntary submersion (Millard et al., 1973; Butler and Woakes, 1975, 1984; Bevan et al., 1992) or from animals in their natural environment but diving through an artificial ice hole (Kooyman et al., 1992b). To our knowledge, only three published studies so far have investigated the full range of changes in f_H during diving in free-ranging marine birds (Bevan et al., 1997, 2002; Green et al., 2003). In the present study, two different cardiac responses to the dive could be observed, and from the diving behaviour it was possible to define two distinct types of dive: short, shallow dives and deep, long dives (i.e. there were no long, shallow dives). Interestingly, this segregation of the dives between short and

long dives is associated with a difference in temporal changes of f_H during the dive. During the first 6 s of the dive, there was a rapid reduction in f_H, similar to that measured in unrestrained freely diving captive tufted ducks (Bevan and Butler, 1992). Then, depending on the duration of the dive, f_H either increased in the subsequent 6 s if the bird was going to perform a long and deep dive, then progressively decreased to a rate similar to the f_H when the birds were ashore. If the dive was short and shallow, f_H stayed low, and again stabilised around the level of f_H recorded when birds were ashore. However, both the values for lowest f_H during diving were significantly (approximately 25%) lower than that for king penguins resting in water (114±7.2, N=5; Fahlman et al., 2004).

The differences in f_H during the first 6 s of submersion in the long and short dives may relate to the effort involved during this period. Sato et al. (2002) indicate that king penguins have to work harder at the beginning of deep dives than at the beginning of shallow dives to overcome the increased buoyancy associated with inhaling more air prior to the longer, deeper dives. However, as depth increases, the buoyant force would decrease due to compression of the air-filled spaces between the feathers, thus progressively reducing energy requirement.

Mean values of heart rate when ashore and during diving bouts (see Table 2) were similar to those obtained for gentoo penguins (90 and 140 beats min^{-1}, respectively; Bevan et al., 2002).

Estimated metabolic rate

When a bird is at its nest incubating, it needs only to supply energy for its maintenance metabolism (basal metabolic rate, BMR), any thermoregulatory costs associated with the maintenance of the egg or the chick at its optimal temperature, and any territorial defence activity. Croxall (1982) estimated in a number of penguin species that the energy cost of incubation was 1.2–1.4 times the BMR, which is similar to that found in the present study (1.3× BMR; calculated from the formula of Ellis, 1984). The estimated, mass-specific metabolic rate of king penguins ashore [3.15 W kg^{-1} (–0.71,

+0.93), where the values within parentheses are the computed S.E.E.; see Table 3], is within the range of data obtained by Kooyman et al. (1992a), who measured values between 2.4 and 4.8 W kg^{-1} using doubly labelled water, and similar to the resting metabolic rate (RMR) obtained from king penguins in a respirometer (2.8±0.1 W kg^{-1}, Le Maho and Despin, 1976; 3.0±0.3 W kg^{-1}, Barré, 1980; 3.7±0.1 W kg^{-1}, Froget et al., 2001). More interestingly, f_H, and thus FMR$_{est}$, was at its lowest when the birds were at sea during the first part of the foraging trip (travelling before the first diving bout), and during a diving bout (Table 3). There was then a large increase in f_H during the first hour following the end of a diving bout. This may have been the result of the increased rate of energy expenditure associated with the rewarming of the body that occurs at the end of a diving bout (Handrich et al., 1997).

At sea, when the bird was at the surface, it was not possible to distinguish between periods when the bird was resting and those when it was porpoising or travelling from one foraging patch to another. However, mass-specific MR$_{est}$ of the birds when at sea [5.77 W kg^{-1} (–0.37, +0.40)] was 25% greater than that of birds resting in water (4.6 W kg^{-1}; Culik et al., 1996; Fahlman et al., 2004), indicating that these animals were quite active when at sea. This level of metabolism included all activities while the bird was at sea, such as the period when the birds were travelling to and from the colony, and activity at night when diving was reduced.

It is of interest to note that at sea, mass-specific FMR$_{est}$ from the present study is 42% lower than the value of 9.95±0.33 W kg^{-1} obtained by Kooyman et al. (1992a) using DLW. This illustrates the apparent overestimation of MR of aquatic birds and mammals when at sea by the DLW method (Butler et al., 2004).

Energy cost of diving and cADL.

The mass-specific $\dot{V}_{O_2est}$ during diving bouts was determined in the present study to be 17.3 (–0.95, +1.01) ml min^{-1} kg^{-1}. When tested against the validation data in Froget et al. (2001), equation 2A in Fahlman et al. (2004) gives an average

Table 3. *Heart rate and estimates of rates of oxygen consumption and mass-specific field metabolic rate determined from these heart rates for birds ashore and while at sea*

Activity	f_H (beats min^{-1})	$\dot{V}_{O_2est}$ (ml O$_2$ min^{-1})	FMR$_{est}$ (W kg^{-1})
FMR ashore	87.2±4.3	122.1 (–28.2, +36.0)[a,b,c,d,e]	3.15 (–0.71, +0.93)
Diving bout	123.5±3.1	216.6 (–11.9, +12.7)[a,f]	5.44 (–0.30, +0.32)
First hour after bout	168.7±10.2	330.1 (–17.2, +18.0)[b,f,g,h,i]	8.29 (–0.43, +0.45)
Rest of the interbout	136.7±8.8	248.5 (–12.9, +13.6)[c,g]	6.24 (–0.32, +0.34)
Total interbout	141.6±8.8	260.6 (–14.5, +15.4)[d,h]	6.54 (–0.36, +0.39)
FMR at sea	130.8±5.1	234.1 (–15.1, +16.1)[e,i]	5.77 (–0.37, +0.40)

f_H, heart rate; $\dot{V}_{O_2est}$, estimated rate of oxygen consumption; FMR$_{est}$, estimated mass-specific field metabolic rate.

$\dot{V}_{O_2est}$ and FMR$_{est}$ were determined from the f_H values using equation 1 [log($\dot{V}_{O_2}$)=–0.279+1.24log(f_H)+0.0237n–0.0157log(f_H)n, r^2=0.81], where n=number of days fasting, or equation 2A [log($\dot{V}_{O_2}$)=1.35log(f_H)–0.488, r^2=0.86] from Fahlman et al. (2004), for birds ashore and while at sea, respectively.

Values are means ± S.E.M., N=8 or 10 (see Table 2). Values in parentheses are the computed S.E.E.

Superscripted letters signify significant differences between estimates for specific activities (P<0.05, Z-test).

algebraic overestimation of 2%. The available oxygen stores in diving king penguins are estimated to be between 45 ml kg^{-1} (Ponganis et al., 1999) and 58 ml kg^{-1} (Kooyman, 1989). Using the estimated $\dot{V}_{O_2}$ during diving bouts and the upper value of usable O_2 stores given above, the calculated aerobic dive limit (cADL) is 3.4 min and approximately 35% of all dives exceed this value (Fig. 2).

If our estimates of $\dot{V}_{O_2}$ are correct, this implies that king penguins are performing most of their foraging dives (those greater than 3 min duration) by exceeding their cADL. Unfortunately, with the exception of those on tufted ducks (see Woakes and Butler, 1983), no study so far has been able to determine $\dot{V}_{O_2}$ during diving itself. The rate of oxygen consumption estimated over a complete dive cycle, as in the present study, is the closest estimate that exists for free-ranging king penguins. Thus either $\dot{V}_{O_2}$ during diving is much lower or/and the oxygen stores are much larger than estimated. In fact, as Butler (2000) points out, even if the $\dot{V}_{O_2}$ during diving is the same as that when the birds are resting in water (14.1 ml min^{-1} kg^{-1}, Culik et al., 1996; and 15.3±1.3 ml min^{-1} kg^{-1}, Fahlman et al., 2004), maximum cADL would be 3.8–4.1 min and still approximately 20% of the dives would exceed this duration (Fig. 2). It is of course possible that $\dot{V}_{O_2}$ during diving itself, i.e. when the bird is actually submerged, is lower than when resting in water (hypometabolism), and one possible mechanism by which this could be achieved is regional hypothermia (Handrich et al., 1997).

Even though there is debate over the physiological significance of such hypothermia (Ponganis et al., 2003), Bevan et al. (2002) calculated that a drop in body temperature of only 2.4°C would reduce resting $\dot{V}_{O_2}$ in water to such a level that all of the dives of gentoo penguins would be within their cADL. This is very pertinent to the present discussion because, like the king penguin, over 20% of the dives of gentoo penguins exceed their cADL, even if $\dot{V}_{O_2}$ during diving is assumed to be the same as that when resting in water (Bevan et al., 2002).

List of symbols and abbreviations

ADL	aerobic dive limit
AOP	active oxygen pulse
BMR	basal metabolic rate
cADL	calculated aerobic dive limit
DLT	diving lactate threshold
DLW	doubly labelled water
ECG	electrocardiogram
f_H	heart rate
FMR	field metabolic rate
FMR$_{est}$	estimated field metabolic rate
HRDDL	heart rate and depth data logger
MR	metabolic rate
MR$_{est}$	estimated metabolic rate
OP	oxygen pulse
ROP	resting oxygen pulse
RQ	respiratory quotient
S.E.E.	standard error of the estimate
S.E.M.	standard error of the mean
TEB	time–energy budget
$\dot{V}_{O_2}$	rate of oxygen consumption
$\dot{V}_{O_2est}$	estimated rate of oxygen consumption

References

Barré, H. (1980). Etude du métabolisme énergétique du Manchot royal (*Aptenodytes patagonicus*) dans les conditions ambiantes naturelles. *CR Acad. Sci. Paris D* **291**, 493-495.

Bevan, R. M., Boyd, I. L., Butler, P. J., Reid, K., Woakes, A. J. and Croxall, J. P. (1997). Heart rates and abdominal temperatures of free-ranging South Georgian shags, *Phalocrocorax georgianus*. *J. Exp. Biol.* **200**, 661-675.

Bevan, R. M. and Butler, P. J. (1992). Cardiac output and blood flow distribution during swimming and voluntary diving of the tufted duck (*Athya fuligula*). *J. Exp. Biol.* **168**, 199-217.

Bevan, R. M., Butler, P. J., Woakes, A. J. and Boyd, I. L. (2002). The energetics of gentoo penguins, *Pygoscelis papua*, during the breeding season. *Func. Ecol.* **16**, 175-190.

Bevan, R. M., Butler, P. J., Woakes, A. J. and Prince, P. A. (1995). The energy expenditure of free-ranging black-browed albatrosses. *Phil. Trans. R. Soc. Lond. B* **350**, 119-131.

Bost, C.-A., Georges, J. Y., Guinet, C., Cherel, Y., Putz, K., Charrassin, J. B., Handrich, Y., Zorn, T., Lage, J. and Le Maho, Y. (1997). Foraging habitat and food intake of satellite-tracked king penguins during the austral summer at Crozet Archipelago. *Mar. Ecol. Prog. Ser.* **150**, 21-33.

Boyd, I. A. (1996).Temporal scales of foraging in a marine predator. *Ecology* **77**, 426-434.

Boyd, I. L., Arnould, J. P. Y., Barton, T. and Croxall, J. P. (1994). Foraging behaviour of Antarctic fur seals during periods of contrasting prey abundance. *J. Anim. Ecol.* **63**, 703-713.

Boyd, I. L., Bevan, R. M., Woakes, A. J. and Butler, P. J. (1999). Heart rate and behavior of fur seals: implications for measurement of field energetics. *Am. J. Physiol.* **276**, H844-H857.

Butler, P. J. (1993). To what extent can heart rate be used as an indicator of metabolic rate in free-living marine mammals? *Symp. Zool. Soc. Lond.* **66**, 317-332.

Butler, P. J. (2000). Energy cost of surface swimming and diving of birds. *Physiol. Biochem. Zool.* **73**, 699-705.

Butler, P. J. (2004). Metabolic regulation in diving birds and mammals. *Resp. Physiol. Neurobiol.* **141**, 297-315.

Butler, P. J., Woakes, A. J. and Bishop, C. M. (1998). The behaviour and physiology of Svalbard barnacle geese, *Branta leucopsis*, during their autumn migration. *J. Avian Biol.* **29**, 536-545.

Butler, P. J., Green, J. A., Boyd, I. L. and Speakman, J. R. (2004). Measuring metabolic rate in the field: the pros and cons of the doubly-labelled water and heart rate methods. *Func. Ecol.* **18**, 168-183.

Butler, P. J. and Jones, D. R. (1997). Physiology of diving of birds and mammals. *Physiol. Rev.* **77**, 837-899.

Butler, P. J. and Woakes, A. J. (1984). Heart rate and aerobic metabolism in humboldt penguins, *Spheniscus humboldti*, during voluntary diving. *J. Exp. Biol.* **108**, 419-428.

Charrassin, J.-B., Bost, C.-A., Pütz, K., Lage, J., Dahier, T., Zorn, T. and Le Maho, Y. (1998). Foraging strategies of incubating and brooding king penguins *Aptenodytes patagonicus*. *Oecologia* **114**, 194-201.

Chastel, O., Weimerskirch, H. and Jouventin, P. (1993). High annual variability in reproductive success and survival of an Antarctic seabird, the Snow Petrel *Pagodroma nivea*. *Oecologia* **94**, 278-285.

Cherel, Y. and Ridoux, V. (1992). Prey species and nutritive value of food fed during summer to king penguin *Aptenodytes patagonica* chicks at Possession Island, Crozet Archipelago. *Ibis* **134**, 118-127.

Croxall, J. P. (1982). Energy cost of incubation and moult in petrels and penguins. *J. Anim. Ecol.* **51**, 177-194.

Croxall, J. P. (1984). Seabirds. In *Antarctic Ecology*, vol. 2 (ed. R. M. Laws), pp. 533-620. London: Academic Press.

Croxall, J. P., Reid, K. and Prince, P. A. (1999). Diet, provisioning and productivity responses of marine predators to differences in availability of Antarctic krill. *Mar. Ecol. Prog. Ser.* **177**, 115-131.

Culik, B. M., Pütz, K., Wilson, R. P., Allers, D., Lage, J., Bost, C.-A. and Le Maho, Y. (1996). Diving energetics in king penguins (*Aptenodytes patagonicus*). *J. Exp. Biol.* **199**, 973-983.

Ellis, H. I. (1984). Energetics of free-ranging seabirds. In *Seabird Energetics* (ed. G. C. Whittow and H. Rahn), pp. 203-234. New York: Plenum Press.

Fahlman, A., Handrich, Y., Woakes, A. J., Bost, C.-A., Holder, R., Duchamp, C. and Butler, P. J. (2004). The effect of fasting on the $\dot{V}_{O_2}$ and f_H relationship in king penguins, *Aptenodytes patagonicus*. *Am. J. Physiol* **287**, R870-R877.

Froget, G., Butler, P. J., Handrich, Y. and Woakes, A. J. (2001). Heart rate as an indicator of oxygen consumption: influence of body condition in the king penguin. *J. Exp. Biol.* **204**, 2133-2144.

Froget, G., Gautier-Clerc, M., Le Maho, Y. and Handrich Y. (1998). Is penguin banding harmless? *Polar Biol.* **20**, 409-413.

Froget, G., Handrich, Y., Le Maho, Y., Rouanet, J.-L., Woakes, A. J. and Butler, P. J. (2002). The heart rate–oxygen consumption relationship during cold exposure of king penguins: a comparison with that during exercise. *J. Exp. Biol.* **205**, 2511-2517.

Green, J. A., Butler, P. J., Woakes, A. J., Boyd, I. L. and Holder, R. I. (2001). Heart rate and rate of oxygen consumption of exercising macaroni penguins. *J. Exp. Biol.* **204**, 673-684. [Erratum: *J. Exp. Biol.* **206**, 3113.]

Green, J. A., Butler, P. J., Woakes, A. J. and Boyd, I. L. (2003). Energetics of diving in macaroni penguins. *J. Exp. Biol.* **206**, 43-57.

Handrich, Y., Bevan, R. M., Charrassin, J.-B., Butler, P. J., Pütz, K., Woakes, A. J., Lage, J. and Le Maho, Y. (1997). Hypothermia in foraging king penguins. *Nature* **388**, 64-67.

Huntley, M. E., Lopez, M. D. and Karl, D. M. (1991). Top predators in the Southern ocean: a major leak in the biological carbon pump. *Science* **253**, 64-66.

Kooyman, G. L. (1989). *Diverse Divers*. Berlin: Springer-Verlag.

Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnett, E. E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J. Comp. Physiol.* **138**, 335-346.

Kooyman, G. L., Cherel, Y., Le Maho, Y., Croxall, J. P., Thorson, P. H., Ridoux, V. and Kooyman, C. A. (1992a). Diving behavior and energetics during foraging cycles in king penguins. *Ecol. Monogr.* **62**, 143-163.

Kooyman, G. L., Ponganis, P. J., Castellini, M. A., Ponganis, E. P., Ponganis, K. V., Thorson, P. H., Eckert, S. A. and Le Maho, Y. (1992b). Heart rate and swim speeds of emperor penguins diving under sea ice. *J. Exp. Biol.* **165**, 161-180.

Le Maho, Y. (1994). New perspectives for research on Antarctic birds and mammals. *Polar Biol.* **14**, 315-318.

Le Maho, Y. and Despin, B. (1976). Réduction de la dépense énergétique au cours du jeûne chez le manchot royal *Aptenodytes Patagonicus*. *CR Acad. Sci. Paris D* **283**, 979-982.

Le Maho, Y., Gendner, J.-P., Challet, E., Bost, C.-A., Gilles, J., Verdon, C., Plumeré, C., Robin, J.-P. and Handrich, Y. (1993). Undisturbed penguins as indicators of changes in marine resources. *Mar. Ecol. Prog. Ser.* **94**, 1-6.

Millard, R. W., Johansen, K. and Milsom, W. K. (1973). Radiotelemetry of cardiovascular responses to exercise and diving in penguins. *Comp. Biochem. Physiol.* **46A**, 227-240.

Montevecchi, W. A. (1993). Birds as indicators of change in marine prey stocks. In *Birds as Monitors of Environmental Change* (ed. R.W. Furness and J. J. D. Greenwood), pp. 217-266. London: Chapman and Hall.

Ponganis, P. J., Kooyman, G. L., Van Dam, R. and Le Maho, Y. (1999). Physiological responses of king penguins during simulated diving to 136 m depth. *J. Exp. Biol.* **202**, 2819-2822.

Ponganis, P. J., Van Dam, R. P., Levensen, D. H., Knower, T., Ponganis, K. V. and Marshall, G. (2003). Regional heterothermy and conservation of core temperature in emperor penguins diving under ice. *Comp. Biochem. Physiol.* **135A**, 477-487.

Sato, K., Naito, Y., Kato, A., Niizuma, Y., Watanuki, Y., Charrassin, J.-B., Bost, C. A., Handrich, Y. and Le Maho, Y. (2002). Buoyancy and maximal diving depth in penguins: do they control inhaling air volume? *J. Exp. Biol.* **205**, 1189-1197.

Schmidt-Nielsen, K. (1997). *Animal Physiology*, 5th edn. Cambridge and New York: Cambridge University Press.

Scholander, P. F. (1940). Experimental investigation on the respiratory function in diving mammals and birds. *Hvalradsets Skr. Nor. Vidensk*, **22**, 1-131.

Speakman, J. R. (1998). The history and theory of the doubly labeled water technique. *Am. J. Clin. Nutr.* **98**, 932S-938S.

Woakes, A. J. and Butler, P. J. (1983). Swimming and diving in tufted ducks, *Aythya fuligula*, with particular reference to heart rate and gas exchange. *J. Exp. Biol.* **107**, 311-329.

Woakes, A. J., Butler, P. J. and Bevan, R. M. (1995). Implantable data logging system for heart rate and body temperature: Its application to the estimation of field metabolic rates in Antarctic predators. *Med. Biol. Eng. Comp.* **33**, 145-151.

Wilson, R. P., Pütz, K., Bost, C.-A., Culik, B. M., Bannasch, R., Reins, T. and Adelung, D. (1993). Diel dive depth in penguins in relation to diel vertical migration of prey: Whose dinner by candlelight? *Mar. Ecol. Prog. Ser.* **94**, 101-104.

Zar, J. H. (1999). *Biostatical Analysis*. 4th edn. Upper Saddle River, New Jersey: Prentice Hall International, Inc.