The Journal of Experimental Biology 208, 2973-2980
Published by The Company of Biologists 2005
doi:10.1242/jeb.01687

Air sac P_{O_2} and oxygen depletion during dives of emperor penguins

T. Knower Stockard[1], J. Heil[2], J. U. Meir[1], K. Sato[3], K. V. Ponganis[1] and P. J. Ponganis[1,*]

[1]*Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0204, USA*, [2]*Anesthesiology Department, US Naval Medical Center, Balboa Hospital, San Diego, CA 92134, USA* and [3]*International Coastal Research Center, The Ocean Research Institute, University of Tokyo, 2-106-1 Akahama, Ostuchi Iwate 028-1102, Japan*

*Author for correspondence (e-mail: pponganis@ucsd.edu)

Accepted 12 May 2005

Summary

In order to determine the rate and magnitude of respiratory O_2 depletion during dives of emperor penguins (*Aptenodytes forsteri*), air sac O_2 partial pressure (P_{O_2}) was recorded in 73 dives of four birds at an isolated dive hole. These results were evaluated with respect to hypoxic tolerance, the aerobic dive limit (ADL; dive duration beyond which there is post-dive lactate accumulation) and previously measured field metabolic rates (FMR*s*). 55% of dives were greater in duration than the previously measured 5.6-min ADL. P_{O_2} and depth profiles revealed compression hyperoxia and gradual O_2 depletion during dives. 42% of final P_{O_2}s during the dives (recorded during the last 15 s of ascent) were <20 mmHg (<2.7 kPa). Assuming that the measured air sac P_{O_2} is representative of the entire respiratory system, this implies remarkable hypoxic tolerance in emperors. In dives of durations greater than the ADL, the calculated end-of-dive air sac O_2 fraction was <4%. The respiratory O_2 store depletion rate of an entire dive, based on the change in O_2 fraction during a dive and previously measured diving respiratory volume, ranged from 1 to 5 ml O_2 kg^{-1} min^{-1} and decreased exponentially with diving duration. The mean value, 2.1±0.8 ml O_2 kg^{-1} min^{-1}, was (1) 19–42% of previously measured respiratory O_2 depletion rates during forced submersions and simulated dives, (2) approximately one-third of the predicted total body resting metabolic rate and (3) approximately 10% of the measured FMR. These findings are consistent with a low total body metabolic rate during the dive.

Key words: air sac, *Aptenodytes forsteri,* dive, electrode, emperor penguin, hypoxia, metabolic rate, oxygen.

Introduction

Diving durations of sea birds and marine mammals are dependent on the magnitude and rate of depletion of the blood, muscle and respiratory O_2 stores. In birds, the air sac/lung volume results in a large respiratory O_2 store (Butler and Jones, 1997). For example, in contrast to phocid seals, in which lung O_2 may comprise only 4% of the total body O_2 store (Ponganis et al., 2003a), the respiratory O_2 store in the lungs and air sacs of penguins has been estimated to contain as much as 40–50% of the total available O_2 (Ponganis and Kooyman, 2000). Despite the potential contribution of the respiratory O_2 store to the determination of dive capacity in penguins, little is known about the rate and magnitude of its depletion during diving. In macaroni (*Eudyptes chrysophalus*) and gentoo (*Pygoscelis papua*) penguins, air sac O_2 declined to as low as 4–5% during 2-min forced submersions (Scholander, 1940). During simulated dives of Adelie (*Pygoscelis adeliae*) and gentoo penguins in a pressure chamber, air sac O_2 decreased to 2–4% during dives of 3.5-min duration (Kooyman et al., 1973). In order to (1) determine the rate and magnitude of air sac O_2 partial pressure (P_{O_2}) and respiratory O_2 depletion during free dives and (2) assess the relationship of those variables to dive duration, hypoxic tolerance and the aerobic dive limit (ADL; dive duration beyond which there is an elevation in post-dive blood lactate concentration), we recorded air sac P_{O_2} during dives of emperor penguins (*Aptenodytes forsteri*) foraging under sea ice at an isolated dive hole. This is a particularly useful model in which to examine respiratory O_2 depletion since both field metabolic rate (FMR) and the ADL have been determined under these conditions (Nagy et al., 2001; Ponganis et al., 1997).

Materials and methods

Emperor penguins (*A. forsteri* Gray) were captured near the McMurdo Sound ice edge in October 2003, were maintained for two months at an isolated dive hole enclosed within a corral on the McMurdo Sound sea ice (77°41′.766, 165°59′.024), and were then released at the end of the study. The birds were allowed daily access to the dive hole, where they dove and foraged as evidenced by guano deposition and weight gain.

A P_{O_2} electrode (Licox C1 Revoxode; Integra LifeSciences, Plainsboro, NJ, USA) and thermistor (model 554; Yellow Springs Instruments, Yellow Springs, OH, USA) were inserted percutaneously 10–14 cm through a peel-away catheter (a slit 14 g Angiocath catheter; Becton Dickinson, Sandy, UT, USA) into the posterior thoracic air sac while birds were under general isoflurane anesthesia (Ponganis et al., 2001). The probes were secured to the feathers with Tesa™ tape and Loctite™ glue as previously described (Ponganis et al., 2001). The insertion site was along the lateral body wall, approximately 20 cm below the axilla, and between two ribs overlying the posterior thoracic air sac. The probes were connected *via* a waterproof cable to a custom-built microprocessor recorder (UFI, Morro Bay, CA, USA), which was attached to feathers of the mid-back with 5-min epoxy glue (Devcon; Danvers, MA, USA), a Velcro™ patch and cable ties. After overnight recovery, the birds were provided access to the dive hole for one day. On the morning following the dive day, the recorder and probes were removed under general anesthesia.

Air sac P_{O_2} and temperature were recorded at 15-s intervals. In addition to the P_{O_2}/temperature recorder (250 g, 15×6×3.5 cm), a Mk9 time depth recorder (TDR; Wildlife Computers, Redmond, WA, USA; sensitive to 0.5 m, 30 g, 6.7×1.7×1.7 cm) was attached with 5-min epoxy glue, Velcro™ and cable ties to feathers along the mid-line back just above the tail. Depth was recorded at 1-s intervals. The TDR pressure output was verified in a pressure chamber at Scripps Institution of Oceanography. The temperature probes (sensitive to 0.05°C, 0.2-s 60% response time) were calibrated as previously described (Ponganis et al., 2001).

The manufacturer's specifications for the P_{O_2} electrode include a zero offset of <1 mmHg, a linear response, probe-sensitivity error of <1%, a probe drift of <2% day^{-1}, a 1-min 90% response time, and a temperature correction factor for probe output of <5% $deg.^{-1}$. P_{O_2} electrode responses were evaluated in 0.9% saline-filled (8 ml) test tubes in a water bath (ThermoNESLAB RTE 7; Portsmouth, NH, USA) at 38°C by bubbling the saline with appropriate gases: 100% N_2 (ultra high purity grade 5.0; minimum purity 99.999%; WestAir Gases, San Diego, CA, USA) for a 0% O_2 value, 3% O_2 (primary standard grade; actual purity 2.99%; WestAir Gases), room air (21% O_2) or 100% O_2 (ultra high purity grade 4.4; minimum purity 99.994%; WestAir Gases).

For probe evaluation, the P_{O_2} electrode output was recorded at 5-s intervals for the first 45 s, 15-s intervals from 45 s to 2 min, and 30-s intervals from 2 min until a stable output was reached. Upon stabilization of output, the probe was transferred to the next tube, progressing through a series that allowed for the recording of each possible transition among the varying oxygen concentrations. This allowed calculation of response time, calculation of regression calibration equations over different ranges of % O_2 and determination of drift over 48 h of continuous use. The temperature correction factor was determined by recording the electrode output at 21% O_2 between 37 and 42°C.

The effect of pressure on electrode output was evaluated with compression in a water-filled pressure chamber. In addition, the electrode response in air was also evaluated in the McMurdo Station recompression chamber with 10-m step compressions to a final depth of 50 m.

For deployment in the field, the P_{O_2} electrode was calibrated with 100% N_2 and room air at 38°C in a sterile, saline-filled test tube. Percentage O_2 was converted to P_{O_2} (mmHg) with the use of the local barometric pressure (available from the McMurdo Station weather service) after subtraction of the vapor pressure of water. All P_{O_2} values are therefore expressed in mmHg; in figures, the corresponding values in kPa are also expressed, assuming 1 mmHg=0.133 kPa.

After removal from the bird, TDR and P_{O_2}/temperature recorder outputs were downloaded to a laptop PC. Recorder output was converted to data in Excel with the use of appropriate calibration equations; P_{O_2} probe output was first corrected to 38°C with a 4% $deg.^{-1}$ temperature correction factor (see Results). Data were analyzed and illustrated graphically using Excel, Origin and SPSS software. Statistical significance was assumed at $P<0.05$. All data are expressed as means ± S.D. unless otherwise stated.

The final P_{O_2} was recorded during the final 15 s of the dive. End-of-dive P_{O_2} was calculated from the final P_{O_2} recorded during a dive by first calculating the O_2 fraction at the depth at which the final P_{O_2} was recorded [i.e. final P_{O_2}/(ambient pressure – vapor pressure of water)] and by then using that fraction to calculate the equivalent P_{O_2} at the surface [O_2 fraction × (barometric pressure – vapor pressure of water)]. These calculated variables were considered the closest possible approximation of end-of-dive P_{O_2} and O_2 fraction available from the data. In O_2 fraction calculations, 100% humidity was assumed in the air sac.

Results

P_{O_2} *electrode evaluation*

Initial exposure of a P_{O_2} electrode to different concentrations of O_2 revealed that a probe's output was constant for a given O_2 concentration (Fig. 1). Evaluation of 15 electrodes at 0, 21 and 100% O_2 yielded mean electrode outputs of 4±1, 493±13 and 2143±54, respectively. The output at 100% O_2 for each electrode was 8.1±1.3% less than expected, based upon a linear response between 0 and 21% O_2. Repeated examination of two electrodes' outputs at 0, 3 and 21% O_2 confirmed that each probe's output was linear between 0 and 21% O_2 and that calibration equations (Table 1), based either upon the 0 and 21% O_2 data or on the 0, 3 and 21% data, resulted in accurate calculations of expected P_{O_2} at those O_2 concentrations (Table 1).

Output of the P_{O_2} electrode was relatively stable over 48 h at 0, 3 and 21% O_2; however, at 100% O_2, electrode output drifted significantly over time from the initial reading (Fig. 2). Percentage changes in output of four electrodes at 0, 21 and 100% O_2 at 24 and 48 h were 0, 7.3±5.1 and 30.8±7.3% and 0, 10.5±5.8 and 35.6±7.7%, respectively. This drift resulted in

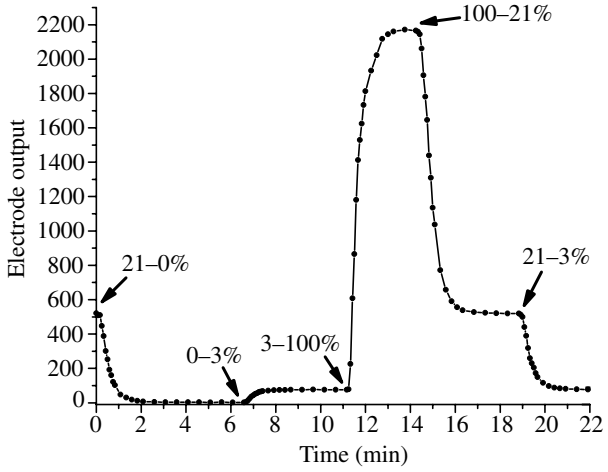

Fig. 1. Response of a P_{O_2} electrode to changes in O_2 concentration. Arrows indicate the time at which the electrode was shifted from one O_2 concentration to another (0, 3, 21, 100% O_2).

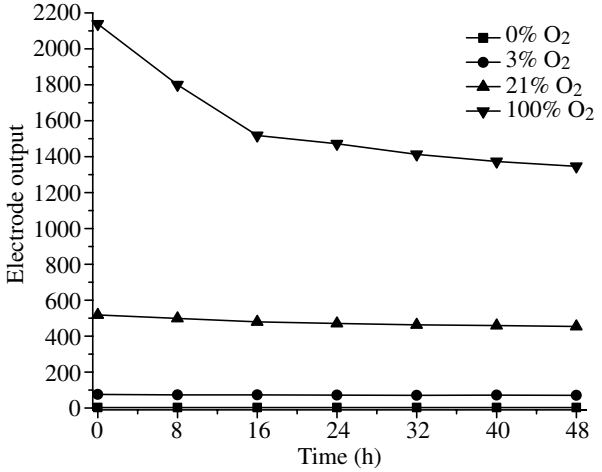

Fig. 2. Drift of a P_{O_2} electrode's output at four different O_2 concentrations over a 48 h calibration.

a decrease in calculated P_{O_2} with time (calculated with the original linear regression equation for initial data points at 0 and 21% O_2 at time 0; see Table 2). The magnitudes of these decreases were: 0 mmHg P_{O_2} at 0% O_2, 11–15 mmHg P_{O_2} at 21% O_2 and 199–229 mmHg P_{O_2} at 100% O_2 at 24 and 48 h.

The 90% response times from 0 to 21% O_2 in four electrodes were 0.8±0.1, 0.8±0.2 and 1.0±0.1 min at 0, 24 and 48 h, respectively. See typical responses to step changes in O_2 concentration in Fig. 1. Detailed analysis of probe 1 for 12 step changes between 0, 3, 21 and 100% O_2 revealed mean 90% response times of 1.1±0.1 min at 0 h, 1.3±0.2 min at 24 h and 1.3±0.4 min at 48 h.

P_{O_2} electrode output of four probes at 21% O_2 changed by 4.2±0.4% deg.$^{-1}$ between temperatures of 37 and 42°C. Electrode output did not change during compression in a water-filled pressure chamber to 51 atmospheres absolute (ATA; 500 m depth). Output of an electrode in air in a recompression chamber increased during compression and returned to baseline with decompression to ambient, surface pressure (Fig. 3). Response time, calibration equations and drift of P_{O_2} electrodes suspended in the gas phase above the saline in the stoppered test tubes were similar to those parameters of the same electrodes immersed in saline.

Diving data

Air sac P_{O_2} and temperature were collected during 73 dives of four birds. Only partial records (5 dives and 3 dives) were obtained from two of the penguins due to saltwater electrical shorting of a cable connection in one case and presumed physical damage (unidentifiable) to the electrode in the other. Saltwater shorting, probe migration out of the air sac and physical damage prevented collection of diving data in seven other penguins. Mean body mass of the four penguins was 24.9±3.6 kg. Maximum depth of dive averaged 26±11 m (range, 9–74 m; Fig. 4). Mean diving duration was 6.0±2.1 min (range, 2.3–10.5 min; Fig. 4).

Mean air sac P_{O_2} ranged from 114 to 130 mmHg during 3-h periods when the penguins were resting overnight in the corral (Table 3). Start-of-dive P_{O_2} (the last surface value prior to the start of a dive) was 136±8 mmHg, corresponding to 19.6±1.2% O_2. Start-of-dive P_{O_2} did not correlate with either dive duration or maximum depth of dive (r=0.09 and 0.12, respectively, P>0.05). Air sac P_{O_2} typically increased during descent and then gradually decreased during the latter portion of the dive (Fig. 5). The final P_{O_2} during a dive (the last recorded value during the dive) ranged from 0 to 90 mmHg (Fig. 6). The end-of-dive P_{O_2} (the final P_{O_2} of a dive corrected to 0-m depth) ranged from 0 to 80 mmHg, corresponding to a

Table 1. P_{O_2} *electrode output and calculated* P_{O_2} *values*

O_2 fraction	Mean probe output	Expected P_{O_2} (mmHg)	Calculated P_{O_2} (0, 21% data)	Calculated P_{O_2} (0, 3, 21% data)
0%	2±1 (N=3)	0	0	0
3%	76±1 (N=3)	21	21	21
21%	519±3 (N=4)	148	148	148

Expected and calculated P_{O_2} values at hour 0 for one O_2 electrode's output. Values are in mmHg (1 mmHg=0.133 kPa). Probe output is expressed as mean ± S.D. N = number of exposures to a given % O_2. Regression equations were calculated from 0 and 21% O_2 data (y=0.29x–0.67, r2=1.0) or from 0, 3 and 21% O_2 data (y=0.29x–0.73, r2=1.0).

Table 2. *Drift of the* P_{O_2} *electrode*

O_2 fraction	0% O_2		21% O_2		100% O_2	
	$P_{O_2,calc}$ (mmHg)	% Drift	$P_{O_2,calc}$ (mmHg)	% Drift	$P_{O_2,calc}$ (mmHg)	% Drift
Hour 0	0	–	143±4	–	648±37	–
Hour 24	0	0	132±7	7.3±5.1	449±63	30.8±7.3
Hour 48	0	0	128±8	10.5±5.8	419±68	35.6±7.7

Data are from four electrodes (means ± S.D.) and are expressed in mmHg (1 mmHg=0.133 kPa). $P_{O_2,calc}$ = P_{O_2} calculated with the electrode output from a given time and with the hour 0 regression equation (generated from the 0 and 21% calibration points). % Drift = [(electrode $output_{hour\ 0}$ – electrode $output_{hour\ n}$)/electrode $output_{hour\ 0}$]×100.

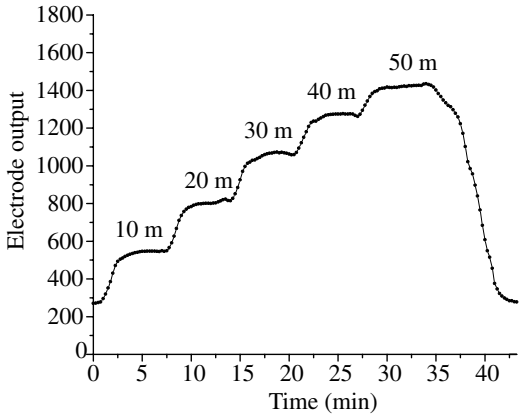

Fig. 3. The output of a P_{O_2} electrode exposed to air in response to step changes in pressure in a recompression chamber. Electrode output, recorded at 15-s intervals, returned to baseline with decompression back to ambient, surface pressure. Equivalent depths (m) are indicated for each step change in pressure.

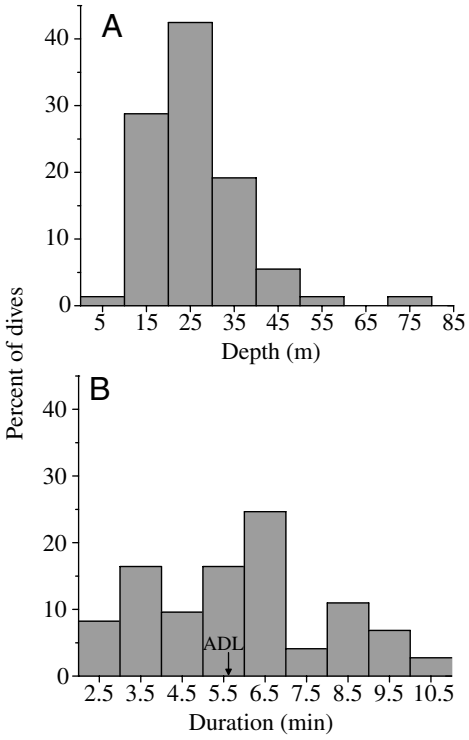

Fig. 4. Distribution of (A) maximum depth and (B) duration of 73 dives of four penguins at the isolated dive hole. The previously measured aerobic dive limit (ADL, 5.6 min) of birds at the isolated dive hole is indicated in B.

0–11.7% air sac O_2 fraction (Fig. 7). End-of-dive P_{O_2} and O_2 fraction decreased exponentially with increasing dive duration (Fig. 7). The mean net change in air sac O_2 fraction during a dive was 16.5±2.8%; the percentage of available air sac O_2 depleted during a dive ranged from 42 to 100% (Fig. 8).

Mean air sac temperature during rest periods at night ranged from 37.1 to 38.3°C (Table 3). Mean air sac temperatures during individual dives ranged from 36.1 to 38.7°C in all birds. Mean temperature during dives of individual birds did not correlate with diving duration (r<0.02, P>0.05). Typically, air sac temperature increased slightly during dives (Fig. 9).

Discussion

P_{O_2} *electrode evaluation*

Evaluation of the P_{O_2} electrode revealed that the probe should be a useful tool to investigate changes in P_{O_2} during diving. The electrode withstood compression in water to 500-m depth, responded appropriately in air during compression/decompression in a pressure chamber, had similar responses to changes in O_2 concentration in liquid and gas phases and had a reproducible temperature correction factor and response time, both of which were similar to that specified by the manufacturer. In addition, the sensitivity of the electrode was linear between 0 and 21% O_2, and the drift of the electrode's sensitivity at those concentrations was acceptable.

The major limitation of the electrode was its response to 100% O_2. During initial calibration, the magnitude of the electrode's output at 100% O_2 was, on average, about 8% less than expected in comparison with the outputs at <21% O_2. In addition, there was a large drift (a decrease as great as 30%) in the electrode's 100% O_2 response over 24–48 h (Fig. 2). Both the decreased sensitivity at 100% O_2 and the drift of that response over time made it impossible to either construct an accurate linear regression calibration over the entire range of 0–100% O_2 or use a second calibration equation between 21% and 100% O_2 for high P_{O_2} values.

We conclude from this evaluation that the P_{O_2} electrode should allow us to achieve the primary goal in this project,

Table 3. *Air sac* P_{O_2} *and temperature values during rest periods*

	Resting P_{O_2} (mmHg)	Resting temperature (°C)
Bird 9	119±9	37.9±0.3
Bird 5	130±9	38.3±0.4
Bird 7	114±6	37.5±0.4
Bird 6	119±7	37.1±0.2

P_{O_2} and temperature recorded in four emperor penguins at rest during a 3-h period at night. Data are means ± S.D. (N=720 for each bird) and are expressed in mmHg (1 mmHg=0.133 kPa).

namely to determine the initial and end-of-dive P_{O_2}. Use of a 0–21% O_2, or 0–3–21% O_2 calibration equation for each electrode yields accurate calculations of P_{O_2} between 0 and 21% O_2, the range of O_2 concentration expected prior to and at the end of a dive. The primary limitations in interpretation of the data in this range are the small amount of drift in the electrode response over time and the 1-min response time of the electrode. The electrode should also provide a qualitative record for P_{O_2} values above the calibration range, such as might occur due to air sac compression at depth. However, such hyperoxic values will not be quantifiable because of the less-than-expected electrode output and the drift of the electrode response at higher P_{O_2} values. Finally, because instantaneous air sac P_{O_2} may change quickly with rapid changes in depth, the response time of the electrode may create a lag between the instantaneous air sac P_{O_2} and the electrode reading.

P_{O_2} *electrode location*

The posterior thoracic air sac was chosen as the location for insertion of the P_{O_2} electrode because of its anatomical access, the safety of the procedure at this site, the lack of effects of the probe at this site on behavior and the use of this air sac in prior studies of penguins (Kooyman et al., 1973; Scholander, 1940). Although gas composition of the cervical air sacs is considered to most closely represent end-parabronchial gas (Powell et al., 1981), the electrode was not placed in those air sacs because of concern over (1) safety, (2) disturbance of behavior due to interference with neck movements and (3) the potential for electrode damage due to body movement and preening.

Placement of the O_2 electrode in the posterior thoracic air sac, or indeed in any air sac, might be considered a limitation in trying to estimate O_2 depletion of the entire respiratory system of a diving penguin. This is because air flow and gas mixing within the avian respiratory system, even during routine respiration, are complex and incompletely understood (Powell and Hempelman, 1985; Scheid, 1979; Torre-Bueno et al., 1980). However, in swimming penguins, interclavicular-air-sac and posterior-thoracic-air-sac pressure oscillations are associated with wing beats and have been considered a mechanism to enhance gas mixing and gas exchange (Boggs et al., 2001). Such pressure differentials within the air sacs may also contribute to air sac gas mixing and movement of air through the lung in emperors diving at the isolated dive hole since these birds stroke continuously in this situation (Van Dam et al., 2002). Therefore, despite incomplete knowledge of air flow patterns in the respiratory system during a dive, we feel that the posterior-thoracic-air-sac P_{O_2} data are a reasonable estimate of the overall, average P_{O_2} of the respiratory system.

Dive behavior

The dive behavior of the birds in this experiment was typical of emperors foraging at an isolated dive hole. The dives were less than 100 m in depth and ranged to almost 11 min in duration. Although similar in duration to many dives at sea, dive depths were only in the shallow range of the depths reached by emperors at sea (Kooyman and Kooyman, 1995). 55% of dives were greater than the 5.6-min ADL previously measured in emperors diving at an isolated dive hole (Ponganis et al., 1997). This type of diving pattern is similar to that in prior studies in which heart rate and swim speed (Kooyman et al., 1992), FMR (Nagy et al., 2001), stroke frequency (Van Dam et al., 2002) and feeding behavior (primary prey; *Pagothenia borchgrevinkii*; Ponganis et al., 2000) have been documented in penguins diving at the isolated dive hole.

Air sac temperature

Air sac temperature at rest was within the range of temperatures measured previously at multiple body sites in emperor penguins (Ponganis et al., 2001, 2003b). Only minor fluctuations occurred during and between dives (Fig. 9), and

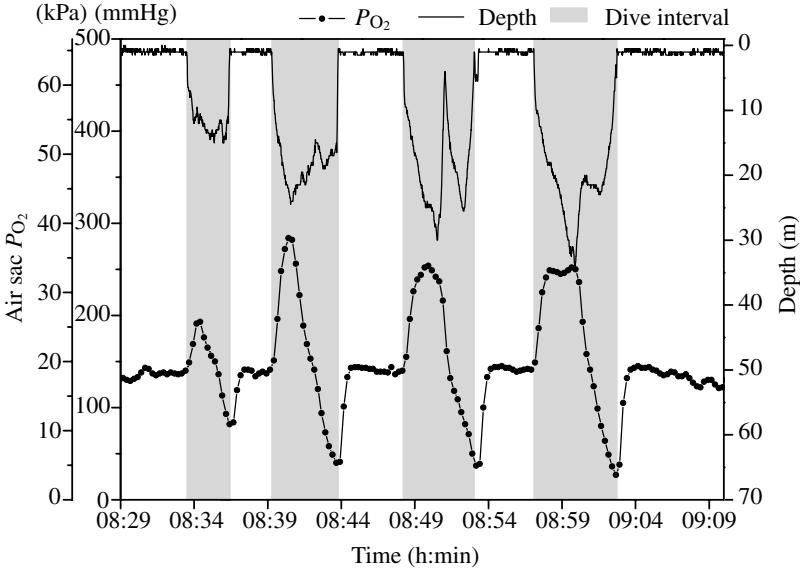

Fig. 5. Air sac P_{O_2} and depth profiles of emperor penguin 5.

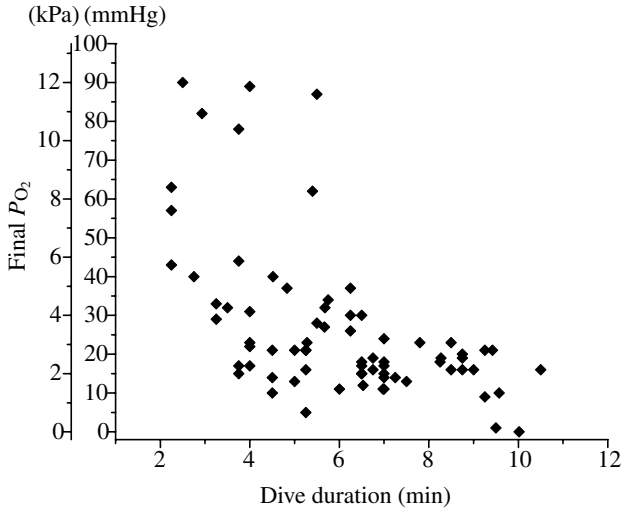

Fig. 6. The final P_{O_2} recorded during dives (within the last 15 s of the dive) decreased with increasing dive duration in the four penguins.

mean dive temperature did not correlate with diving duration. As in past studies of temperature regulation during diving of emperor penguins at the isolated dive hole, there was no evidence for a hypothermic extension of aerobic dive time.

Air sac P_{O_2}

Compression hyperoxia and O_2 depletion

Air sac P_{O_2} during rest periods and prior to diving was similar to that previously reported in other penguins (measured as % O_2; Kooyman et al., 1973; Scholander, 1940). There was no correlation between start-of-dive P_{O_2} and dive duration. P_{O_2} profiles during dives demonstrated the net effect of both ambient pressure changes and continued O_2 consumption during a dive (Fig. 5). The peak P_{O_2} values in these profiles are probably an underestimate of the actual peak air sac P_{O_2} because of the decreased electrode sensitivity at high P_{O_2} values and because of the lag time of the electrode response. Nonetheless, the profiles provide an excellent example of compression hyperoxia and O_2 depletion during diving. Continued oxygen depletion from the respiratory system of these penguins should be expected since (1) these dives are shallow, (2) gas exchange continues during simulated dives in other penguin species to depths as great as 136 m (Kooyman et al., 1973; Ponganis et al., 1999) and (3) a continuous stroke–glide swim pattern (Van Dam et al., 2002) should maintain movement of air through the lungs and enhance gas exchange (Boggs et al., 2001).

Hypoxic tolerance

The final P_{O_2} values of dives (Fig. 6) were recorded at depth during the last 15 s of diving, ranged from 0 to 90 mmHg and are the closest available approximation of the instantaneous air sac P_{O_2} during the latter phases of ascent. The final air sac P_{O_2} was less than 20 mmHg in 42% of all dives. As such, these data demonstrate significant hypoxic tolerance in the emperor

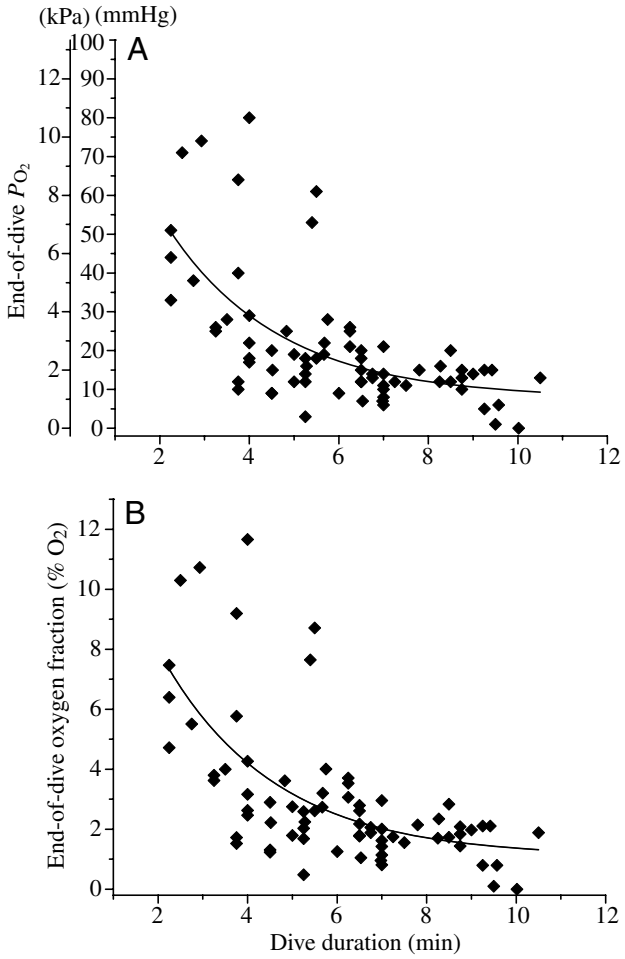

Fig. 7. (A) Air sac end-of-dive P_{O_2} (final P_{O_2} corrected to 0-m depth) and (B) end-of-dive O_2 fraction (% O_2) decreased with increasing dive duration. Regression equations: P_{O_2}: $y=(105.3\times e^{-x/2.50})+7.7$, $r^2=0.39$, $P<0.0001$; O_2 fraction: $y=(15.3\times e^{-x/2.52})+1.7$, $r^2=0.40$, $P<0.0001$.

penguin. For, if air sac P_{O_2} is representative of the P_{O_2} in the parabronchial air capillary of the penguin lung, these values are the maximum arterial P_{O_2} values near the ends of these dives. Depending on the degree of shunt through the lung, the actual arterial P_{O_2} value may be even lower. For example, in bar-headed geese (*Anser indicus*) exposed to 5% inspired O_2, arterial P_{O_2} was 29 mmHg, which is 5 mmHg less than the inspired P_{O_2} of 34 mmHg (Black and Tenney, 1980).

How emperors avoid shallow water blackout under such conditions remains a mystery. The final P_{O_2} values in 42% of all dives were less than air sac and intravascular P_{O_2} values in maximally force-submerged ducks (Hudson and Jones, 1986). Since the P_{50} of emperor penguin hemoglobin (Hb) is similar to those of the Hbs of high-altitude birds (Black and Tenney, 1980; Tamburrini et al., 1994), Hb saturation at a given P_{O_2} should not be exceptional. It is conceivable that venous P_{O_2} might be greater than the extremely low air sac values during long dives and that a 100% pulmonary shunt would avoid gas exchange and preserve available O_2 for delivery to the brain. However, there is no evidence, especially at shallow depth, for this mechanism of

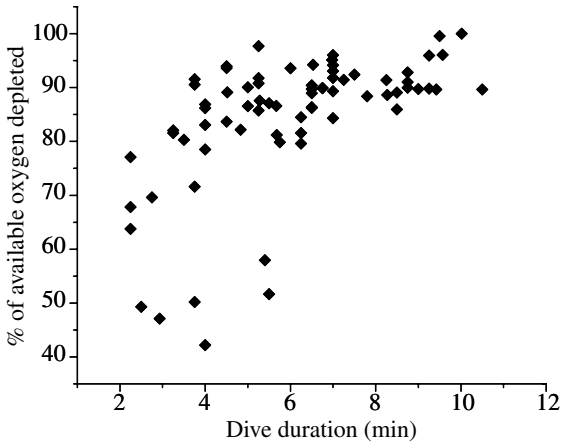

Fig. 8. The percentage of total available air sac O_2 depleted during a dive increased with dive duration. Percentage of O_2 depleted = [(start-of-dive O_2 fraction – end-of-dive O_2 fraction)/start-of-dive O_2 fraction]×100.

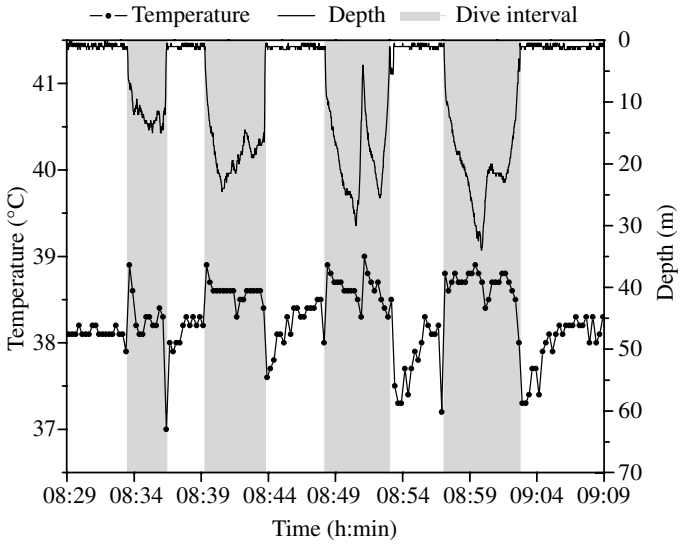

Fig. 9. Air sac temperature and depth profiles during four dives of emperor penguin 5.

cessation of gas exchange (Kooyman et al., 1973). Oxygenation of carotid blood *via* the avian ophthalmic rete (Bernstein et al., 1984; Pinshow et al., 1982) is unlikely since continued passage of fresh air over nasal and oral mucosa will not occur during a dive. On the other hand, adaptations in the brain may also contribute to increased cerebral hypoxemic tolerance. For instance, increased capillary density in the brains of harbor seals (*Phoca vitulina*) may account for the harbor seal's tolerance of arterial P_{O_2} as low as 10 mmHg (Kerem and Elsner, 1973). In this regard, birds, in general, have high brain capillary densities (Faraci, 1986). Elevated concentrations of neuroglobin, the recently discovered O_2-binding protein in the brain (Burmester et al., 2000), may also represent another potential mechanism for increased cerebral hypoxemic tolerance.

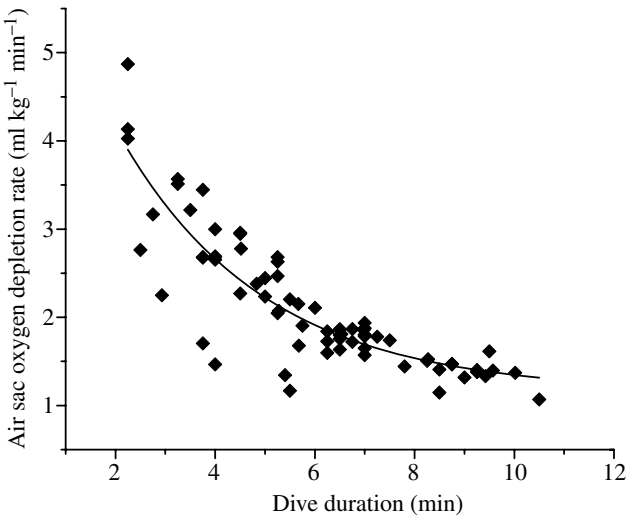

Fig. 10. The estimated air sac O_2 depletion rate based on the previously measured diving respiratory volume of 69 ml kg^{-1} and the change in air sac O_2 fraction during dives. Regression equation: $y=(5.95\times e^{-x/2.91})+1.15$, $r^2=0.76$, $P<0.0001$.

Respiratory O_2 store depletion

End-of-dive P_{O_2} values (final P_{O_2} during a dive corrected to 0-m depth) were calculated to provide an estimate of the end-of-dive respiratory O_2 fraction (% O_2) and examine the magnitude and rate of respiratory O_2 depletion during diving. The end-of-dive air sac O_2 fraction decreased exponentially with dive duration (Fig. 7). Assuming that these air sac O_2 fractions are representative of the entire respiratory system, these calculations indicate that, depending on the duration of the dive, 42–100% of available air sac O_2 is consumed during the dive (Fig. 8). In dives with durations greater than the ADL, the end-of-dive air sac O_2 fraction ranged from 0 to 4%, corresponding to P_{O_2} values of 0–28 mmHg. Although an absolute value of 0% O_2 may be secondary to sampling or measurement artifact, these low values are consistent with significant depletion of the respiratory O_2 store during diving. The data from these long dives also demonstrate, as has been previously emphasized (Kooyman and Ponganis, 1998), that the onset of post-dive lactate accumulation in emperors is not associated with the complete depletion of all O_2 stores.

The rate of depletion of the entire respiratory O_2 store can be estimated from the diving respiratory volume (DRV) and the change in air sac O_2 fraction. For DRV, we have chosen the 69 ml kg^{-1} value measured during simulated dives of another deep-diving penguin, the king penguin (*A. patagonicus*; Ponganis et al., 1999). Although mean DRV during free dives may be greater than that during simulated dives in a pressure chamber (Sato et al., 2002), DRV in free-diving penguins appears to be variable and to increase with maximum depth of dive (Sato et al., 2002). Since the dives of emperors in this study were shallow, and since the 69 ml kg^{-1} value from the pressure chamber study was in the lower range

of values recorded in free-diving king penguins (Sato et al., 2002), we chose to use that value in our calculations.

The DRV and O_2 fraction calculations resulted in a mean respiratory O_2 store depletion rate of 2.1±0.8 ml O_2 kg^{-1} min^{-1} (Fig. 10). This value is about one-third the measured and predicted resting metabolic rate for a penguin of this body mass (Kooyman and Ponganis, 1994; LeMaho et al., 1976; Pinshow et al., 1977) and is much less than the 5–11 ml O_2 kg^{-1} min^{-1} respiratory O_2 depletion rates previously measured during forced submersions and simulated dives of other penguin species (Kooyman et al., 1973; Scholander, 1940). This supports the concept that respiratory O_2 consumption is low during diving, most likely regulated by reductions in heart rate and peripheral blood flow (Kooyman et al., 1992; Scholander, 1940). In addition, the respiratory O_2 store depletion rates of dives of emperor penguins decreased exponentially with diving duration and ranged from 1 to 5 ml O_2 kg^{-1} min^{-1} (Fig. 10), demonstrating that the rates of respiratory O_2 store depletion of individual dives were variable and dependent on the duration of the dive and probably also on dive behavior.

These calculations represent the first estimation of one component of the total O_2 store depletion rate and metabolic rate during the dive of an emperor penguin. They are consistent with hypothesized low diving metabolic rates, which were based on oxygen consumption during flume swimming, and on observed differences between wing beat frequency during flume swimming *versus* diving (Kooyman and Ponganis, 1994). These values also suggest that the O_2 consumption rate during a dive is much less than the FMR of emperors at the isolated dive hole (21 ml O_2 kg^{-1} min^{-1}; Nagy et al., 2001). Such a low diving metabolic rate would be consistent with the inability to distinguish differences in the Nagy et al. (2001) study between FMR of hand-fed penguins and FMR of birds that foraged for food.

Supported by NSF grant OPP-0229638. J.U.M. was supported by a NDSEG Fellowship. We thank Y. Habara for assistance in the field, H. Hanish and M. Loughry of UFI for design and construction of the P_{O_2} recorders, R. Robbins and the McMurdo dive chamber staff for use of the compression chamber, and G. L. Kooyman for manuscript review.

References

Bernstein, M. H., Duran, H. L. and Pinshow, B. (1984). Extrapulmonary gas exchange enhances brain oxygen in pigeons. *Science* **226**, 564-566.

Black, C. P. and Tenney, S. M. (1980). Oxygen transport during progressive hypoxia in high-altitude and sea-level waterfowl. *Res. Physiol.* **39**, 217-239.

Boggs, D. F., Baudinette, R. V., Frappell, P. B. and Butler, P. (2001). The influence of locomotion on air-sac pressures in little penguins. *J. Exp. Biol.* **204**, 3581-3586.

Burmester, T., Weich, B., Reinhardt, S. and Hankein, T. (2000). A vertebrate globin expressed in the brain. *Nature* **407**, 520-523.

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

Faraci, F. M. (1986). Circulation during hypoxia in birds. *Comp. Biochem. Physiol. A* **85**, 613-620.

Hudson, D. M. and Jones, D. R. (1986). The influence of body mass on the endurance to restrained submergence in the Pekin duck. *J. Exp. Biol.* **120**, 351-367.

Kerem, D. and Elsner, R. W. (1973). Cerebral tolerance to asphyxial hypoxia in the harbor seal. *Res. Physiol.* **19**, 188-200.

Kooyman, G. L. and Kooyman, T. G. (1995). Diving behavior of emperor penguins nurturing chicks at Coulman Island, Antarctica. *Condor* **97**, 536-549.

Kooyman, G. L. and Ponganis, P. J. (1994). Emperor penguin oxygen consumption, heart rate and plasma lactate levels druing graded swimming exercise. *J. Exp. Biol.* **195**, 199-209.

Kooyman, G. L. and Ponganis, P. J. (1998). The physiological basis of diving to depth: birds and mammals. *Ann. Rev. Physiol.* **60**, 19-32.

Kooyman, G. L., Schroeder, J. P., Greene, D. G. and Smith, V. A. (1973). Gas exchange in penguins during simulated dives to 30 and 68 m. *Am. J. Physiol.* **225**, 1467-1471.

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

LeMaho, Y., Delclitte, P. and Chatonnet, J. (1976). Thermoregulation in fasting emperor penguins under natural conditions. *Am. J. Physiol.* **231**, 913-922.

Nagy, K. A., Kooyman, G. L. and Ponganis, P. J. (2001). Energetic cost of foraging in free-diving emperor penguins. *Physiol. Biochem. Zool.* **74**, 541-547.

Pinshow, B., Fedak, M. A. and Schmidt-Nielsen, K. (1977). Terrestrial locomotion in penguins: it costs more to waddle. *Science* **195**, 592-594.

Pinshow, B., Bernstein, M. H., Lopez, G. E. and Kleinhaus, S. (1982). Regulation of brain temperature in pigeons: effects of corneal convection. *Am. J. Physiol.* **1982**, R577-R581.

Ponganis, P. J. and Kooyman, G. L. (2000). Diving physiology of birds: a history of studies on polar species. *Comp. Biochem. Physiol. A* **126**, 143-151.

Ponganis, P. J., Kooyman, G. L., Starke, L. N., Kooyman, C. A. and Kooyman, T. G. (1997). Post-dive blood lactate concentrations in emperor penguins, Aptenodytes forsteri. *J. Exp. Biol.* **200**, 1623-1626.

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

Ponganis, P. J., Van Dam, R. P., Marshall, G., Knower, T. and Levenson, D. H. (2000). Sub-ice foraging behavior of emperor penguins. *J. Exp. Biol.* **203**, 3275-3278.

Ponganis, P. J., Van Dam, R. P., Knower, T. and Levenson, D. H. (2001). Temperature regulation in emperor penguins foraging under sea ice. *Comp. Biochem. Physiol. A* **129**, 811-820.

Ponganis, P., Kooyman, G. L. and Ridgway, S. H. (2003a). Comparative Diving Physiology. In *Physiology and Medicine of Diving* (ed. A. O. Brubakk and T. S. Neuman), pp. 211-226. Edinburgh: Saunders.

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

Powell, F. L. and Hempelman, S. C. (1985). Sources of carbon dioxide in penguin air sacs. *Am. J. Physiol.* **248**, R748-R752.

Powell, F. L., Geiser, J., Gratz, R. K. and Scheid, P. (1981). Airflow in the avian respiratory tract: variations of O2 and CO concentrations in the bronchi of the duck. *Res. Physiol.* **44**, 195-213.

Sato, K., Naito, Y., Kato, A., Niizuma, Y., Watanuki, Y., Charassin, 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.

Scheid, P. (1979). Mechanisms of gas exchange in bird lungs. *Rev. Physiol. Biochem. Pharmacol.* **86**, 137-186.

Scholander, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalradets skrifter* **22**, 1-131.

Tamburrini, M., Condo, S. G., di Prisco, G. and Giardina, B. (1994). Adaptation to extreme environments: structure-function relationships in emperor penguin hemoglobin. *J. Mol. Biol.* **237**, 615-621.

Torre-Bueno, J. R., Geiser, J. and Scheid, P. (1980). Incomplete gas mixing in the air sacs of the duck. *Res. Physiol.* **42**, 109-122.

Van Dam, R. P., Ponganis, P. J., Ponganis, K. V., Levenson, D. H. and Marshall, G. (2002). Stroke frequencies of emperor penguins diving under sea ice. *J. Exp. Biol.* **205**, 3769-3774.