Energetics of diving in macaroni penguins

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Summary

Heart rate (f_H), abdominal temperature (T_{ab}) and diving depth were measured in thirteen free-ranging breeding female macaroni penguins. Measurement of these variables allowed estimation of the mass-specific rate of oxygen consumption ($\dot{V}_{O_2}$) while diving and investigation of the physiological adjustments that might facilitate the diving behaviour observed in this species. In common with other diving birds, macaroni penguins showed significant changes in f_H associated with diving, and these variables accounted for 36% of the variation in dive duration. When $\dot{V}_{O_2}$ was calculated for dives of different durations, 95.3% of dives measured were within the calculated aerobic dive limit (cADL) for this species. Mean f_H for all complete dive cycles was 147 ± 6 beats min^{-1}. When this f_H is used to estimate $\dot{V}_{O_2}$ of 26.2 ± 1.4 ml min^{-1} kg^{-1} then only 92.8% of dives measured were within the cADL. Significant changes in abdominal temperature were not detected within individual dives, though the time constant of the measuring device used may not have been low enough to record these changes if they were present. Abdominal temperature did decline consistently during bouts of repeated diving of all durations and the mean decrease in T_{ab} during a diving bout was $2.32\pm0.2°$C. There was a linear relationship between bout duration and the magnitude of this temperature drop. There was no commensurate increase in dive duration during dive bouts as T_{ab} declined, suggesting that macaroni penguins are diving within their physiological limits and that factors other than T_{ab} are important in determining the duration of dives and dive bouts. Lowered T_{ab} will in turn facilitate lower metabolic rates during diving bouts, but it was not possible in the present study to determine the importance of this energy saving and whether it is occurs actively or passively.

Key words: energetics, diving, macaroni penguin, heart rate, abdominal temperature, rate of oxygen consumption, calculated aerobic dive limit.

Introduction

Penguins are among the most accomplished of divers. Numerous studies of their diving behaviour have shown that penguins have remarkable dive performances (Kooyman et al., 1992a; Williams, T. D. et al., 1992; Bengston et al., 1993). The emperor penguin *Aptenodytes forsteri*, the largest species at 25–30 kg, can reach depths of 524 m (Kooyman and Kooyman, 1995) for durations of up to 22 min (Robertson, 1994). Even the considerably smaller (3–4 kg) Adélie penguin *Pygoscelis adéliae* can dive to 98 m for up to 160 s (Wilson et al., 2002).

Further investigations examined how physiological and behavioural adjustments might permit such impressive diving behaviour (Butler and Jones, 1997; Kooyman and Ponganis, 1998). The extent to which diving animals balance the use of aerobic and anaerobic metabolism during natural dives is unclear. The majority of evidence suggests, however, that most dives are essentially aerobic (Butler and Jones, 1997). Anaerobic metabolism may be used in some circumstances (Kooyman et al., 1980; Ydenberg and Clark, 1989; Carbone and Houston, 1996; Mori, 1998; Butler, 2001), but within any

dive there must be oxygen available for the central nervous system (CNS), heart and active muscles, even after lactate begins to accumulate. Observations of diving behaviour confirm that most dives are within bouts of repeated diving with relatively low ratios of post-dive surface interval duration to dive duration (dive:pause ratio).

The aerobic dive limit (ADL), the diving duration beyond which post-dive blood lactate levels increase above resting values, was first determined experimentally in Weddell seals (Kooyman et al., 1980) and defined by Kooyman et al. (1983). Since then, ADL or diving lactate threshold (DLT; Butler and Jones, 1997) has been determined in two more species of seal (Ponganis et al., 1997a,c) under captive conditions and in freely diving emperor penguins (Ponganis et al., 1997b) and bottlenose dolphins (Williams, T. M. et al., 1999). In emperor penguins the DLT was 5–7 min, which agreed quite closely with an ADL of 8 min estimated from observations of natural diving behaviour (behavioural ADL; Kooyman and Kooyman, 1995). This behavioural ADL was calculated as the dive

duration above which recovery times at the surface were proportionately longer in duration, suggesting that dives had a substantial anaerobic component. Only 4% of natural dives exceeded this behavioural ADL, therefore it was concluded that most diving was aerobic.

ADL has also been calculated (cADL) for many diving animals, including several penguin species, by dividing an estimate of usable body oxygen stores by an estimate of the rate of oxygen consumption ($\dot{V}_{O_2}$) while submerged (Butler and Jones, 1997). When compared to observed patterns of diving in different penguin species, these studies have found that 2–50% of dives exceed the cADL (Culik et al., 1994, 1996a; Boyd and Croxall, 1996; Bethge et al., 1997; Bevan et al., 2002; Wilson et al., 2002). In these studies, examination of the dive:pause ratio suggests that it is unlikely that so many dives use predominantly anaerobic metabolism. In order for a large proportion of natural dives by many species of penguins to be aerobic, the cADL must be greater. Both usable oxygen stores and $\dot{V}_{O_2}$ are difficult to measure while submerged, and other pathways such as the metabolism of phosphocreatine might provide energy under these conditions (Butler and Jones, 1997). Submerged $\dot{V}_{O_2}$ is particularly difficult to measure (Costa, 1988). If estimates of the usable oxygen stores for penguins are approximately correct, then $\dot{V}_{O_2}$ during diving needs to be as low as that recorded from penguins at rest on the water surface for most dives to be within the cADL (Butler, 2000).

In the present study we measured heart rate (f_H), abdominal temperature (T_{ab}) and depth in macaroni penguins *Eudyptes chrysolophus* diving freely while foraging in their natural environment, using purpose-built implantable data loggers (Woakes et al., 1995). Heart rate can be used to estimate $\dot{V}_{O_2}$ in diving animals (Fedak, 1986; Bevan et al., 1992; Butler, 1993) and a relationship between heart rate and $\dot{V}_{O_2}$ has been established for macaroni penguins (Green, J. A. et al., 2001). This approach allows us to consider the effects of the suite of physiological and behavioural adaptations that have been found to contribute to the maximising of cADL while submerged. These adaptations include variation of heart rate and circulation (Butler and Woakes, 1979; Fedak et al., 1988; Kooyman et al., 1992b; Davis and Kanatous, 1999), regional hypothermia (Bevan et al., 1997, 2002; Handrich et al., 1997) and the use of passive gliding during the ascent and descent phases of dives (Williams, T. M. et al., 1999, 2000). Thus these measurements enabled us to relate the energetic costs and physiological responses to diving with the observed patterns of diving behaviour.

The present study, therefore, had four main aims: (1) to estimate from heart rate the energy cost of free-ranging diving behaviour in macaroni penguins, (2) to determine if macaroni penguins dive within their cADL and establish therefore whether they predominantly use aerobic respiration, (3) to examine heart rate changes on a fine scale (measured every 2 s) in order to assess whether circulatory adjustments made during diving might extend dive duration (Butler and Jones, 1997; Davis and Kanatous, 1999), (4) to measure abdominal

temperature and investigate the hypothesis that lowered body temperature contributes to the extension of diving duration (Culik et al., 1996b; Handrich et al., 1997; Bevan et al., 2002).

Materials and methods
Study animals

The study was undertaken at the British Antarctic Survey (BAS) base on Bird Island, South Georgia during the austral summer of 1998/99. We followed the requirements of the UK Animal (Scientific Procedures) Act 1986, especially those set out by the Home Office in the Official Guidance on the operation of the Act. As our benchmark, we followed guidance to researchers using similar methods in the UK. Our procedures also conformed to the Code of Ethics of Animal Experimentation in Antarctica. The macaroni penguins used in the study were breeding females from the colony at Fairy Point on the north side of the island. The population at this colony has been monitored for many years (Williams, T. D. and Croxall, 1991) and has also been the subject of more intensive studies (Davis et al., 1983, 1989; Croxall et al., 1988, 1993, 1997; Williams, T. D., 1989). 15 penguins *Eudyptes chrysolophus* Brandt were used in the present study, all of which were engaged in provisioning a growing chick. Where possible, birds were caught for implantation away from the nesting area of the colony after they had fed their chick. After capture, the birds were removed to the surgical facility and kept in an outdoor enclosure for 2–3 h before the surgery to allow digestion of food.

Implantation procedure

Implantation of the data logger into the abdominal cavity allows data to be recorded without compromising the swimming, foraging and breeding performance of animals, as has been observed with the use of externally mounted devices on the morphometrically identical royal penguin (Hull, 1997). The implantation procedure was basically the same as described for similar studies (Bevan et al., 1995a). Briefly, the sterilised data logger was implanted into the abdominal cavity *via* a mid-line incision made in the skin and body wall muscle in the brood patch while the bird was anaesthetised with halothane. The logger design incorporates a low power radio frequency transmitter, which emits a short pulse on each QRS wave of the electrocardiogram (ECG). Detection of this signal on a radio receiver was used to indicate when the data logger was in the correct position. Once in position, the body wall and skin were sutured, antibiotic powder (Woundcare, Animalcare Ltd, York, UK) applied to the wound and a long-acting antibiotic (LA Terramycin, Pfizer, Sandwich, UK) and analgesic (Vetergesic, Reckitt and Colman Products Ltd, Hull, UK) injected intramuscularly. Aseptic conditions were maintained wherever possible. The time at which the data logger was implanted was noted to the nearest second.

All birds were weighed immediately before surgery using a spring balance (10±0.1 kg, Pesola, Switzerland) and a passive implantable transponder (PIT) tag, mounted on a plastic cable

tie, was secured around their ankle. Birds were put into a large darkened box to recover from the surgery. Once the birds were alert and responsive, usually after 1–2 h, they were returned to the colony where behaviour varied between individuals. Some would go swimming within a few hours, whereas others made their way to the nest site or stood alone elsewhere in the colony. Around the time at which the data logger memory was predicted to be full, implanted birds were recaptured after returning from a foraging trip and having fed their chicks. The data logger was removed using the same procedure as during implantation, and the bird was released back in to the colony once it had recovered.

Heart rate data loggers

The data loggers could record heart rate, hydrostatic pressure (diving depth) and abdominal temperature every 2 s and, at this sampling rate, could store data over 30.3 days. Before use, the devices were encased in paraffin wax and encapsulated in silicon rubber to provide waterproofing and biocompatability. The hydrostatic pressure sensor in the data logger could detect diving depth to within 1.2 m. The temperature sensor of the encapsulated data logger was calibrated by immersing the device in water baths of known temperature. This procedure was also used to determine the time constant (τ) of the temperature sensor, which was 74 s. Unfortunately, given the relatively short dive durations of macaroni penguins, this meant that changes in abdominal temperature could only be analysed within diving bouts, not within individual dives. The time of removal of the data logger was noted and the precise times of implantation and removal were later used to establish the time base of the data downloaded from the data logger. The heart rate, abdominal temperature and depth data from within the data logger memory were downloaded onto a computer (Acorn RISC PC) using purpose-designed software.

Data analysis

The data were prepared and analysed using purpose-written computer programs within the SAS statistical package (version 6.11, SAS institute) on a UNIX workstation. Further analyses were performed with the statistical packages Minitab 12 (Minitab Inc.), SPSS 10.0.8 (SPSS) and Excel 97 (Microsoft). The recovery period following the implantation procedure (Bevan et al., 2002) was excluded from the analysis by ignoring data collected during the period from implantation to the start of the first foraging trip. In the present study the duration of this period was 55.5±5 h (mean ± S.E.M.).

Time at-sea on foraging trips was estimated from the depth data, supported with data from field observations and a PIT tag recorder (FSI Ltd, Cambridge, UK) situated in a gate at the edge of the colony. Each record of heart rate, abdominal temperature and dive depth was also marked with the daylight conditions (light or dark). These were calculated using the times for civil sunrise and sunset calculated for the longitude and latitude of Bird Island (54°00′S, 38°02′W). In examining dive records, dives with maximum depths of <2.4 m were ignored during analyses, since wave action and recorder noise degraded depth accuracy for shallower dives. In all analyses, dives were treated as independent events. While accepting that this assumption may not be strictly correct, it is necessary in order to perform further statistical analyses.

A dive cycle was defined as a dive and the following interval spent at the water surface prior to the next dive. Bouts of dives were defined following the iterative statistical method of Boyd et al. (1994), which relies on searching the dive sequence for a change in behaviour that differs significantly from the previous set of behaviours since the last significant change. A minimum dive bout was formally defined as a group of at least three dives occurring within a period of 10 min. The dive record for each penguin was searched sequentially from the start, and once a group of dives had satisfied this minimum requirement, a search was made through the subsequent dives to find the end of the diving bout. This was done by calculating the mean and standard deviation (S.D.) of the surface intervals between dives, within the diving bout, and comparing these with the next surface interval in the sequence. If the next surface interval was significantly greater than the previous surface intervals in the bout (*t*-test, *P*<0.01) then the bout was deemed to have ended. If the duration of the surface interval was not significantly different from those in the current bout, then the dive was included within the bout, the mean ± S.D. of the surface intervals for the bout were recalculated, and the analysis then moved onto the next dive in the sequence.

The f_H data were used to estimate mass specific rate of oxygen consumption, $\dot{V}_{O_2}$, using the relationship obtained from macaroni penguins walking on a treadmill (Green, J. A. et al., 2001). For breeding female penguins, which were the subjects of the present study, the equation was:

$$\dot{V}_{O_2} = (0.297 \times f_H) - 17.40, \tag{1}$$

$r^2 = 0.84$, $\dot{V}_{O_2}$ is in ml min^{-1} kg^{-1} at standard temperature and pressure, dry (STPD), and f_H is in beats min^{-1}.

This technique is normally calibrated when the animals' metabolism is in steady state and hence cannot be used to estimate $\dot{V}_{O_2}$ while the animal is submerged. However, if f_H and $\dot{V}_{O_2}$ are averaged over a number of complete dive/surface cycles, then f_H is an accurate and reliable predictor of $\dot{V}_{O_2}$ in aquatic birds and mammals (Fedak, 1986; Bevan et al., 1992; Butler, 1993). The S.D. of an estimate made using Equation 1 was calculated using equation 11 of Green et al. (2001), which includes the variability within and between calibration and field animals, and is quoted in the text where estimates have been made.

Oxygen stores have not been measured in macaroni penguins, or indeed any of the crested penguins, but have been calculated for other species of penguins (Kooyman, 1989; Kooyman and Ponganis, 1990; Chappell et al., 1993; Bethge et al., 1997), usually following the assumptions of Stephenson et al. (1989) and Croll et al. (1992). These studies have detected differences between species and within species between different studies. However, the range of estimates is not large, varying from 45 ml O$_2$ kg^{-1} in little blue penguins

Table 1. *Deployment details and simple parameters of diving for 13 breeding female macaroni penguins from which data were obtained*

Bird	Mass (kg)	Duration of data record (days)	Proportion of time at-sea	Proportion of time submerged while at-sea	Number of dives recorded
H02	3.6	8.26	0.61	0.61	2656
H15	3.3	12.95	0.80	0.58	6367
H17	3.1	4.65	0.78	0.58	1926
H25	3.9	11.56	0.69	0.56	4466
H29	3.6	6.17	0.79	0.47	2341
H53	3.3	28.01	0.49	0.52	7182
H59	4.0	1.29	0.83	0.48	466
H61	3.4	6.29	0.70	0.50	2339
H69	3.8	4.18	0.73	0.17	639
H73	3.8	3.48	0.54	0.42	857
H79	4.1	26.81	0.51	0.58	6932
H93	3.6	9.09	0.54	0.62	3316
H95	3.4	13.26	0.58	0.64	4535
Mean ± S.E.M.	3.6±0.3	10.46±2.32	0.67	0.52	3386±649

(Bethge et al., 1997) to $63\,ml\,O_2\,kg^{-1}$ for Adélie penguins (Culik et al., 1994). In the present study it was not possible to collect the data necessary to calculate oxygen stores for macaroni penguins, so a value of $58\,ml\,O_2\,kg^{-1}$ was used, which is in the middle of the range of most of the calculated values for other species and has been used previously as an estimate to compare different penguin species (Butler, 2000). Stephenson et al. (1989) discuss the influence of training on the composition of oxygen stores but there is no reason to assume that the birds in the present study were not fit and acclimated for intensive diving.

Data were analysed using analysis of variance (ANOVA) with Tukey *post-hoc* testing, linear regression and stepwise multiple linear regression. Results were considered significant at $P<0.05$ and the significance level is quoted in the text. Unless stated otherwise, mean values are the grand mean of the mean value for each penguin and are ± 1 S.E.M. Percentage values were arcsine-transformed before comparisons were made (Zar, 1999). All times are given in local time (GMT $-3\,h$) unless otherwise stated.

Results

Deployments, diving behaviour and dive bouts

Data were obtained from 13 penguins. Failure in the encapsulation led to battery failure in the other two deployments. Table 1 shows details of the 13 birds from which data were obtained. Diving activity was greater during daylight (Fig. 1), when dives were deeper (two-way ANOVA, $F_{23,276}=45.19$, $P<0.001$), more frequent (two-way ANOVA, $F_{23,276}=15.43$, $P<0.001$) and of longer duration (two-way ANOVA, $F_{23,276}=51.38$, $P<0.001$).

When dives were classified into bouts, 98% of all dives were part of a bout consisting of at least three dives (Table 2). Only dives within bouts were considered for further analyses. When considering post-dive surface intervals, the last dive of
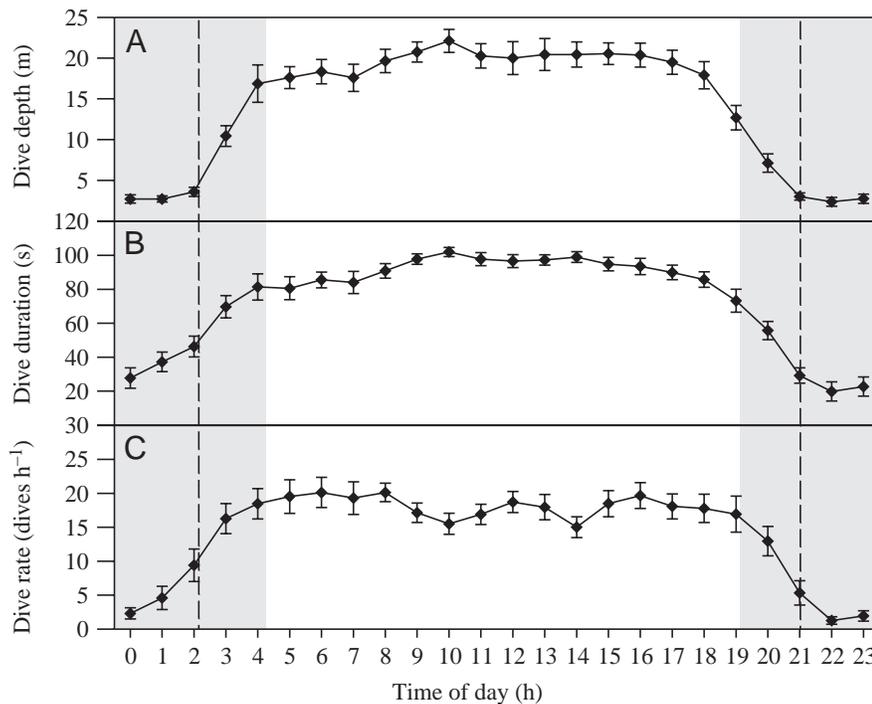

Fig. 1. Influence of time of day on (A) dive depth, (B) dive duration and (C) dive rate, recorded from 13 breeding female macaroni penguins. All values are means ± S.E.M. Shaded areas indicate hours of darkness, and broken vertical lines indicate dawn and dusk at the beginning of the season.

a bout was discarded. Individual distributions of both dive depth and duration were not normal, so Kruskal–Wallis tests with Dunn's multiple comparisons were used to examine differences between individuals. There were significant differences between individuals in both dive depth (Kruskal–Wallis statistic$_{(13)}$=964.3, $P<0.001$) and duration (Kruskal–Wallis statistic$_{(13)}$=1088, $P<0.001$) (Table 2). Fig. 2 shows the mean frequency distributions of dive depth and duration, calculated by taking an average of the individual frequencies of occurrence of each dive depth or duration interval from all 13 penguins. These distributions were not substantially different from those of all dives from all penguins but this approach treats all individuals equally, despite large differences in the number of dives recorded from individual penguins (Table 2). 21% of all dives were to a maximum depth of 4.8 m (Fig. 2A), with declining frequencies to 94.8 m, the maximum dive depth recorded. This dive was recorded by penguin H79, which was responsible for most of the deeper and longer dives, including all those deeper than 70 m. Dive durations were more normally distributed (Fig. 2B), though slightly negatively skewed.

Abdominal temperature during diving

The mean T_{ab} while on-shore was 40.1±0.9°C, and the mean T_{ab} during diving bouts and while at-sea but not diving were 34.8±1.2°C and 38.2±1.0°C, respectively. Two-way analysis of variance with Tukey *post-hoc* testing ($F_{2,38}$=31.6, $P<0.001$) revealed significant differences between all three measurements of T_{ab}. Further analyses were performed to investigate the decrease in T_{ab} associated with diving and what effect it might have in improving diving performance. Average diving temperature (DT_{ab}) was calculated for each dive as the mean temperature while submerged. Linear regressions were used to determine whether DT_{ab}, dive duration and mean diving f_H varied progressively during the course of each diving bout (Table 3). 63.4% of all dive bouts showed a significant change in DT_{ab} through the course of the bout and 76.2% of these (i.e. 48.3% of all dive bouts) were significant declines, with a mean r^2 of
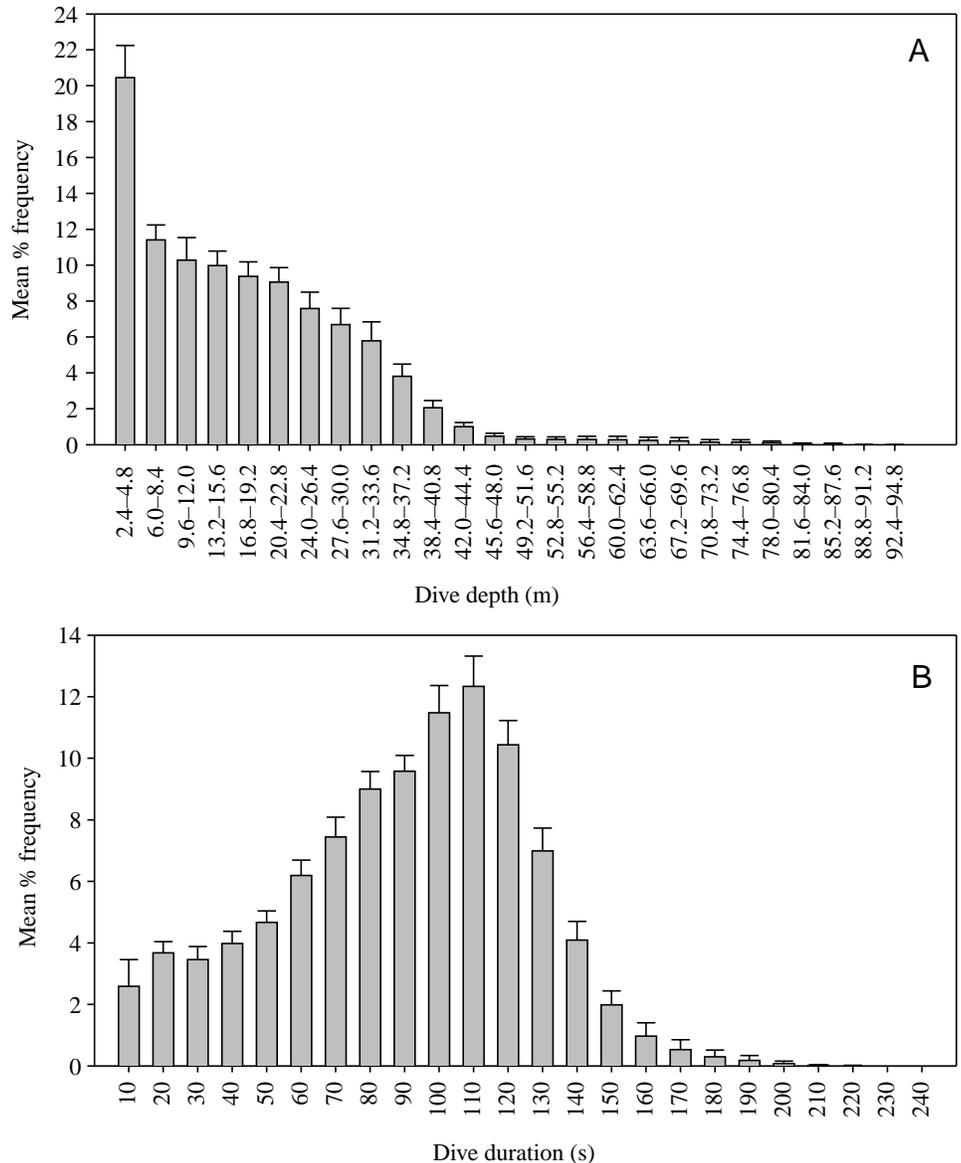

Fig. 2. Mean frequency distributions from 13 breeding female macaroni penguins of (A) dive depth and (B) dive duration. All dives were within bouts of three or more dives. Values are means + S.E.M.

0.76 (Table 3). However, only 35.0% and 35.4% of bouts showed a significant change in dive duration and f_H, respectively, over the course of the bout, and the average r^2 of these relationships was only 0.37 and 0.34, respectively. The decline in T_{ab} (ΔT_{ab}) during each dive bout was calculated as the difference between the maximum and minimum values of DT_{ab} from that bout. Mean ΔT_{ab} from all 13 penguins was 2.32±0.20°C, range 0–13.51±1.1°C. ΔT_{ab} increased with the duration of the diving bout for each individual (mean r^2=0.55, all $P<0.001$) and for all diving bouts pooled (r^2=0.46, $P<0.001$, Fig. 3).

Heart rate and rate of oxygen consumption while diving

Mean heart rates while the penguins were on-shore and at-

Table 2. *Characteristics of diving behaviour within diving bouts in 13 breeding female macaroni penguins*

Bird	Number of diving bouts	Number of dives			Dive depth (m)				Dive duration (s)			
		Within bouts	Not within bouts	Average per bout	Mean	Mode	Median	Maximum	Mean	Mode	Median	Maximum
H02	53	2615	41	49.34	21.7±0.3	7.2	19.2	69.6	100.3±0.7	124	102	180
H15	171	6251	116	36.56	17.3±0.1	2.4	14.4	57.6	81.4±0.4	78	82	224
H17	43	1879	47	43.70	18.1±0.2	15.6	16.8	45.6	95.4±0.7	108	102	164
H25	141	4371	95	31.00	19.0±0.2	2.4	18.0	60.0	88.1±0.5	112	96	168
H29	66	2281	60	34.56	18.3±0.2	2.4	20.4	42.0	85.6±0.6	102	94	142
H53	186	7091	91	38.12	14.9±0.1	2.4	12.0	69.6	84.5±0.4	109	86	178
H59	24	456	10	19.00	13.0±0.4	2.4	10.8	54.0	95.4±1.4	109	98	186
H61	66	2262	77	34.27	16.2±0.2	2.4	14.4	51.6	83.5±0.7	114	90	156
H69	20	618	21	30.90	18.1±0.5	2.4	19.2	44.4	68.6±1.6	104	82	180
H73	35	845	12	24.14	14.2±0.4	2.4	12.0	44.4	80.3±1.1	100	84	172
H79	216	6814	118	31.55	23.4±0.3	2.4	13.2	94.8	95.4±0.6	58	92	232
H93	71	3277	39	46.15	16.1±0.2	2.4	15.6	46.8	79.8±0.6	68	82	174
H95	98	4484	51	45.76	17.5±0.1	2.4	18.0	58.8	87.8±0.5	112	92	174
Mean ± S.E.M.	91.54±19.20	3326±640	59.85±10.24	35.77±2.46	17.5±0.8	3.8±1.0	15.7±0.9	56.9±4.1	86.6±2.4	99.8±5.4	90.9±2.0	179.2±6.8

Table 3. *Characteristics of the linear regressions describing changes in abdominal temperature, heart rate and dive duration within diving bouts*

Variable	Dive bouts with decreasing abdominal temperature, heart rate or dive duration			Dive bouts with increasing abdominal temperature, heart rate or dive duration			Bouts with no change in abdominal temperature, heart rate or dive duration		
	Mean proportion of bouts (%)	Mean r^2	Number of dives/bout (mean ± S.E.M.)	Mean proportion of bouts (%)	Mean r^2	Number of dives/bout (mean ± S.E.M.)	Mean proportion of bouts (%)	Mean r^2	Number of dives/bout (mean ± S.E.M.)
Abdominal temperature	48.3	0.76±0.04	54±4	15.1	0.64±0.04	27±3	36.6	0.33±0.03	14±1
Heart rate	12.0	0.31±0.04	72±9	23.31	0.36±0.02	60±7	64.6	0.16±0.01	21±1
Dive duration	16.1	0.36±0.02	80±6	18.9	0.38±0.03	45±4	65.0	0.02±0.01	22±2

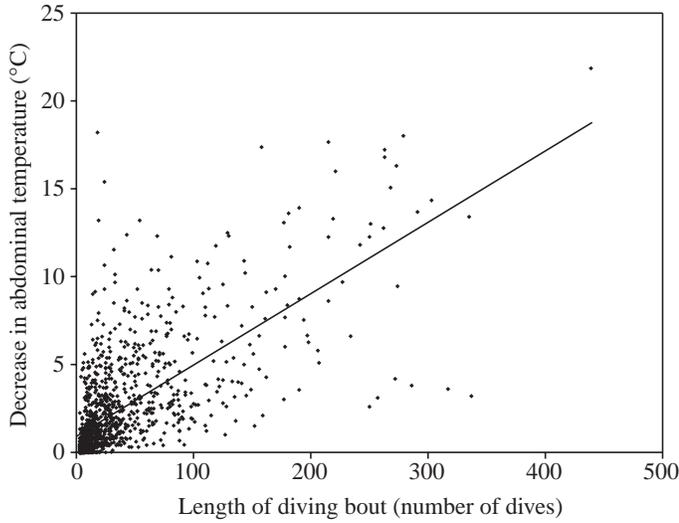Fig. 3. Relationship between the drop in abdominal temperature (ΔT_{ab}) and duration of diving bouts recorded from 13 breeding female macaroni penguins. $y=0.04x+0.91$.

sea were 116 ± 6 and 148 ± 7 beats min^{-1}, respectively. While the penguins were at-sea, mean heart rate during diving bouts (Df_H) was 147 ± 6 beats min^{-1}, whereas mean heart rate while the birds were at-sea but not diving (NDf_H), calculated from f_H between diving bouts, was 154 ± 8 beats min^{-1}. Two-way ANOVA with Tukey *post-hoc* testing ($F_{2,38}=38.1$, $P<0.001$) showed that Df_H was not significantly different from NDf_H, but both were significantly greater than f_H while on-shore. During the dive cycle, macaroni penguins showed increases and decreases in f_H associated with dives of all durations. The extent of these changes in f_H associated with diving were related to dive duration. Table 4 shows mean, maximum and minimum f_H at different stages of the diving cycle for dives of different durations and for dives of all durations, while Fig. 4 shows how heart rate varied during dives lasting 102–110 s, the most frequently observed category of dive duration (Fig. 2B). A similar pattern was observed in dives of both longer and shorter durations and can be described as follows. (1) Prior to diving, f_H was elevated above Df_H and started to decrease just before submergence. (2) Upon submerging, f_H immediately decreased before recovering slightly. f_H then decreased more slowly to a level below Df_H. (3) At the bottom of the dive f_H tended to stabilise. (4) As the penguin started to ascend to the surface, f_H increased slowly. (5) After the penguin surfaced, f_H then increased more rapidly to a level above Df_H. (6) This high heart rate was usually followed immediately by another dive, if the dive was part of a dive bout, otherwise f_H declined to Df_H. ANOVA showed that if dives of all durations were averaged together, there were significant differences between Df_H and f_H at different stages of the dive cycle (two-way ANOVA, $F_{4,64}=97.8$, $P<0.001$). Further Tukey *post-hoc* tests showed that mean pre-dive and post-dive f_H values were significantly higher than mean Df_H, mean f_H while submerged and minimum f_H while submerged.

Table 4. *Mean, maximum and minimum values of heart rate (f_H) associated with different stages of the diving cycle, for dives of different duration, recorded from 13 breeding female macaroni penguins*

Dive duration (s)	Number of penguins	Number of dives	f_H (beats min^{-1})								
			Pre-dive (mean)	Pre-dive (max)	During dive (mean)	During dive (min, mean)	During first 10 s of dive (min)	Post-dive (mean)	Post-dive (max)	Pre-dive – during dive (max, min)	Pre-dive – during dive (mean)
0–20	13	1913	185±11	242±9	156±8	105±9	107±8	179±10	263±10	30±7	137±8
22–40	13	3048	194±12	255±10	143±8	89±9	107±8	186±11	264±10	51±7	166±8
42–60	13	5253	203±15	269±11	139±7	81±8	109±8	200±14	274±12	65±11	188±8
62–80	13	7111	209±16	273±12	133±5	71±7	105±7	207±16	276±12	76±12	203±9
82–100	13	8206	214±17	278±12	129±4	62±6	95±7	212±17	278±12	84±14	215±9
102–120	13	9005	216±18	281±12	127±4	56±5	89±6	214±18	281±12	89±15	225±10
122–140	13	5162	217±17	289±12	125±3	53±5	93±8	215±16	288±10	91±15	236±11
142–160	12	1562	209±19	284±11	122±4	51±6	91±11	208±19	282±12	87±19	233±9
162–180	7	554	208±26	264±26	119±11	53±8	93±14	266±35	319±25	89±22	211±20
182 +	2	240	210±30	248±8	153±54	47±11	156±60	182±11	283±23	57±24	201±19
All dives	13	42054	208±15	273±10	132±5	67±6	97±7	206±15	275±10	76±12	206±7

Values are means ± S.E.M.
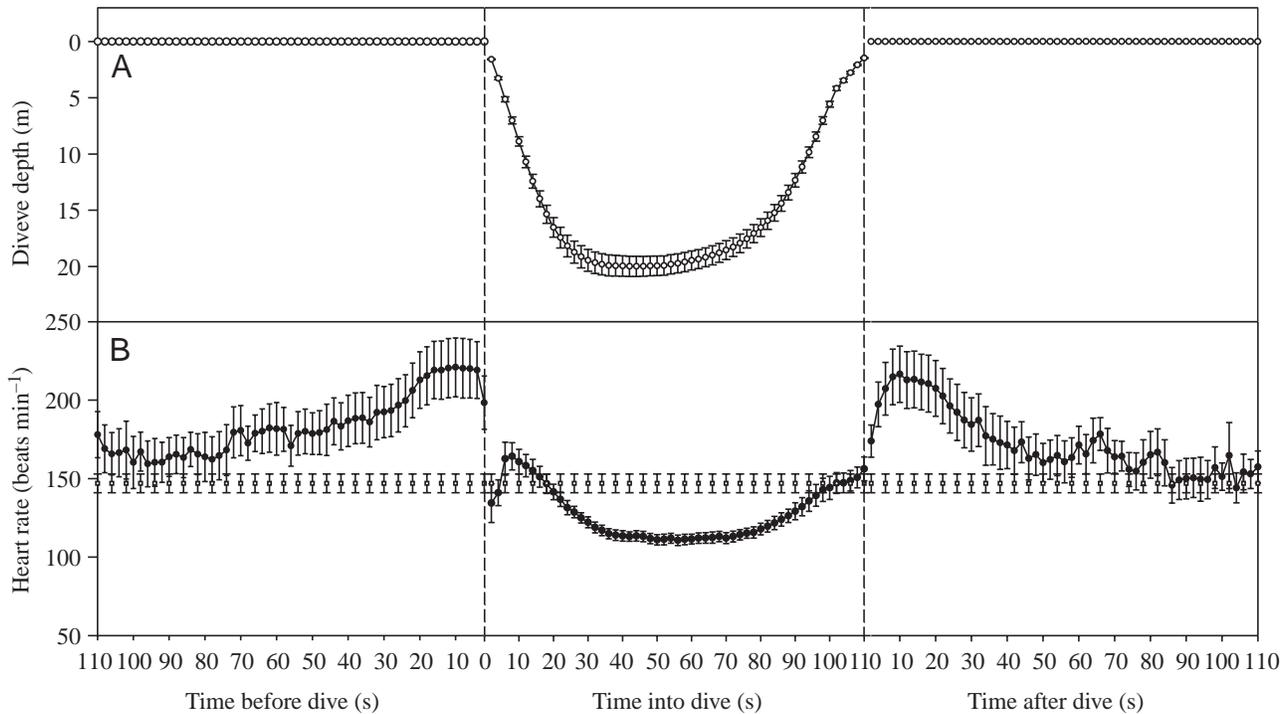Max, maximum; min, minimum.

Fig. 4. (A) Changes in depth during dives of 102–110 s and (B) associated changes in heart rate (filled circles) with overall heart rate during diving bouts (open circles), recorded from 13 breeding female macaroni penguins. Values are means ± S.E.M.

Furthermore, minimum f_H while submerged was significantly lower than Df_H and mean f_H while submerged. There was no significant difference between mean f_H while submerged and Df_H.

In order to investigate further how the marked changes in heart rate during the dive cycle might be related to dive duration, stepwise multiple linear regression analysis was used. Two multiple regressions were performed. In the first analysis, the dependent variable was dive duration and the independent variables were measurements of f_H made during the corresponding dive cycle, thought to be those that characterised the major features of the f_H changes during a dive. These variables were: mean diving f_H, minimum diving f_H, minimum f_H within the first 10 s of submersion, pre-dive mean f_H, pre-dive maximum f_H, post-dive mean f_H and post-dive maximum f_H. The analysis was performed for each penguin using all of its dives, and for all dives from all the penguins pooled (Table 5). The analysis indicated that, on average, 36% of the variation in dive duration could be predicted by the adjustments in f_H. There was considerable variation between individuals but, as shown in Table 5, the most consistent influences on dive duration of the individual penguins were minimum f_H while submerged, followed by minimum f_H shortly after submersion and mean post-dive f_H. When all the dives from all penguins were pooled, the three most important influences were minimum f_H when submerged, mean pre-dive f_H and minimum f_H shortly after submersion.

The second analysis used differences in f_H between different phases of the dive cycle, as the magnitude of these

changes also appeared to vary with changes in dive duration. In this regression, the dependent variable was again dive duration and the independent variables were: the difference in f_H from mean pre-dive to mean during diving, the difference in f_H from maximum pre-dive to minimum during diving and the difference in f_H from maximum pre-dive to minimum within 10 s of submersion. Again, the analysis was performed for each penguin using all of its dives, and for all dives from all the penguins pooled (Table 6). This analysis explained on average 22% of the variation in dive duration, and for each individual and all dives pooled the r^2 value was lower than in the corresponding first analysis. This analysis was clearly of less value than the first and was not considered further.

The resulting multiple regression equations for each individual penguin (Table 5) could be used to predict dive duration from measurements of heart rate for that animal. Though all of the individual relationships were significant (Table 5), the reliability of such a prediction would vary considerably from individual to individual as there was considerable variation in the r^2 values of the relationships (0.05–0.71). The relationship for all of the penguins pooled could be used to predict dive duration for an individual from outside this study, from measurements of f_H. However, the r^2 value of this relationship was relatively low (0.20, Table 5), meaning that the confidence intervals around such a prediction would be large and the prediction of limited value.

$\dot{V}_{O_2}$ while on-shore and at-sea, estimated using Equation 1, was 16.9 ± 1.4 and 26.3 ± 1.4 ml min^{-1} kg^{-1}, respectively. $\dot{V}_{O_2}$

was not calculated from Df_H and NDf_H since these were not significantly different from each other or f_H while at-sea. Equation 1 was, however, used to estimate $\dot{V}_{O_2}$, and the 95% confidence limits of these estimates, using f_H from completed dive cycles. Since f_H varied with dive duration (Table 4), it was necessary to estimate $\dot{V}_{O_2}$ and the confidence limits at each different dive duration for the full range observed by macaroni penguins (Table 7). As mean f_H decreased with dive duration, then so did estimated $\dot{V}_{O_2}$ (Table 7).

Discussion

Diving behaviour

Two previous studies have investigated the diving behaviour of macaroni penguins breeding at Bird Island, using externally mounted devices (Croxall et al., 1988, 1993). The first of these studies used a simple depth histogram recorder on eight breeding males. The second used a more sophisticated dive depth recorder, but this was heavy, bulky and was only used on two female penguins. Another more comprehensive study was completed on breeding males and females at Heard Island (Green et al., 1998), and used time depth recorders measuring depth every 3 s to give more detailed dive profiles. Despite these differences in methodology and location, the patterns in diving behaviour shown by these studies were similar and these, in turn, are similar to the patterns observed in the present study. Similarities were observed in distributions of dive depth and duration, with many short dives to less than 5 m and other longer dives to approximately 40–50 m. In all four studies, there was considerable individual variation in diving behaviour.

In all four of the above studies, macaroni penguins tended to dive predominantly in daylight. Dives at night were less frequent, to shallower depths and of shorter duration (Fig. 1). For macaroni penguins foraging in waters around Bird Island, a suggested cause for this is the diurnal migration of Antarctic krill (Croxall et al., 1993). Krill are found near the top of the water column at night but are more widely dispersed through the water column during daylight. For penguins feeding near Heard Island, the reasons are

Table 5. *Multiple linear regression equations describing how heart rate (f_H) in female macaroni penguins during different stages of their dive cycle is related to dive duration*

| | | Variables and parameters | | | | | | | |
| | | f_H during dive (beats min^{-1}) | | f_H post-dive | | Min f_H during first 10 s | f_H pre-dive | | |
Bird	Intercept	Mean	Min	Mean	Max		Mean	Max	r^2
H02	−1.47±6.16ns	ns	[1] −0.660±0.029***	[2] 0.418±0.031***	ns	[4] −0.088±0.022***	[3] 0.235±0.030***	ns	0.45
H15	67.26±1.67***	[3] 0.246±0.018***	[1] −0.727±0.022***	[2] 0.126±0.018***	[6] −0.018±0.008*	[5] 0.042±0.016**	[4] 0.081±0.013***	ns	0.27
H17	106.72±1.98***	ns	[1] −0.638±0.035***	ns	[4] 0.026±0.012*	[3] 0.060±0.024*	ns	[2] 0.032±0.011**	0.18
H25	100.78±4.53***	[4] −0.274±0.034***	[1] −0.707±0.024***	[7] 0.090±0.018***	[5] 0.029±0.012*	[3] 0.120±0.015***	[2] 0.090±0.018***	[6] 0.062±0.012***	0.43
H29	105.38±2.47***	ns	[1] −0.135±0.021***	[4] 0.039±0.013**	[2] −0.252±0.012***	[3] −0.045±0.014**	ns	ns	0.05
H53	31.47±3.32***	[2] −0.362±0.023***	[5] −0.102±0.011***	[3] 0.222±0.010***	ns	[4] −0.165±0.011***	[1] 0.285±0.010***	ns	0.40
H59	43.71±13.13***	ns	[1] −0.402±0.046***	[2] 0.182±0.056***	ns	ns	[3] 0.142±0.053**	ns	0.28
H61	95.08±2.13***	[4] 0.115±0.023***	[1] −0.577±0.033***	ns	[5] −0.024±0.008**	[2] 0.144±0.017***	[3] −0.092±0.015***	ns	0.18
H69	24.28±5.46***	[5] −0.099±0.035**	[1] −0.497±0.032***	[2] −0.195±0.017***	ns	[3] 0.186±0.020***	[4] 0.116±0.018***	ns	0.71
H73	42.66±5.93***	[5] −0.200±0.052***	[1] −0.495±0.033***	[2] 0.205±0.023***	ns	[4] 0.133±0.025***	[6] 0.063±0.027*	[3] 0.081±0.014***	0.59
H79	87.94±1.31***	ns	[2] −1.133±0.033***	ns	[5] 0.061±0.010***	[4] 0.199±0.025***	[3] 0.126±0.016***	[1] 0.078±0.012***	0.27
H93	66.19±4.14***	[5] −0.085±0.012***	[1] −0.492±0.022***	[2] 0.296±0.023***	[3] −0.179±0.016***	[4] 0.185±0.015***	[6] 0.200±0.023***	[7] −0.079±0.015***	0.49
H95	130.61±2.75***	[1] −0.637±0.025***	[3] −0.373±0.019***	[5] −0.090±0.013***	[4] 0.116±0.009***	ns	[6] −0.030±0.013*	[2] 0.151±0.010***	0.38
Mean ± S.E.M.									0.36± 0.04
All dives	92.40± 0.71 ***	[5] −0.078±0.007***	[1] −0.388±0.006 ***	[4] 0.073±0.005***		[3] −0.076±0.005***	[2] 0.107±0.006***	[6] 0.011±0.003***	0.20

Values are means ± S.E.M.

Max, maximum; min, minimum.

The equations are of the format: dive duration=intercept+(parameter$_1$×variable$_1$) +….+ (parameter$_n$×variable$_n$).

*A significant factor at $P<0.05$ (Wald test); **a significant factor at $P<0.01$; ***a significant factor at $P<0.001$; ns, a non-significant factor.

A superscript number before the parameter describes the order in which that variable was found to improve the fit of the stepwise regression (F-test) and added to the model.

Table 6. *Multiple linear regression equations describing how changes in heart rate (f_H) in female macaroni penguins during different stages of their dive cycle are related to dive duration*

		Variables and parameters			
Bird	Intercept	Max pre-dive f_H–min f_H during first 10 s	Max pre-dive f_H–min f_H during dive	Mean pre-dive f_H–mean f_H during dive	r^2
H02	–22.63±3.95***	ns	[1] 0.349±0.020***	[2] 0.407±0.024***	0.29
H15	64.01±1.08***	ns	[1] 0.133±0.008***	[2] –0.066±0.008***	0.06
H17	77.09±2.24***	[3] –0.149±0.026***	[1] 0.248±0.027***	[2] –0.130±0.017***	0.06
H25	17.80±1.89***	[3] –0.084±0.017***	[1] 0.375±0.020***	[2] 0.056±0.010***	0.28
H29	ns	ns	ns	ns	0.00
H53	23.49±1.51***	[2] 0.053±0.010***	[3] 0.023±0.009**	[1] 0.377±0.008***	0.33
H59	9.78±8.41 ns	ns	[1] 0.297±0.039***	[2] 0.215±0.047***	0.22
H61	83.19±1.95***	[1] –0.151±0.016	[2] 0.125±0.017***	[3] 0.041±0.019*	0.05
H69	19.81±2.45***	[3] –0.172±0.026***	[1] 0.262±0.026***	[2] 0.221±0.025***	0.56
H73	21.82±2.66***	[2] –0.219±0.025***	[1] 0.351±0.026***	[3] 0.211±0.025***	0.48
H79	61.22±1.35***	[2] –0.210±0.027***	[1] 0.412±0.028	[3] –0.082±0.013***	0.13
H93	33.14±1.92***	[3] –0.178±0.017***	[2] 0.222±0.020***	[1] 0.291±0.010***	0.35
H95	49.75±1.75***	[3] –0.048±0.017**	[1] 0.245±0.018***	[2] –0.110±0.009***	0.11
Mean ± S.E.M.					0.22±0.05
All dives	64.79±0.50***		[1] 0.109±0.003***	[2] 0.020±0.003***	0.07

Values are means ± S.E.M.

Max, maximum; min, minimum.

The equations are of the format: dive duration=intercept+(parameter$_1$×variable$_1$) +….+ (parameter$_n$×variable$_n$).

*A significant factor at $P<0.05$ (Wald test); **a significant factor at $P<0.01$; ***a significant factor at $P<0.001$; ns, a non-significant factor.

A superscript number before the parameter describes the order in which that variable was found to improve the fit of the stepwise regression (F-test) and added to the model.

Table 7. *Mean heart rate (f_H), mass-specific rate of oxygen consumption ($\dot{V}_{O_2}$) with 95% confidence intervals and calculated aerobic dive limit (cADL) of macaroni penguins for dives of different durations*

			$\dot{V}_{O_2}$ estimate (ml min^{-1} kg^{-1})			
Dive duration (s)	Percentage of dives	f_H during dive cycle (beats min^{-1})	During dive cycle	Lower 95% confidence limit	Upper 95% confidence limit	cADL, calculated using $\dot{V}_{O_2}$ estimate (s)
2–10	2.2	172±10	33.6±1.5	30.7	36.5	104
12–20	3.0	169±9	32.6±1.4	29.8	35.5	107
22–30	3.1	161±9	30.4±1.4	27.6	33.2	114
32–40	3.8	158±9	29.4±1.4	26.6	32.2	118
42–50	4.6	158±9	29.4±1.4	26.6	32.1	118
52–60	6.2	153±8	28.1±1.4	25.3	30.8	124
62–70	7.4	151±8	27.3±1.4	24.6	30.1	127
72–80	9.1	148±7	26.5±1.4	23.8	29.2	131
82–90	9.8	146±6	26.0±1.4	23.3	28.7	134
92–100	11.8	146±6	26.0±1.4	23.3	28.7	134
102–110	12.7	145±6	25.6±1.4	22.9	28.3	136
112–120	10.8	144±6	25.2±1.4	22.5	27.9	138
122–130	7.2	143±5	25.0±1.4	22.3	27.7	139
132–140	4.2	144±4	25.3±1.4	22.6	28.0	138
142–150	2.0	141±6	24.4±1.4	21.7	27.1	143
152–160	1.0	137±5	23.2±1.4	20.4	26.0	150
162–170	0.5	133±13	22.2±1.5	19.2	25.2	157
172 +	0.6	137±12	23.3±1.9	19.6	27.0	149
All dives	100	149±7	26.8±1.4	24.1	29.6	130

Values are means ± S.E.M.

less clear, though little is known about the myctophid icefish on which the penguins feed and a reliance on visual foraging was suggested as the explanation for decreased diving at night (Green et al., 1998). Such a reliance on daylight for successful foraging has also been proposed in other penguin species feeding on a variety of prey in different locations (Wilson et al., 1993).

Heart rate changes within dives

Fig. 4 shows the average change in heart rate associated with dives of 102–110 s duration. Heart rate during diving has been recorded previously in diving birds, but only within laboratory conditions (Butler and Woakes, 1979, 1984; Stephenson et al., 1986), semi-natural conditions (Culik, 1992; Kooyman et al., 1992b) or in the field at a lower resolution (Bevan et al., 1997, 2002). These studies showed similar patterns in the change of heart rate to those of the present study, with f_H higher than the resting level before and after dives, then falling to a level close to or lower than the resting level during dives. Such a response is now widely accepted to be a trade-off between the 'classic dive response', which conserves oxygen stores while the animal is deprived of access to air, and the 'exercise response', which prioritises blood flow and oxygen uptake to active muscles when exercising (Butler, 1988).

In the present study, the mean NDf_H was not significantly different from the mean heart rate during bouts of diving (Df_H). It is not possible to state exactly what activities the penguins were engaged in when not diving, but it seems likely that they were travelling between the feeding sites and the colony. Swimming or porpoising while travelling is energetically more expensive than resting either in water or air (Culik and Wilson, 1991; Bevan et al., 1995b), and hence NDf_H cannot necessarily be considered to be the f_H while resting on water. In gentoo penguins, f_H while resting on the water in a respirometer was the same as f_H averaged over complete free-ranging dive cycles (Bevan et al., 1995b), and we have assumed that the same is true for macaroni penguins.

Adjustments in f_H allow dive duration to be extended by ensuring full loading of oxygen stores before the dive, then by reducing aerobic metabolism during the dive (Butler and Jones, 1997) and ensuring the full and effective use of oxygen stores while submerged (Davis and Kanatous, 1999). Changes in heart rate, blood flow and perfusion during diving have been proposed ever since the early physiological experiments on forcibly submerged animals (Scholander, 1940) and have subsequently been observed in freely diving penguins (Millard et al., 1973) and other diving birds (Bevan and Butler, 1992). Data on these circulatory adjustments are limited (Kooyman and Ponganis, 1998), but they could have a very great effect on reducing aerobic metabolism and maximising the effective use of oxygen stores (Davis and Kanatous, 1999). The stepwise multiple linear regression showed that minimum f_H had the strongest relationship to dive duration followed by minimum f_H during the first 10 s of the dive and mean f_H after the dive. Since the minimum heart rate occurs relatively early in the dive (Fig. 4), this might suggest that the penguins are to some extent

setting the duration of the dive when the minimum f_H is reached, though the importance of mean f_H post-dive suggests that penguins adjust f_H as a response to the previous dive rather than to prepare for the next one. This idea would contradict the apparent prediction of the duration and depth of the following dive and adjustment of the volume of inhaled air (Sato et al., 2002; Wilson et al., 2002) and clearly this subject requires further investigation. Currently the multiple regression analysis is instructive, but it is difficult to determine whether, within the penguin, dive duration is dependent on the cardiac and circulatory adjustments or *vice versa*. What can be stated with certainty is that in macaroni penguins, the cardiac adjustments become more exaggerated as dive duration increases.

Rate of oxygen consumption during diving

Heart rate cannot be used to estimate $\dot{V}_{O_2}$ while submerged. In tufted ducks *Aythya fuligula*, estimation of submerged $\dot{V}_{O_2}$ using values for mean submerged f_H at mean dive duration, actually underestimated mean submerged $\dot{V}_{O_2}$ at mean dive duration, as calculated from a multiple linear regression (Woakes and Butler, 1983). However, if f_H is averaged over complete dive cycles, then it is an accurate and reliable predictor of $\dot{V}_{O_2}$ for the dive cycle (Fedak, 1986; Bevan et al., 1992; Butler, 1993). This approach was adopted in the present study and $\dot{V}_{O_2}$ during dive cycles was estimated using mean f_H recorded from completed dive cycles. If we assume that $\dot{V}_{O_2}$ while submerged is equivalent to this mean value, then it is possible to determine the cADL for macaroni penguins. As the observed dive duration increased, $\dot{V}_{O_2}$ decreased and hence cADL increased (Table 7). For all dive durations up to 138 s (95.3%) of dives), the cADL was greater than the observed dive duration (Fig. 5). The 95% confidence limits can also be used to calculate cADLs for the potential minimum and maximum estimates of $\dot{V}_{O_2}$. If the upper confidence limit is used, then for a given dive duration, cADL will be lower and only dives up to 126 s (89.2% of dives) would be within the cADL. In contrast, at the lower confidence interval, for a given dive duration the cADL will be higher and all dives would be within the cADL. These results imply that most natural dives within diving bouts by macaroni penguins are aerobic. $\dot{V}_{O_2}$ calculated from Df_H of 147 beats min^{-1} would be 26.2±1.4 ml min^{-1} kg^{-1}, with upper and lower confidence limits of 28.9 and 23.5 ml min^{-1} kg^{-1}, respectively. The resultant cADL would be 133 s with limits of 120–148 s. This would translate to 92.8% of observed dives being within the cADL with 95% confidence limits of 84.5–97.6%. This approach demonstrates the importance of including the variation in heart rate associated with dives of different durations. Calculating cADL at different durations suggests that 95.3% of observed dives used aerobic metabolism, whereas the more straightforward approach using overall mean Df_H to calculate cADL suggests that only 92.8% of observed dives used aerobic metabolism.

cADL has been calculated using $\dot{V}_{O_2}$ while resting on water for three other penguin species (Butler, 2000), though in each case, $\dot{V}_{O_2}$ was measured using respirometry, rather than
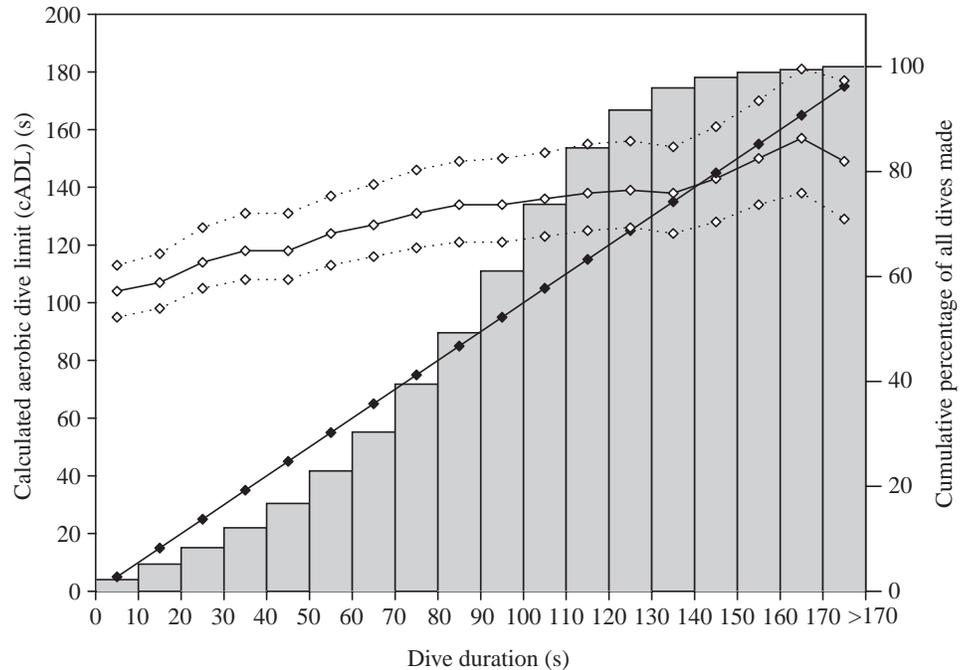
Fig. 5. Change in observed calculated aerobic dive limit (cADL, open diamonds, with 95% confidence limits) with increasing dive duration (grouped into 10 s bins). The line marked by filled diamonds represents the line of equality where cADL equals dive duration. Dive durations (on the line of equality) above the observed cADLs probably involve an anaerobic component. Also shown are the cumulative percentage frequency of dive durations (grey bars).

estimated from the field. In emperor penguins, 96% of foraging dives in the field would be within the cADL, whereas in king penguins *Aptenodytes patagonicus* and gentoo penguins *Pygoscelis papua*, only 80% of dives in the field would be within the cADL. Given that the oxygen stores are assumed to be the same for these four species, there must be a difference in diving behaviour or $\dot{V}_{O_2}$ while submerged between species. Food density, availability and location will fluctuate, causing variation in ecological conditions between populations and species, which are more likely to be the causes of variability in diving performance than differences in physiology. Ecological differences between gentoo and macaroni penguins breeding at Bird Island have been described previously (Croxall et al., 1997), and the breeding success of gentoo penguins is far more vulnerable than macaroni penguins to variations in their food availability (Croxall et al., 1999). Perhaps gentoo penguins are under greater pressure to gather enough food to provision their two chicks, leading to a higher proportion of anaerobic diving.

Similarly, emperor penguins are substantially larger than king penguins (approx. 25–30 and 10–15 kg, respectively) (Pütz et al., 1998), yet their diving performance is similar (Kooyman and Ponganis, 1990; Kooyman et al., 1992a; Kooyman and Kooyman, 1995). As would be expected, with their greater size and oxygen stores, emperor penguins are capable of superior maximum dive depth and duration than king penguins, but a large proportion of the foraging dives of both species are to 100–200 m depth and up to 5–6 min duration (Kooyman et al., 1992a; Kooyman and Kooyman, 1995). This implies that emperor penguins operate well within their physiological limits, whereas king penguins dive to depths and for durations that are close to the maximum of their capabilities.

Abdominal temperature changes during dive bouts

Abdominal temperature showed a progressive decline during most dive bouts. Similar decreases in body temperature have been observed in other diving birds including king penguins (Culik et al., 1996b; Handrich et al., 1997), gentoo penguins (Bevan et al., 2002), king cormorants (Kato et al., 1996) and blue-eyed shags (Bevan et al., 1997) as well as in marine mammals (Hill et al., 1987). The mean decrease during a diving bout (ΔT_{ab}) in macaroni penguins was 2.32±0.2°C, similar to that in gentoo penguins of 2.6°C (Bevan et al., 2002). Mean ΔT_{ab} was considerably less than the mean maximum ΔT_{ab} of 13.5±1.1°C, as most individuals performed many short diving bouts where ΔT_{ab} was low. This also explains why the mean T_{ab} during diving bouts was 4.7°C lower than the mean T_{ab} while not diving, as long bouts with large values of ΔT_{ab} account for a large proportion of the time spent within diving bouts.

The decline in T_{ab} may be the inevitable consequence of the ingestion of cold food or of conduction to cold seawater from exposed surfaces on the feet and flippers. Local changes in circulation may effect the dissipation of heat from the abdominal region. Animals may attempt to reduce this heat loss or simply allow it to continue. Alternatively, in an effort intentionally to lose or 'dump' heat, animals may increase blood flow to the abdomen and/or exposed surfaces. These alternative mechanisms for heat loss, and determination of whether this an active or passive process, are still subject to investigation (Kooyman et al., 1980; Hill et al., 1987; Kooyman, 1989; Handrich et al., 1997; Ponganis et al., 2001). However, studies of the barnacle goose *Branta leucopsis*, a non-diving bird, have shown that it is possible for birds to experience anapyrexia (Cabanac and Brinnel, 1987), a resetting of their body temperature to a lower level when

conservation of energy may be important, even if the animal is active and food is not scarce (Butler and Woakes, 2001).

Data from king penguins suggest that the decrease in T_{ab} is in some way facilitated and not just the consequence of ingesting cold food, as the T_{ab} of foraging king penguins was lower than that in the stomach (Handrich et al., 1997). It has been proposed that this reduction in T_{ab} leads to lowered metabolic rates in diving birds (Boyd and Croxall, 1996; Culik et al., 1996b; Butler, 2000), through the effect of cold temperatures on metabolically active tissues (Heldmaier and Ruf, 1992) and reduced thermoregulatory costs. Barnacle geese engaged in a long, energetically costly migration were found to allow their T_{ab} to fall progressively by 4.4°C, and it is proposed that if this hypothermia extended to the whole body, an amount of fat could be saved equivalent to up to 25% of that used for migration (Butler and Woakes, 2001). In diving birds, a lowering of T_{ab} and metabolic rate is suggested to be sufficient to bring most natural dives observed in the field within the cADL (Boyd and Croxall, 1996; Butler, 2000). This is not the only mechanism that might account for the discrepancies between observed diving behaviour and cADL. For example, phosphocreatine may be a source of energy that animals use while submerged (Butler and Jones, 1997) and further research into this possibility should be a priority.

In the present study, it was not possible to detect variation in T_{ab} within dives. In king penguins, fluctuations in temperature in localised parts of the body were found to vary between consecutive dives (Culik et al., 1996b). Similar experiments investigating changes in T_{ab} of emperor penguins diving from man-made holes in sea-ice using a thermistor with a much smaller time constant (0.2 s) showed that T_{ab} can drop quite considerably within individual dives (Ponganis et al., 2001). However, in the same study (Ponganis et al., 2001), another thermistor placed in the inferior vena cava, which receives blood drained from core organs such as the kidneys, liver and gastrointestinal tract, registered no significant changes in temperature during diving. The authors concluded that there was no evidence to suggest that reduction in T_{ab} facilitates diving durations greater than the cADL or DLT, as core temperature did not vary during diving and there was no relationship between the magnitude of T_{ab} fluctuation and dive duration. Further work, involving more sensitive and faster responding temperature sensors at multiple locations around the body, may cast more light on the extent of this regional hypothermia and its possible importance in extending dive durations in different species.

Though it was not possible to detect differences in T_{ab} within individual dives in the present study, T_{ab} did decline progressively during diving bouts. The shape and gradient of this temperature decline varied between individuals (which may be attributable to the position of the data logger) and between diving bouts performed by the same individual. However, in each case the decline was progressive throughout the bout, and abdominal temperature only increased after or at the very end of the bout. The magnitude of the temperature drop did increase consistently with the duration of diving bouts (Fig. 3). If diving

behaviour was determined only by physiological capacity, and lowered abdominal temperature was essential to facilitate increased diving duration, then we might expect to see dive duration increasing and/or mean f_H decreasing progressively through bouts as abdominal temperature decreases. However, as Table 4 shows, nearly as many diving bouts showed a progressive decrease in dive duration during bouts as showed a progressive increase, and over 64% showed no significant change at all. In addition, nearly all dives were within the cADL. This supports the suggestion that, for macaroni penguins, factors other than physiological ones are likely to be more important in determining average diving behaviour. Such factors could include progressive satiation during dive bouts and the location and density of patches of food within the water column, especially since Antarctic krill are found in swarms (Everson, 2000). In gentoo and king penguins, which may be pushing the physiological limits of aerobic diving more than macaroni penguins, patterns of increasing dive duration within bouts might be observed.

The progressive decrease in T_{ab} of macaroni penguins is likely to be the result of many smaller decreases associated with individual dives. The abdomen may not have sufficient time to return to its initial temperature during the surface interval between dives, and the overall decrease in temperature may be the result of an accumulation of these cycles. This pattern was found to occur in diving emperor penguins (Ponganis et al., 2001) where T_{ab} started to decrease as soon as a dive commenced and continued to decrease until the animal surfaced. Upon surfacing, T_{ab} immediately increased until the next dive commenced. However, the increase while at the surface was not sufficient to match the decrease while diving and the net effect was a progressive decline in T_{ab} during diving bouts.

Conclusions

The present study suggests that most dives by macaroni penguins are likely to be aerobic. Circulatory adjustments and the associated reduction of heart rate during dives permit a sufficiently low level of oxygen consumption such that even the longest observed dives performed by these animals may be supported by aerobic metabolism. Bouts of repeated diving are also associated with a reduction in abdominal temperature, which is probably a result of the accumulation of many smaller decreases during individual dive/surface cycles. Decreased temperature in the abdomen will further contribute to a reduction in metabolic rate, but further work would be required to determine the extent of cooling in the penguins' bodies and to what extent this might lead to a significant reduction in metabolic rate during dives.

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