

## Patterns of respiration in diving penguins: is the last gasp an inspired tactic?

Rory P. Wilson<sup>1,\*</sup>, Alejandro Simeone<sup>1</sup>, Guillermo Luna-Jorquera<sup>2</sup>, Antje Steinfurth<sup>1</sup>,  
Sue Jackson<sup>3</sup> and Andreas Fahlman<sup>3</sup>

<sup>1</sup>*Institut für Meereskunde, Düsternbrooker Weg 20, D-24105 Kiel, Germany*, <sup>2</sup>*Depto. de Biología Marina, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile* and <sup>3</sup>*Human and Animal Physiology, Stellenbosch University, Private Bag XI, Matieland 7602, South Africa*

\*Author for correspondence

Accepted 24 February 2003

### Summary

Humboldt penguins *Spheniscus humboldti* in captivity and free-living Magellanic penguins *S. magellanicus* were fitted with loggers to determine beak angles during breathing. The Humboldt penguins were also fitted with masks for determining rates of air flow during breathing. During periods of higher gas exchange requirement, Humboldt penguins opened their beaks during inspiration, where tidal volume was linearly correlated with both change in beak angle and maximum beak angle, closed them slightly during the final stages of inspiration and finally closed them during expiration. Substantial differences were apparent between individuals. Contrary to the condition proposed for most birds, our data suggest that expiration is passive during periods of high respiratory tidal volumes, and that the increased resistance of the respiratory pathway serves to slow air flow so as to maximize gas exchange in the lungs. During foraging, Magellanic penguins at the surface between dives showed similar breathing patterns but maximum

beak angles were much higher and breath cycle time shorter, as would be expected for animals attempting to maximize gas exchange. Both maximum beak angle per breath and breath frequency changed systematically over the surface pause; both were initially high, then decreased to a low before rising again to a maximum just before diving. Based on known changes in tidal volume with beak angle derived from Humboldt penguins, a simple model is proposed to examine rates of gas exchange over the surface pause. This indicates that the observed patterns do not maximize the rate of transfer of oxygen over the whole of the surface pause but are rather concerned with an initial rapid accumulation of oxygen in the tissues followed by effective carbon dioxide release.

Key words: respiration, tidal volume, penguin, *Spheniscus humboldti*, *Spheniscus magellanicus*, surface interval between dives, breathing.

### Introduction

Air-breathing marine animals that find their food underwater must divide their time between periods spent underwater to seek food and intervals at the surface, where gas exchange takes place (Kooyman, 1989). Since, during foraging, time spent at the surface does not contribute directly to prey acquisition, animals can increase their efficiency by minimizing the time spent at the surface (Le Boeuf et al., 2000). This is a complex matter since the energetic, and thus the oxygen, demands of the animal underwater are highly variable, and depend, among other things, on dive depth (Kooyman, 1989; Williams et al., 1999), duration (Williams et al., 1992; Castellini et al., 1992) and swim speed (Culik and Wilson, 1991). Since the rate at which gas exchange between the air and body tissues takes place is dependent on the difference in the gas partial pressure between the two media, animals should dive with just enough oxygen to provide for fully aerobic dives where the oxygen supply is exhausted at the end of the dive (Kramer, 1988). This solution optimizes time

(Schoener, 1971; Carbone and Houston, 1996), but necessitates that animals be able to predict their own performance. If this were the case, all inter-dive intervals could be assessed as preparation for the dive to come. In fact, however, many authors have examined the duration of surface intervals on the assumption that they are solely a recovery response to the preceding dive (e.g. Croxall et al., 1991; Wanless et al., 1993). The reality is likely to be twofold: (i) intervals at the surface between dives nominally comprise a recovery period from the previous dive and a preparation for the dive to come, and (ii) we may, in any case, be erring in attempting to derive body oxygen norms on the basis of data obtained for resting animals. Clearly, non-exercising animals with virtually unlimited access to oxygen will have higher body oxygen concentrations than animals with high energy expenditure and limited access to oxygen. A similar parallel may be drawn using carbon dioxide. There is no obvious disadvantage to working with lower body oxygen concentrations apart from the question of reserves, and

indeed Kramer's premise indicates that there is much to be gained by so doing (Kramer, 1988). Ultimately, the higher the  $P_{O_2}$  gradient within and between tissues and inhaled air, the more rapidly oxygen will move into the tissues.

Although a great deal is known about the process of gas exchange in diving marine endotherms (e.g. Butler and Jones, 1997), there are few data that might illuminate the utility of different gas-exchange strategies on genuinely free-living animals (but see Le Boeuf et al., 2000), due to the logistic difficulties of this type of study. We used a new type of logger (Wilson et al., 2002) to quantify voluntary breathing patterns in captive Humboldt penguins *Spheniscus humboldti* and free-living, foraging Magellanic penguins *S. magellanicus*, in order to improve our understanding of respiration during surface intervals between dives. Acquired data were examined using a simple model to determine the extent to which rates of gas exchange might be affected and enhanced by observed breath frequency and derived tidal volume data.

## Materials and methods

### Laboratory work

Work was conducted on nine captive Humboldt penguins *Spheniscus humboldti* Meyen in the marine facility at the University Católica del Norte in Coquimbo, Chile. The birds were captured at Isla Pájaros (29°35'S), northern Chile, during June 2001, in accordance with the guidelines of the Chilean Undersecretariat for Fisheries (Subpesca) under scientific permit to G. L.-J. They were housed in two pens (each 4.5 m×3 m), each supplied with a small seawater pool (1.7 m×3 m×0.5 m depth) in which the birds could bathe and preen. Birds were fed daily *ad libitum* on anchovy *Engraulis ringens* and released at the site of capture after these (and other) experiments ceased in August 2001. The experiments below were the first conducted on these penguins.

### Measurement of beak angle

Individual penguins were removed from the group and fitted with the Inter-Mandibular Sensor (IMASEN) (Wilson et al., 2002). This consisted of a small Hall sensor (0.8 mm×2.5 mm diameter), highly sensitive to magnetic field strength, which was glued with two-component epoxy resin to the upper beak of the bird close to the tomium, approximately 30 mm from the rictus. This sensor was linked by a thin (0.95 mm diameter), teflon-coated cable to a logging unit placed on the bird's back and held in place by tape, following details given in Wilson et al. (1997). Attachment of the device took approximately 10 min, primarily determined by the drying time of the glue. The dimensions of the logger were 12.5 cm×2 cm. The logger itself consisted of electronics potted in resin powered by a lithium 3.6 V battery (DK Log-IM; Driesen und Kern GmbH, Am Hasselt 25, D-24576 Bad Bramstedt, Germany). The unit had a memory of 4 Mb and was set to store data on magnetic field strength (with 16 bit resolution) at a frequency of 5 Hz. Increasing the distance between the Hall sensor on one beak half and the magnet on the other beak half (as the beak opened)

resulted in a corresponding decrease in magnetic field strength that was sensed by the Hall device. Thus, the Hall sensor output was related to the beak angle. The Hall sensor output was calibrated to allow conversion into beak angle by setting the IMASEN to record and then allowing the penguin to bite wooden rods of known diameter at defined positions along the beak. The distance between the articulation and the point on the beak at which the rod was bitten was measured and the beak angle calculated *via* simple trigonometry. The Hall sensor output was then plotted against beak angle and curve-fitted to derive a general equation for the relationship between recorded output and beak angle.

### Measurement of rates of inspiration and expiration

The rates of air flow in breathing penguins were determined using a specially constructed blown-plastic mask, fashioned to fit snugly over a Humboldt penguin head. The joins between bird and mask were sealed with neoprene. With this arrangement the volume of air in the mask was approximately 350 ml, although free access to outside air was possible *via* a 60 mm long tube of initial i.d. 23 mm. A tiny plastic door (7 mm×5 mm) was located within this tube. This door was joined at its base to a finely turned stainless steel tube (0.6 mm e.d., 0.3 mm i.d.) pivoted on a nylon line to allow it to move freely, and hinged about its base. It was held nominally in the vertical position using the magnetic fields of three different neodymium boron magnets. One magnet (2 mm×2 mm×1 mm) was glued to the top of the door with the North-South fields facing parallel to the main surface of the door perpendicular to the long axis of the breathing tube. The two other magnets (each 7 mm×1.5 mm diameter) were placed longitudinally along the floor of the breathing tube, one on each side of the door, and so orientated that the closest poles to the door were the same as the adjacent pole on the door-associated magnet. This configuration held the door in a stable, central position irrespective of the orientation of the mask, without having to incur the vagaries of mechanical spring systems. However, air movement resulting from slight pressure differences on one side of the door caused it to lean to one side, the door position being relative to the direction and rate of air flow along the tube. The door position was recorded using a Hall sensor placed next to the door on the outside of the breathing tube. This sensor was connected to a DK Log-IM logger identical, in principle, to that used to measure penguin beak angle. Here, door position resulted in particular magnetic fields being produced in the vicinity of the sensor. Door position *via* the Hall sensor output relative to air flow rate and direction was calibrated by pumping air through the breathing tube (in the absence of a penguin) at known rates (0.25–18 l min<sup>-1</sup>) in the laboratory (0.05 l min<sup>-1</sup>).

Nine birds were equipped with IMASENs and fitted with the mask for periods of 9–30 min (some penguins tended to bite the mask and thus proved unsuitable for measuring beak opening solely as a function of breathing – see below) while rates of air in- and out-flow were logged with the Hall sensor at a frequency of 5 Hz. IMASENS were also set to record at

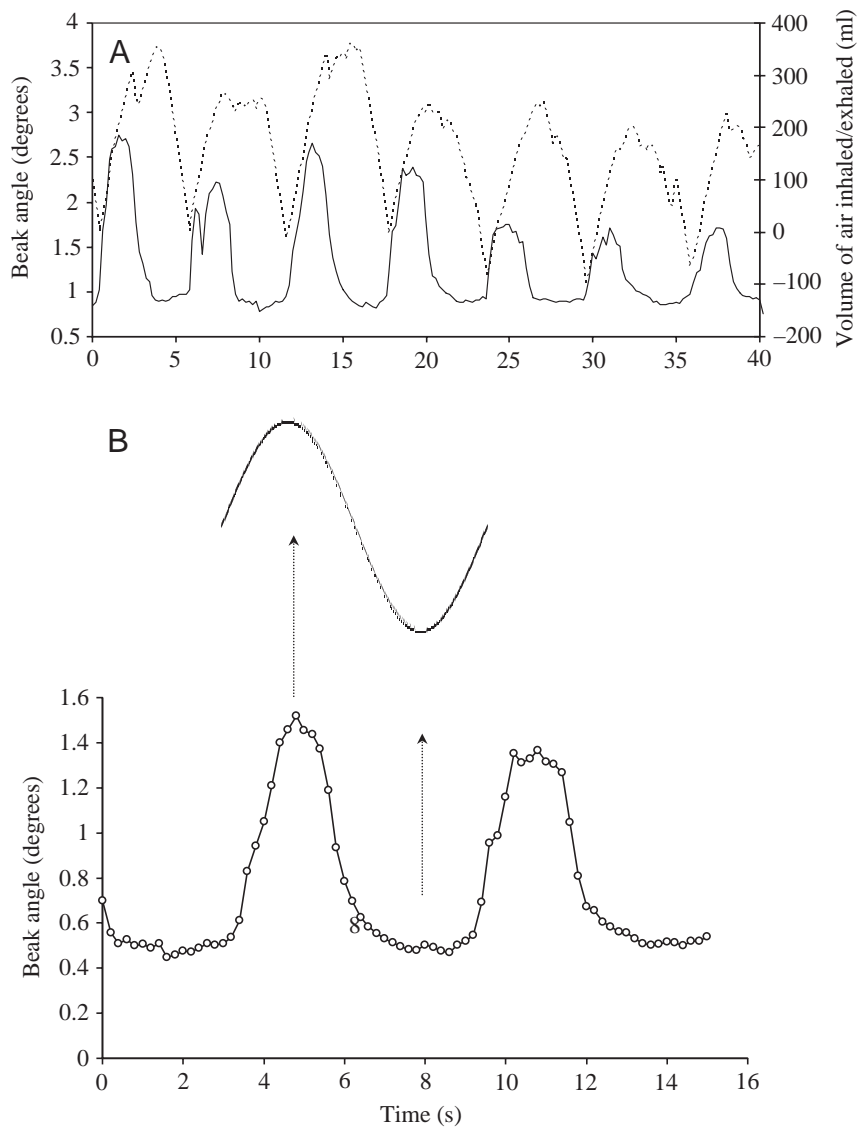


Fig. 1. (A) Continuous line: example of beak movement in a Humboldt penguin associated with approximately 7 breaths. The dotted line shows the increase and decrease of the air in the respiratory system over the course of the breath cycle. (B) Example of the beak movement of a Humboldt Penguin associated with 2.5 breaths. The line shows a peak and a trough translated (arrows) onto a pure sine wave (above) for comparison.

nest and equipped with IMASENs, set to record at 10 Hz, as described for the captive birds. Here, however, the cable linking the logger to the sensor was secured at two sites on the head (the top of the head and the nape of the neck) using a spot of epoxy resin enclosing the cable and binding it to the feathers (Wilson et al., 2002). In addition, birds were equipped with Lotek LTD 100 loggers (Lotek Marine Technologies, 114 Cabot Street, St John's, NF, A1C1Z8 Canada). These units recorded, among other things, depth (12 bit resolution) at 1 s intervals. The units were 57 mm long and 18 mm diameter, weighed 16 g in air (1.8 g in seawater) and were attached to the birds by neoprene-backed plastic leg rings. Birds were left to go on a single foraging trip before the devices were removed and the data downloaded.

Beak and depth data were treated simultaneously using the software MT-beak (Jensen Software Systems, Laboe, Germany). This software displayed the data on the screen in the form of two graphs against a time axis. The program identified deviations in beak opening events (with a threshold set by the user) and determined the start and end time of

the events and maximum values within the defined periods. These values were stored in a separate file together with data on depth.

## Results

### Laboratory work

During the experiments the penguins opened their beaks for a variety of reasons including preening, biting, vocalizing or swallowing (cf. Wilson et al., 2002). The following results, however, are confined to periods when the birds did none of these things. During breathing in penguins that were fully rested no beak movement was observed or recorded by the IMASEN. This constituted about 60% of the time that the birds were equipped with the unit. However, during periods of increased metabolic rate (see below), the beak opened and closed systematically so that inter-mandibular angle (hereafter referred to as beak angle) described a waveform over time (Fig. 1A). This waveform was essentially bilaterally

the same frequency, both devices being perfectly synchronized and double checked for time using external magnets brought into close contact with both Hall sensors simultaneously. The results obtained on the door positions within the mask during breathing were converted into air flow rates using the calibration data (see above). The point at which inspiration or expiration began was clear as a swing in the door position. Flow rates, starting at the beginning of each inspiration or expiration, were summed so as to derive the total amount of air inspired or expired, respectively, representing the tidal volume.

### Field work

Field work was conducted on Magellanic penguins *Spheniscus magellanicus* Forster between 24 November and 15 December 2000 and between 25 November and 3 December 2001 at Cabo Virgenes (52°24'S, 68°26'W), Santa Cruz, Argentina, with the approval of the Consejo Agrario de Santa Cruz. Five individuals brooding chicks were removed from the

Table 1. Comparison of time for one cycle and beak amplitude for 3 breathing Humboldt penguins

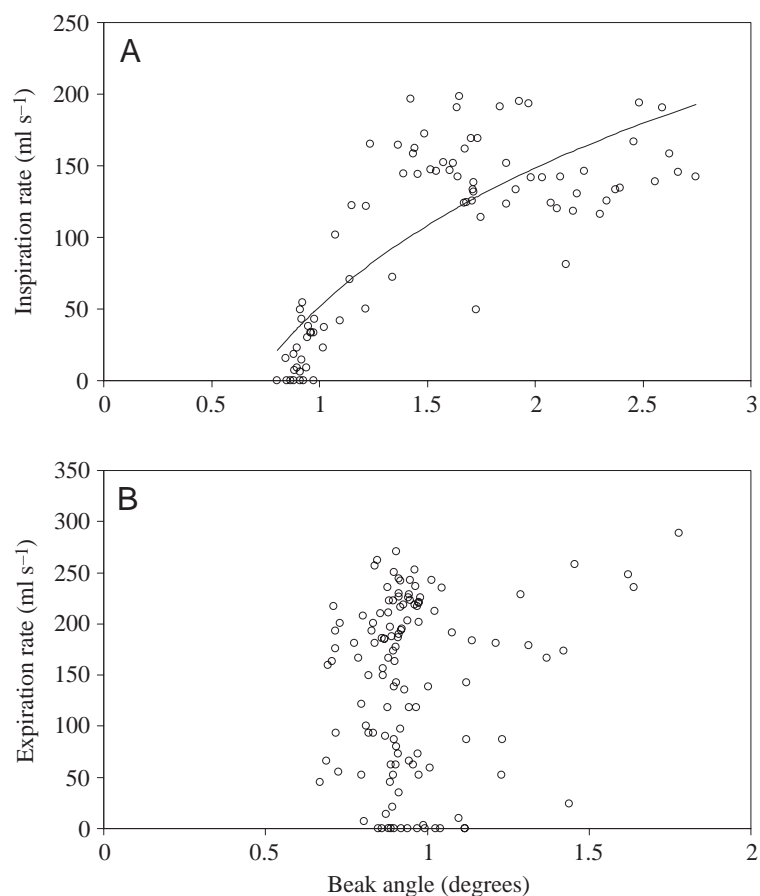
Bird number	Parameter	Mean±S.D.	N
4	Maximum beak angle (degrees)	4.25±1.32	81
4	Change in beak angle (degrees)	1.09±0.87	81
4	Cycle time (s)	5.02±1.25	81
6	Maximum beak angle (degrees)	1.58±0.56	35
6	Change in beak angle (degrees)	1.04±0.63	35
6	Cycle time (s)	2.64±0.67	35
7	Maximum beak angle (degrees)	1.06±0.37	58
7	Change in beak angle (degrees)	0.45±0.27	58
7	Cycle time (s)	4.51±1.76	58

Parameter relationships		a	b	r	F	P
4	Max beak angle <i>versus</i> cycle time	3.98	0.24	0.26	5.60	<0.05
4	Change in beak angle <i>versus</i> cycle time	–	–	0.01	0.00	>0.05
6	Max beak angle <i>versus</i> cycle time	–	–	0.14	0.60	>0.05
6	Change in beak angle <i>versus</i> cycle time	–	–	0.20	1.34	>0.05
7	Max beak angle <i>versus</i> cycle time	3.64	–0.94	–0.52	20.20	<0.001
7	Change in beak angle <i>versus</i> cycle time	3.01	–0.83	–0.34	7.00	<0.01

All birds that breathed at least 30 cycles consecutively, without biting, calling or swallowing.

S.D. standard deviation; N, number of readings.

When calculating the regression equations  $y$  is always taken to be the cycle time,  $x$  the other parameter,  $a$  the intercept and  $b$  the gradient.



symmetrical about its peak (Fig. 1B) but was not, however, sinusoidal, having long flat troughs and a narrow peak (Fig. 1). Typically, the width of the peak at half peak height was  $0.1\lambda$  (where  $\lambda$  is wavelength), this value being  $0.5\lambda$  in a sine wave function. It was notable that the beak was never completely closed during exhalation. We were able to record at least 30 cycles of breathing uninterrupted by changes in beak angle due to other factors such as swallowing, vocalisations, biting etc., in only three birds (numbers 4, 6 and 7), so that a reasonable frequency distribution could only be built up for these (lower numbers lead to poor definition of the distributions). Subsequent discussion refers to data only from these three birds, unless stated otherwise. All frequency distributions were normally distributed ( $P < 0.001$ ; Shapiro–Wilk or Kolmogoroff–Smirnov test, as appropriate) with mean cycle times in these individuals varying between being 2.64 and 5.02 s (Table 1). Both maximum beak angle (range of mean values 1.06–4.25°) and change in beak angle (range 0.45–1.09°) were also normally

Fig. 2. Rate of (A) inspiration and (B) expiration of air relative to beak angle during breathing in Humboldt penguins equipped with inter-mandibular sensors. The rate of inspiration was best described by the equation  $y = 51.6 + 149.7 \ln(\text{beak angle})$ .

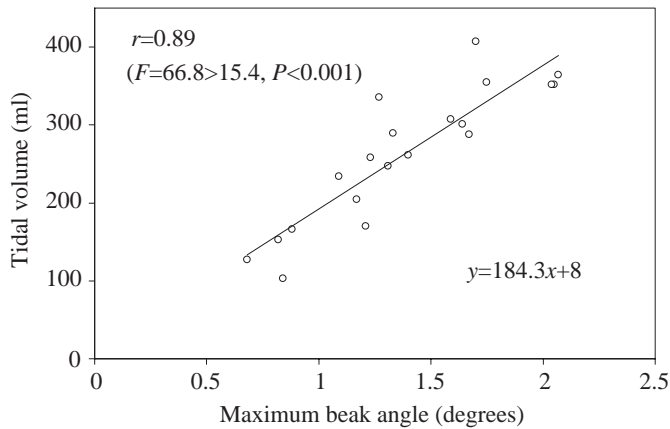


Fig. 3. Tidal volume per breath relative to maximum beak angle for a Humboldt penguin (Bird 3) equipped with an inter-mandibular sensor. Each point represents a single breathing cycle. Note that this bird only breathed for 20 consecutive breaths and thus is not included in Tables 1 and 2 (see text).

distributed in these individuals ( $P < 0.001$ ; Shapiro–Wilk or Kolmogoroff–Smirnov test, as appropriate) (Table 1). Breathing cycle time was related to maximum beak angle in two of three birds (albeit one positively and the other negatively) and change in beak angle over a cycle was (negatively) related to breathing cycle time in one individual (Table 1).

Air was inhaled just after the beak began its opening movement and continued until just before the beak closed (Fig. 1A). Here, the rate at which air was inhaled was dependent on beak angle, with the best fit being inspiration rate =  $51.6 + 139.7 \ln(\text{beak angle})$  ( $r = 0.80$ ,  $F = 150.0$ ,  $P < 0.001$ ) (Fig. 2A). Air was expired at the time the beak angle was minimal and there was no relationship between beak angle and the rate at which air was exhaled (Fig. 2B).

The result of the movements of air meant that the peak in beak angle was offset from the peak corresponding to the moment of maximum volume of air inspired (Fig. 1). However, there was a clear relationship between the tidal volume of any one breath and the maximum beak angle for the beak motion associated with that breath, as well as between the tidal volume of any one breath and the change in beak angle devoted to that breath (here the minimum beak angle is taken as zero) (Fig. 3). This relationship was not the same between individuals (Table 2).

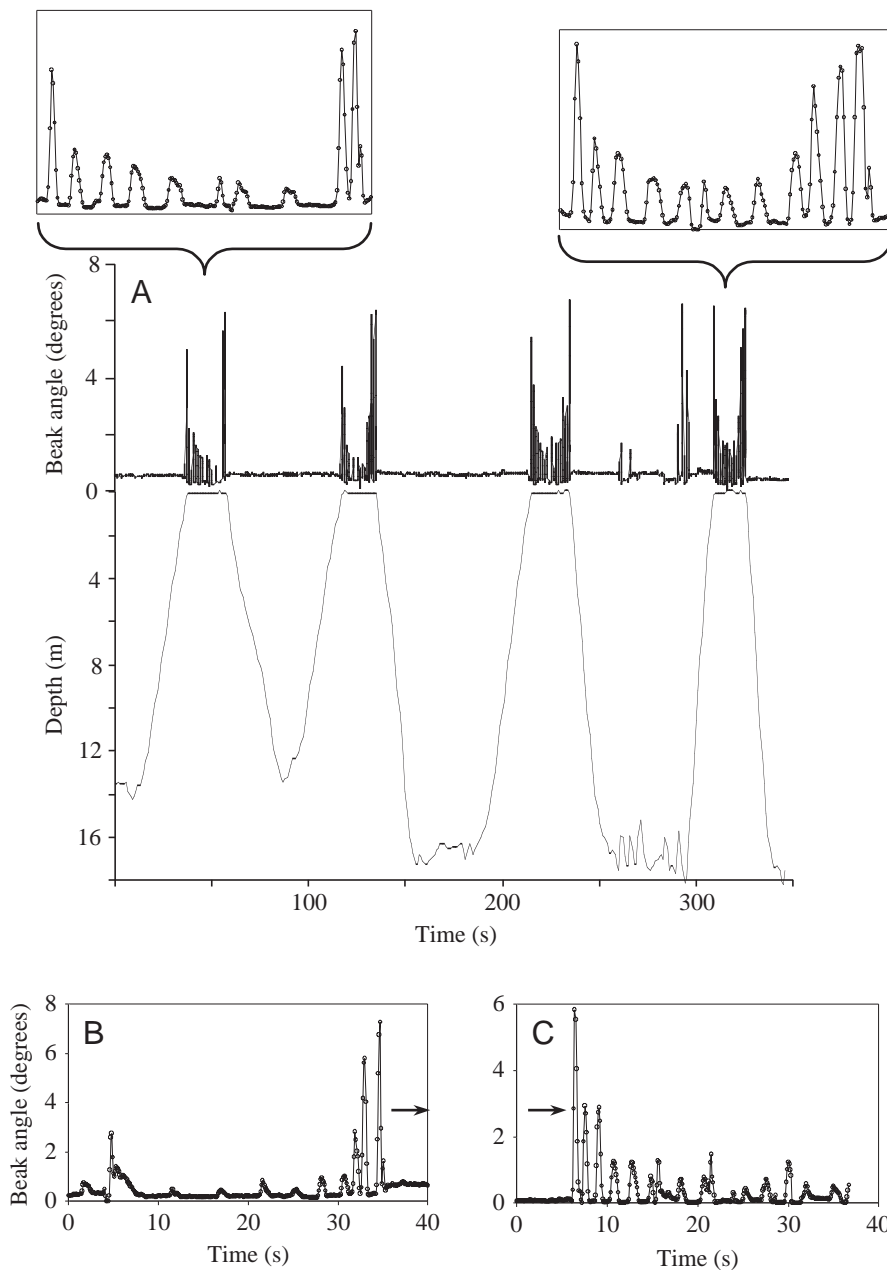


Fig. 4. Example of changes in beak angle in a Magellanic penguin during a foraging trip. (A) Beak angle in relation to depth over time with major systematic changes occurring whenever the bird was at the surface (see enlarged quadrats in insets at the top of the figure). The beak movement during the fourth dive is due to feeding. Beak angle (B) just prior to a dive after an extended period of rest at the surface and (C) after a dive and followed by extended rest at the surface. Note that in these two examples the trace of beak angle over time is not symmetrical about its mid-point (cf. A). Arrows show periods when the bird was underwater.

Table 2. Relationship between maximum beak angle or difference between maximum and minimum beak angles during a breath cycle and the tidal volume for four Humboldt penguins

Bird number	Peak angle (degrees)	Gradient (b)	Intercept (a)	<i>r</i>	<i>F</i>	<i>P</i>
4	Maximum	126.7	-179.6	0.75	14.37	<0.01
4	Difference	72.2	28.5	0.93	76.59	<0.001
6	Maximum	136.6	-10.8	0.82	24.02	<0.001
6	Difference	147.1	84.7	0.78	19.4	<0.001
7	Maximum	71.5	107.6	0.88	31.63	<0.001
7	Difference	73.7	156.8	0.91	40.98	<0.001
8	Maximum	171.2	23.2	0.93	37.02	<0.001
8	Difference	159.6	159.2	0.84	12.98	<0.05

Note that one bird (no. 8) did not breathe for more than 30 consecutive cycles (see text and Table 1) but is included for comparison.

$N=29$  consecutive breaths.

The regression takes the form: tidal volume (ml)=b(angle)+a, where b is gradient and a is intercept.

#### Field work

All five Magellanic penguins fitted with IMASENs in the wild were recovered in good health with the units having logged data for the full duration of the foraging trip. The birds executed 366–1707 dives, in total, during which they fed extensively (cf. Wilson et al., 2002). All the birds showed remarkably similar patterns of respiration, although individual differences in absolute beak angles were apparent (see below).

Two major types of beak movement were apparent when the birds were at the surface: (i) irregular beak openings and closing, considered to be due to preening and calling (see Wilson et al., 2002), which will not be further discussed here, and (ii) a highly regular series of beak opening and closing cycles (Fig. 4). In the surface intervals during normal dive bouts, however, beak angle amplitude changed systematically; immediately on surfacing beak angle amplitude was high, but this decreased to a minimum before increasing again to a maximum just before the bird dived (Fig. 4). This pattern was apparent irrespective of the number of breaths taken by the birds during the surface period, although the initial and final beak angle amplitudes increased with increasing number of breaths taken during the surface pause (Fig. 5).

#### Beak amplitudes during extended pauses at the surface

The U-shaped pattern of maximum beak angle per breath (see Figs 4, 5) was not apparent immediately prior to dives that occurred after a long pause at the surface, where beak amplitude per breath increased from a minimum to a maximum before the dive commenced, nor in surface pauses that followed dives but which were extended for some minutes or more, where the initial high beak amplitude was apparent but

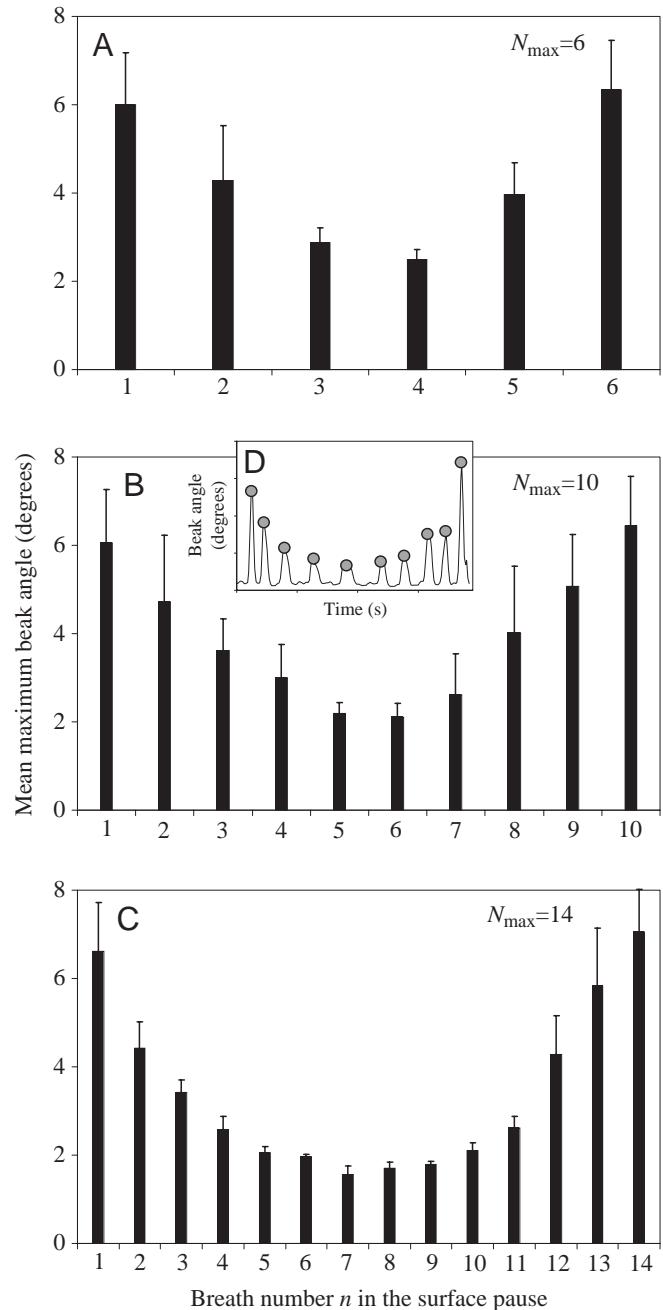


Fig. 5. Maximum beak angles as a function of breath sequence number ( $n$ ) in surface pauses consisting of three different total breath numbers;  $N_{\max}=6$  (A), 10 (B) or 14 (C). Values are derived from the means of five free-living Magellanic penguins and are means  $\pm$  S.E.M. Values shown are derived from a minimum of 5 readings per bird in all cases. The inset (D) shows how these values relate to a single typical 10-breath beak opening trace.

no second peak occurred. In other words, the initial high values decreased to a minimum immediately following a dive and appeared to be associated with recovery from the dive (Fig. 4C), whereas the minimum increasing to a maximum just prior to a dive appeared to be associated with dive preparation (Fig. 4B).

Table 3. Summary statistics for five Magellanic penguins equipped with intermandibular sensors

Bird number	Equation	$r^2$	$F$	$P$
y = breath cycle time (s), x = maximum beak angle (degrees)				
1	$y = -0.16x + 2.27$	0.11	26.2	<0.001
2	$y = -0.08x + 2.44$	0.22	154.5	<0.001
3	$y = -0.14x + 2.09$	0.36	133.4	<0.001
4	$y = -0.19x + 2.35$	0.13	52.1	<0.001
5	$y = -0.13x + 1.95$	0.16	1510.1	<0.001
y = length of surface pause (s), x = number of breaths in surface pause				
1	$y = 4.83\exp(0.129x)$	0.88	571.2	<0.001
2	$y = 5.07\exp(0.117x)$	0.83	545.5	<0.001
3	$y = 7.46\exp(0.081x)$	0.78	240.5	<0.001
4	$y = 6.04\exp(0.102x)$	0.82	399.2	<0.001
5	$y = 5.8\exp(0.09x)$	0.49	285.7	<0.001
y = number of post-dive breaths, x = dive duration (s)				
1	$y = 0.10x + 3.1$	0.52	80.8	<0.001
2	$y = 0.10x + 4.4$	0.4	75.4	<0.001
3	$y = 0.15x + 1.9$	0.37	37.8	<0.001
4	$y = 0.12x + 3.6$	0.62	157.6	<0.001
5	$y = 0.17x + 1.2$	0.45	236.4	<0.001

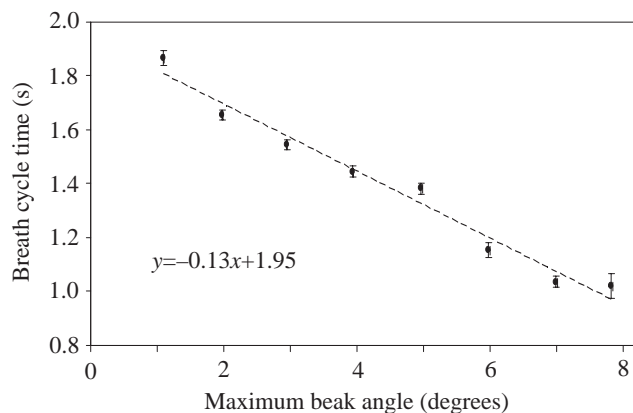


Fig. 6. Relationship between breath cycle time and maximum beak angle for that breath for a single Magellanic penguin foraging off Cabo Vírgenes, Argentina ( $r^2=0.16$ ,  $F=1510.1$ ,  $P<0.001$ ). Values are means  $\pm$  S.E.M. The relationships for the other individuals are shown in Table 3.

#### Breath cycle time and breath number during the surface pause

Breath cycle time changed systematically over the duration of the surface interval; cycle times were initially short, increasing to a maximum (that corresponded to the minimum in beak angle) before decreasing again to a minimum immediately prior to diving. As a consequence of the systematic changes in beak angle amplitude and breath cycle time over the surface interval, there was a clear relationship between breath cycle time and maximum beak angle for that

breath, with higher beak angle amplitudes being associated with short breath cycle times (Fig. 6, Table 3). In addition, the effect of the changing breath cycle time with breath number led to a roughly exponential relationship between the total number of breaths taken during the surface pause and time (Fig. 7, Table 3). Finally, the number of breaths taken during any surface interval within a dive bout (i.e. this does not include long periods of rest at the surface cf. Kooyman, 1989) was positively correlated with the duration of the preceding dive (Table 3).

#### Discussion

Examination of ventilation in endotherms is not a new concept, and studies have been conducted on ventilation rates (e.g. Cohn and Shannon, 1968; Schmidt-Nielsen et al., 1969; Berger et al., 1970; Butler and Woakes, 1979) and volumes (e.g. Smith, 1972). Typically, studies of this type require that the experimental subjects be restrained (Bech et al., 1972; Keijer and Butler, 1982) or at least in captivity (Lasiewski, 1972). Given the stress experienced by these experimental subjects (Le Maho et al., 1992) and the attendant physiological complications (Axelrod and Reisine, 1984), care must be exercised in interpreting the results. Recently, and to our knowledge for the first time, Le Boeuf et al. (2000) equipped free-living elephant seals *Mirounga angustirostris* with a system for recording rates of respiration during normal diving and travelling behaviour (cf. Chu, 1987). While animals equipped with external devices are known to display aberrant behaviour (e.g. Taylor et al., 2001), it is unlikely to be the same

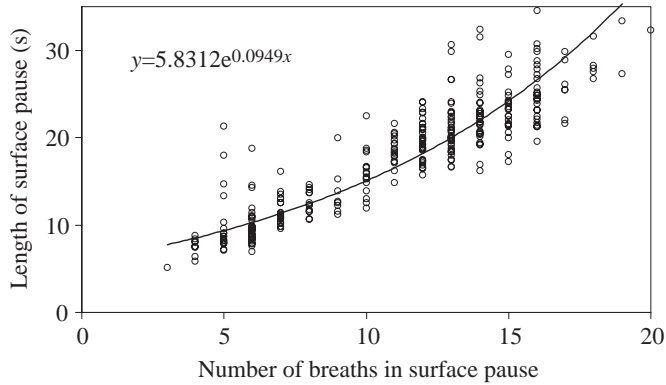


Fig. 7. Length of the surface pause as a function of the number of breaths taken during that pause in a single Magellanic penguin foraging off Cabo Vírgenes, Argentina ( $r^2=0.49$ ,  $F=285.7$ ,  $P<0.001$ ). The relationships for the other individuals are shown in Table 3.

as that seen in restrained animals or those in captivity. There is little information about the effects of the devices we used on penguins. The units constituted less than 2% of the body mass of Magellanic penguins (Gandini et al., 1992) and, more importantly, were for the most part located posteriorly to minimize the deleterious effects of increased drag (Bannasch et al., 1994). All equipped birds continued to breed normally but were only equipped for a single foraging trip. Although we believe that the devices we used were unlikely to affect the breathing patterns of the penguins substantially, further critical work will have to be conducted to assess the potential effects of the devices, particularly with respect to the cable used to connect the sensor to the logger.

#### *Breathing cycle of penguins on land*

The anatomy of the avian respiratory system is complex. Birds may breathe through either the nares or mouth, and have complicated oro-nasal structures that tend to filter out large particles (Powell, 2000); the oro-nasal structures are separated from the trachea by the larynx, which opens into the trachea through the slit-like glottis (McLelland, 1989). The laryngeal muscles contract during breathing so as to open the glottis during inspiration and decrease resistance to inspiratory air flow (Powell, 2000). The complexity of the air passages in the upper airways tends to increase the resistance to air flow through them. The rate of air flow can be approximated by Poiseuille's equation, whereby the volume of air per unit time  $dV/dt$  is given by:

$$dV/dt = (\pi r^4 \Delta P) / (8 \eta l), \quad (1)$$

where  $r$  is the radius of the tube,  $\Delta P$  is the pressure difference along the length of the tube,  $l$  is the length and  $\eta$  is the coefficient of viscosity. It is thus apparent that the radius, or a proxy for it (for example half the minimum passage width at any one point) is crucial in determining resistance to flow, this latter determining the energy that need be invested during inspiration and expiration.

In order to minimize the energy needed for the act of

inspiration, and the time taken for the air to be inspired, the resistance of air being drawn in through the beak must therefore also be minimized. This can be presumably achieved by opening the beak and breathing in through the mouth rather than the nares. However, the appropriate degree of opening is also dependent on the energy expended in the opening process (see below for an explanation of why the beak needs to be opened and closed with each respiratory cycle) and the extent of the desired tidal volume per unit time; greater flow rates require wider breathing apertures (see Equation 1 above). This interpretation is at least loosely supported by our results (Fig. 2A), although we believe that it is naive to assume that beak angle might be the sole factor in determining the width of the respiratory aperture. Inside a penguin's beak is highly complex, consisting of a large spiny tongue and numerous spiny and fleshy sections (Zusi, 1975; Wilson and Duffy, 1986), so that the relative position of these features is likely to prove critical in determining the resistance offered to the air during inspiration, particularly when the beak angle is small.

During rest, both inspiration and expiration are reported to require active contraction of the respiratory muscles, and the relaxed resting volume of the avian respiratory system is midway between the inspiratory and expiratory volumes (Seifert, 1896). An increase in ventilatory volume is probably achieved by recruiting more motor units in active muscles and additional respiratory muscles, both in inspiration and expiration (e.g. Kadono et al., 1963; Fedde et al., 1964). Exhalation in little penguins *Eudyptula minor* does not involve the abdominal muscles (Boggs et al., 2001). This, plus the fact that in penguins the interclavicular air sac is larger than the posterior air sac and undergoes greater pressure fluctuations which are coordinated with flipper movements during swimming, suggests that exhalation may be passive, or at least aided by swimming movements, in this and other species of penguins. We propose that when Humboldt penguins are breathing during periods of elevated metabolic rate, beak closure serves to regulate the flow of air, based on the principles covered by Poiseuille's equation (Equation 1). Were expiration to be active, beak closure, by reducing the effective radius of the exhalatory passage, would necessitate that birds work harder to achieve an appropriate rate of air flow which, in turn, would require an investment of energy. This is counter-intuitive, suggesting that exhalation may indeed be passive in this species. Our observations that penguins in the laboratory invariably exhaled with the beak minimally open and that the wave form of the beak angle in the breathing cycle deviated from a true sine wave by having long, low troughs, may reflect control of the speed of exhalation. Slow ventilation in the little penguin probably increases the efficiency of oxygen extraction (Stahel and Nicol, 1988), and slowing exhalation may prevent reflex inhibition of ventilation by intrapulmonary chemoreceptors that are stimulated when airway  $CO_2$  levels are reduced, as by rapid air passage (Furilla and Bernstein, 1995). Finally, partial beak closure during exhalation when tidal volumes are high may also reduce water and heat losses substantially by forcing air to exit through the nasal turbinates,



reclaiming both water and heat that would otherwise be lost (Geist, 2000). In herring gulls *Larus argentatus*, comparison of respiratory evaporative water loss between birds breathing oropharyngeally and through the nares has led to estimates of water savings by narial breathing as high as 71% and heat savings of 5.6% (Geist, 2000). The lack of a relationship between beak angle and expiration rate may be due to the  $r^4$  term in Poiseuille's equation, because when the respiratory aperture is very small, as was the case during expiration, minute changes can affect air flow rate substantially. This would further be compounded by small changes in, for instance, tongue orientation (see above).

The fact that, in Humboldt penguins at least, beak angle is related to, and probably determines, inspiration rate, and therefore tidal volume, means that respiration can be assessed in free-living birds. It is unfortunate, though not unexpected, that the relationship between tidal volume and beak angle shows such considerable individual variability (Fig. 3, Table 2), necessitating that all free-living penguins to be studied using this methodology need be subject to the mask calibration procedure in order to determine absolute values. Despite this, the linear relationship between beak angle and tidal volume in all individuals studied means that the situation for birds in the wild can at least be modelled using an appropriate equation.

#### *Breathing cycle of penguins at sea*

The most obvious difference between the patterns of breathing demonstrated by the free-living Magellanic penguins and the captive Humboldt penguins was one of degree. In general, the range of beak angles of the foraging birds was much higher (cf. Figs 3, 5). In that the change in beak angles of the Magellanic penguins on land was also very small, it seems likely that this is due to a difference in metabolic rates (or apparent metabolic rates, due to gas exchange occurring during the reduced surface interval corresponding to a relatively long period underwater) rather than some fundamental difference in bill construction or breathing apparatus between Magellanic and Humboldt penguins. In fact, the species are congeneric and remarkably similar in morphology and behaviour (Williams, 1995) so, although it would be unwise to use derived values inter-specifically, trends in measured parameters are likely to be the same.

#### *Can we allude to oxygen acquisition over the surface pause period?*

We have shown that there are a number of relationships between the parameters measured in the free-living birds (see above) which suggest causal links between them. In addition, data from captive Humboldt penguins show that tidal volume can be approximated by examining at maximum beak angle. If we assume that maximum beak angle in Magellanic penguins is also related to tidal volume, we can qualitatively examine the tidal volumes in sequential breaths for the whole of the surface pause in these free-living birds. Furthermore, the time that each inspiration is allowed to remain within the respiratory

system can be determined for the whole of the surface pause. If this information is coupled with a few assumptions based on what is known about gas diffusion rates under defined circumstances, we can even attempt to examine the rate at which oxygen might be expected to be acquired by the birds over the surface pause. The steps involved in this are described below.

Firstly, we need to know the maximum beak angle per breath ( $BA_{max}$ ) in relation to breath number within the sequence of breaths taken in the surface pause ( $n$ ) and the total number of breaths taken in the surface pause ( $N_{max}$ ). These values can be taken directly from means derived from the birds (e.g. Fig. 5).

The actual number of sequential breaths ( $N_{max}$ ) following dives of a particular duration ( $D_d$ ) can be predicted from the linear relationship between these two variables (Table 3), duration being a proxy for energy expenditure and therefore oxygen debt:

$$N_{max} = mD_d + c, \quad (2)$$

where  $m$  and  $c$  are constants. Since breath cycle time ( $T_{BC}$ ) is linearly related to maximum beak angle:

$$T_{BC} = c_1 - m_1 \times BA_{max}, \quad (3)$$

(Fig. 6, Table 3), the timing of each particular beak angle from each breath can be determined for all breaths in the sequence.

Assuming that the linear relationship between tidal volume ( $V_T$ ) and maximum beak angle observed in Humboldt penguins (Table 2) holds good for Magellanic penguins, so that:

$$V_T = m_2 \times BA_{max} + c_2, \quad (4)$$

then the tidal volume per breath can be calculated, as can the total amount of air that passes through the bird's lungs during the surface pause. Combining terms, the relationship between this total amount of air ( $A_T$ ) and the previous dive duration has the approximate form:

$$A_T = \sum_{n=0}^{n=N_{max}} V_T n = \sum_{n=0}^{n=N_{max}} (m_2 \times BA_{max} + c_2) n. \quad (5)$$

The rate of air passage can also be described by the individual terms within this form because tidal volume is related to breath cycle time. Furthermore, if we assume that the oxygen and carbon dioxide levels in the bird's body at the end of the surface pause always have particular values, as must be the case during 'steady state' diving where dive durations are relatively constant and the bird does not exceed its aerobic dive limit (cf. Butler and Woakes, 1984; Butler and Jones, 1997), we can allude to potential rates of oxygen absorption and carbon dioxide elimination over time.

At any one time, and assuming that blood flow to the lungs is constant, the rate of oxygen uptake into the body tissues ( $\dot{V}b_{O_2}$ ) is dependent on the difference in the partial pressure of oxygen between body tissues and lungs (Butler and Jones, 1997) so that:

$$\dot{V}b_{O_2} \propto (P_{lO_2} - P_{bO_2}), \quad (6)$$

where  $Pb_{O_2}$  is the partial pressure of oxygen in the body tissues and  $Pl_{O_2}$  that of the lungs. Thus,

$$\dot{V}b_{O_2} = k1(Pl_{O_2} - Pb_{O_2}), \quad (7)$$

where  $k1$  is a constant. Therefore, specifically, during any one breath, the amount of oxygen removed from the lungs ( $\Sigma O_2 =$  rate of acquisition of oxygen to the body) is given by:

$$\Sigma O_2 = V - \int_{t=0}^{t=T_{BC}} k1(Pl_{O_2} - Pb_{O_2})dt, \quad (8)$$

where  $V$  is the volume of oxygen inhaled per breath. Note that  $k1$  in this equation is often expressed as the time constant ( $\tau$ ) that is physiologically relevant and is directly related to the solubility of the gas, the blood perfusion and the volume of the tissue concerned. The change in  $Pb_{O_2}$  during breathing is assumed to be instantaneous and therefore equal to  $Pl_{O_2}$ . In turn,  $Pl_{O_2}$  is assumed to be equal to the ambient partial pressure for  $O_2$ , although this is simplistic because there is dead space mixing in the trachea and the addition of water vapour also affects values (cf. Powell, 2000). Furthermore, we have simplified the bird lung here to the situation found in the mammals, namely that of a fixed volume of air from which the oxygen is being removed. In reality the contact time of the air with the lung tissues may be considerably less, with new air, and consequently higher concentrations of oxygen, being continuously moved into the lungs from e.g. the caudal air sacs. However, we judge the mathematical expression of continuous air renewal, being subject to a particular rate of oxygen removal according to locality during the passage through the lungs, to be unnecessarily complex for a process which, in effect though not degree, amounts to the same as the situation found in mammalian lungs. Also implicit in this formulation is the assumption that the higher the breath frequency, the shorter the time that oxygen can be absorbed. While it is clear that the passage of air through the lungs is dependent on the complexities of the avian respiratory system (for a review, see Powell, 2000) and that the air might pass through the lungs over a longer period of the breath cycle time, the pattern for oxygen absorption over time will not change in form, even if it does in extent.

The overall increase in total oxygen  $O_{2,tot}$  within the body, therefore, over  $N_{max}$  breaths during a surface pause can be given by:

$$O_{2,tot} = \sum_{n=0}^{n=N_{max}} \left[ V - \int_{t=0}^{t=T_{BC}} k1(Pl_{O_2} - Pb_{O_2})dt \right]. \quad (9)$$

The pattern of the change in body oxygen incurred, therefore, by a Magellanic penguin during a surface pause consisting of a particular number of breaths can be derived by considering the energy expended during the dive and the amount of oxygen corresponding to this. For example, during normal swimming at a speed resulting in the lowest cost of transport, Humboldt penguins expend energy at a rate of  $10.8 \text{ W kg}^{-1}$  (Luna-Jorquera and Culik, 2000). The

respiratory quotient is 0.78 for Humboldt penguins (Luna-Jorquera and Culik, 2000) and a conversion factor of  $20 \text{ J ml}^{-1} O_2$  is appropriate for deriving  $\text{W kg}^{-1}$  (Eckert, 1993; Schmidt-Nielsen, 1993). Assuming this to be typical for the genus, a 4 kg *Spheniscus* penguin expends approximately  $2.2 \text{ ml } O_2 \text{ s}^{-1}$  swimming underwater. Thus, during a typical dive of approximately 74 s, a Magellanic penguin operating according to the patterns displayed in our results will expend approximately 163 ml  $O_2$  and then take 14 breaths (Table 3; e.g. bird 5) during a surface pause of 22 s (Fig. 7) to pay back this debt (plus the debt incurred for the metabolism at the surface during this time, which is likely to be of the order of  $1.1 \text{ ml s}^{-1}$ ; Luna-Jorquera and Culik, 2000). Reference to mean beak angles associated with each breath over the pause period and Fig. 7, above, allows calculation of breath cycle times so that tidal volume and hence the volume of oxygen ( $V$ ; see Equation 4 above) inhaled per breath (for convenience we shall ignore the relatively small amount of oxygen used for metabolic purposes during the surface pause). Assuming that the oxygen debt of the dive of 163 ml is paid back during the surface pause, we can present a scenario for the rate of uptake of oxygen during the surface pause using Equations 8 and 9 (Fig. 8). Note, however, that if the bird's oxygen deficit following the dive is only that incurred during the dive (163 ml in our example), this deficit can never be made good in the time available at the surface, irrespective of the value of the constant  $k1$ . This is due to the perpetually reducing difference in oxygen partial pressures between lungs and blood. In fact, for the bird to be able to dive and rest in a steady state, it must always dive with an oxygen deficit (Fig. 8). Kramer (1988) pointed out that this is a desirable state of affairs in a slightly different context because, in order to minimize time at the surface and increase foraging efficiency, diving animals should only submerge with enough oxygen to cover their needs for the duration of the dive; substantial (non-anaerobic) oxygen debts subsequent to a dive result in a rapid rate of oxygen uptake. In this general context, greater oxygen deficits result in faster oxygen uptake rates, which allows birds to have a highly variable pause duration (cf. Wanless et al., 1993) yet still operate effectively. Shorter pauses result in a greater deficit, which is automatically and iteratively corrected during the next surface pause due to the higher rate of transfer of oxygen from the air to the tissues.

#### *Why is the breathing pattern during the surface pause so shaped?*

The breathing pattern apparent during the surface pause, with an initially high tidal volume and rapid breathing rate followed by a reduction in both these parameters before rising again until the subsequent dive (Fig. 5), is not the pattern that should be observed if the penguin were only trying to maximize rate of oxygen gain into the tissues. For example, taking the model used to derive Fig. 8A, to simulate the rate of oxygen uptake in the tissues during consistently high

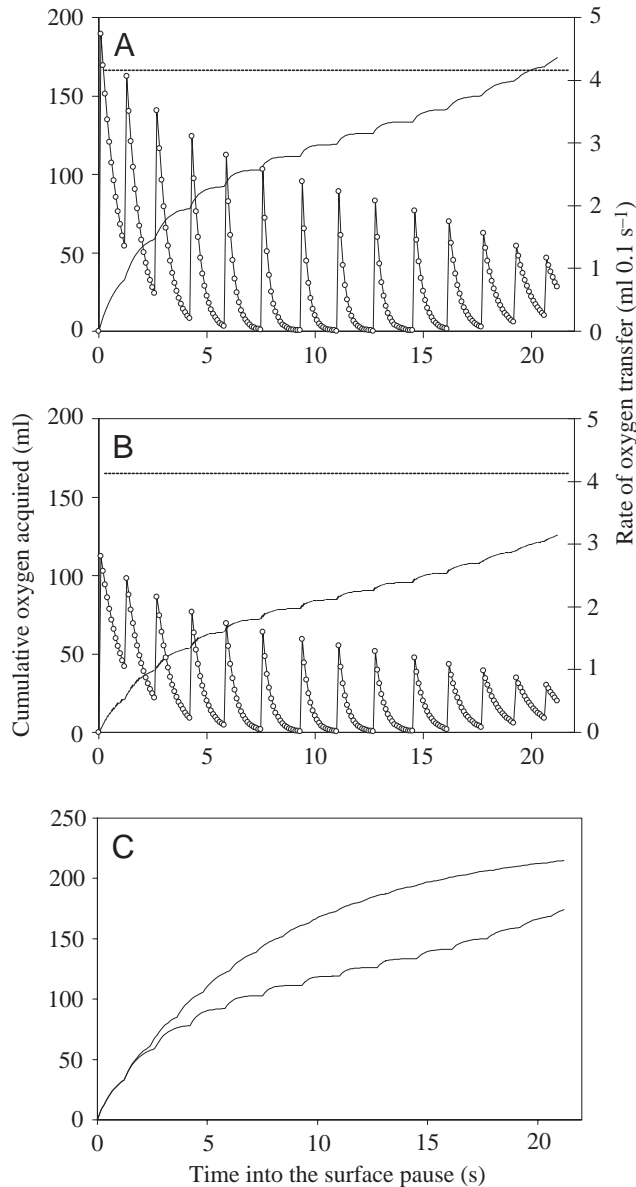


Fig. 8. Derived rate of oxygen transfer from the lungs to the blood (open circles) and cumulative oxygen acquired by the blood (ascending line) during 14 breaths in a single Magellanic penguin that was recovering from a dive lasting 74 s. Tidal volume was taken to be linearly related to maximum beak angle (Table 2) and equivalent to 50 ml deg<sup>-1</sup>. Maximum beak angle per breath was taken from the means of presented data (cf. Fig. 5) and corresponding breath cycle times were taken from curve fits derived from Fig. 6 (Table 3). The bird was assumed to breathe through the lungs for the complete cycle time. Total body oxygen storage was taken to be 232 ml (see text). The bird was calculated as having an oxygen debt due to the dive of 163 ml (see text – dotted horizontal lines) and  $k_1$  in the equation describing the rate of oxygen transfer (see Equation 7 in text) was nominally given the value of 0.05. (A) A bird having an oxygen debt at the onset of the dive of 57 ml, increasing to 220 ml at the end of the dive. Here, the bird manages to repay the oxygen debt incurred during the dive in full by the end of the surface period. (B) The bird surfacing with an oxygen debt due uniquely to the energy expended during the dive. Here, the bird cannot repay this oxygen debt within the surface pause. (C) Oxygen acquisition as in A (lower line) compared to the acquisition of oxygen by a bird breathing according to the conditions given by the first breath (tidal volume 277 ml and breath cycle 1.2 s) for the full duration of the rest period (upper line). Note here the change in scale on the y-axis.

breathing frequencies and high tidal volumes (Fig. 8C), the overall rate of oxygen transfer is higher. Apparently, therefore, penguins could reduce time spent at the surface by deep, fast breathing. Why do they not do this instead of consistently showing the characteristic double modality in tidal volumes with reduced ventilation rates between (Fig. 5)?

In order to explain this we need to invoke another factor, the most likely candidate being CO<sub>2</sub> elimination. If the rate of CO<sub>2</sub> elimination is directly proportional to the partial pressure difference between body CO<sub>2</sub> and inspired CO<sub>2</sub>, in a manner similar to that postulated for oxygen, there can be no real advantage in the breathing pattern observed since the amount of CO<sub>2</sub> in the body decreases over the surface pause in a manner approximating to exponential decay (Fig. 9). However, two things make this scenario unlikely. Firstly, haemoglobin oxygen saturation is a major factor affecting the position of the CO<sub>2</sub> equilibrium curve (the Haldane effect) (Scheipers et al.,

1975; Powell, 2000). The physiological effect of this is to promote CO<sub>2</sub> unloading into the lungs when the blood is oxygenated. Secondly, the CO<sub>2</sub> contained within the body exists in the form of a bicarbonate ion, or combined with terminal amine groups in haemoglobin or in solution. Release of CO<sub>2</sub> is complicated, being partly catalysed by carbonic anhydrase (Maren, 1967), and certainly incurs a greater time lag than does the release of oxygen. Since CO<sub>2</sub> can only be released when the blood is associated with the lungs, we postulate that at any one time the oxygen entering blood *via* the lungs does not induce CO<sub>2</sub> release fast enough for it to enter the lungs immediately, but that the more oxygenated blood circulates, during which time the processes releasing the CO<sub>2</sub> take place so that this gas is then finally liberated the next time the unit of blood containing it passes the vicinity of the lungs

During the course of the surface pause between dives, the breathing patterns exhibited by Magellanic penguins will tend to lead to initial log-type, followed by linear, increases in blood oxygen concentrations (Fig. 8). However, if CO<sub>2</sub> loss is proportional to the degree of oxygenation of the tissues, high rates of CO<sub>2</sub> elimination may only occur after a substantial amount of the oxygen debt has been repaid. As in the case of O<sub>2</sub> uptake, high tidal volumes coupled with high breathing frequencies will tend to increase rates of gaseous diffusion so that the second peak in beak angle, which occurs just before the dive, might be primarily devoted to elimination of CO<sub>2</sub>, whose liberation is facilitated by the previous increment in blood oxygen (Fig. 9). This possibility is supported by pre-dive increases in respiratory exchange ratios that were reported recently for captive freely diving grey seals (Boutillier et al., 2001), reflecting elevated end-tidal  $P_{CO_2}$  and supplying

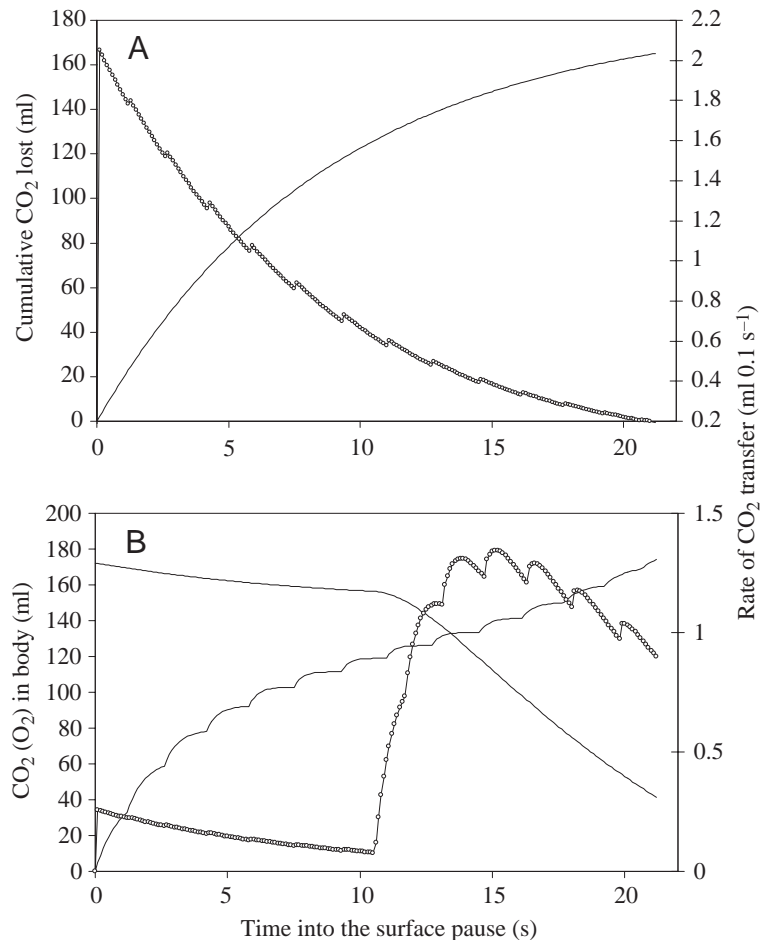


Fig. 9. Carbon dioxide lost by a Magellanic penguin over the course of a surface pause lasting 22 s after having completed a dive of 74 s. Conditions are as in Fig. 8 and total CO<sub>2</sub> in the body at the onset of the pause is taken to be  $220 \times 0.78 = 172$  ml (derived from a respiratory quotient RQ in penguins of 0.78; see text). (A) The rate of CO<sub>2</sub> transfer is taken to be directly proportional to the difference in CO<sub>2</sub> partial pressure between total body stores and air in the respiratory system, with the rate constant  $k_2$  nominally taken to be 0.04. (B) The rate of CO<sub>2</sub> transfer is initially taken to be constant (with a rate constant of 0.005) until tidal volume reaches a minimum (at 10.6 s into the surface pause), whereupon the rate constant is then taken to be directly proportional to the oxygen levels in the body (cf. Fig. 8A) with  $k_2$  set at 0.0005.

evidence for the 'flushing out' of CO<sub>2</sub> immediately before dives. These authors emphasised that readjustment of body CO<sub>2</sub> stores is slower than that of O<sub>2</sub> stores, and may govern inter-dive intervals. Earlier work on captive Humboldt penguins likewise showed increased ventilation just before dives (Butler and Woakes, 1984).

Note that, in a steady state situation, the bird must balance both O<sub>2</sub> and CO<sub>2</sub> losses with gains and it would seem, superficially, that only one of these parameters may dictate the length of the surface pause. This may be achieved, in part, as a result of the iterative and automatic balancing of O<sub>2</sub> and CO<sub>2</sub> according to concentration; the rate of O<sub>2</sub> replacement increases with increasing difference between lungs and tissues and CO<sub>2</sub> exchange is favoured by high blood O<sub>2</sub> levels. Any bird that does not equilibrate its gas levels appropriately during a particular surface pause following a dive is more likely to manage at the next due to the change in body gas concentrations, which affect the subsequent rate of gas exchange accordingly.

Overall, therefore, we reason that diving penguins should attempt to minimize surface time (Schoener, 1971, 1986) although the energetic consequences of so doing should also be considered (Perry and Pianka, 1997). With this as a basis, and since simple replacement of oxygen cannot account for the

patterns we see (Fig. 9), we propose that carbon dioxide release, operating under particular time- and blood oxygen saturation-dependent factors, is a major factor in determining the length and form of penguin inter-dive ventilation behaviour. High breathing frequencies and tidal volumes, typical of the initial and final breaths in a surface pause, are presumably coupled with increased energy expenditure due to the physical process of shunting air around the respiratory system as defined by Poiseuille's formulation, and will result in an appreciable quantity of the oxygen gained per unit time having to be used to operate the respiratory mechanism. In a complete formulation, patterns observed by us must also incorporate this in the solution so that where high respiration rates occur, the gain in oxygen (for example at the beginning of the pause) or loss of carbon dioxide (at the end of the pause) can act to justify the increase in both oxygen usage and carbon dioxide production that this respiratory pattern entails. Reduced tidal volumes and respiratory frequencies, such as occur in the middle of the surface pause, would auger for the execution of some time-dependent process. However, the increase in the length of this relatively relaxed period with increasing number of breaths in any particular surface pause (Figs 5, 6), which appears to be coupled with initial and final tidal volumes and ultimately with oxygen use during the dive (Fig. 8), would appear to be linked to body oxygen and carbon dioxide levels both pre- and post-dive. We have no information on this and thus, despite a relatively complex analysis, still cannot equate our observations with the predictions made by Kramer (1988). Miniaturization of appropriate sensors will allow us to resolve this key element in the question of time optimization in diving endotherms.

Support for this work was given by the grant FONDECYT 1010250 (to G.L.-J.), grants from the Chicago Zoological Society, the Riverbanks Zoological Park and Botanical Garden Conservation Support Fund, the German Academic Exchange Service (to A.S.) and ICSU (to R.P.W.). We are also grateful to Felipe Sepúlveda, Julieta Muñoz and Silvina Sturzenbaum for logistic and other help.

## References

- Axelrod, J. and Reisine, T. D.** (1984). Stress hormones: their interaction and regulation. *Science* **224**, 452-459.
- Bannasch, R., Wilson, R. P. and Culik, B.** (1994). Hydrodynamic aspects of design and attachment of a back-mounted device in penguins. *J. Exp. Biol.* **194**, 83-96.
- Bech, C., Johansen, K. and Maloiy, G. M. O.** (1972). Ventilation and expected gas composition in the flamingo, *Phoenicopterus ruber*, during normal respiration and panting. *Physiol. Zool.* **52**, 313-328.
- Berger, M. J., Hart, J. S. and Roy, O. Z.** (1970). Respiration, oxygen consumption and heart rate in some birds during rest and flight. *Z. vergl. Physiol.* **66**, 201-214.
- Boggs, D. F., Baudinette, R. V., Frappell, P. B. and Butler, P. J.** (2001). The influence of locomotion on air-sac pressures in little penguins. *J. Exp. Biol.* **204**, 3581-3586.
- Boutillier, R. G., Reed, J. Z. and Fedak, M. A.** (2001). Unsteady-state gas exchange and storage in diving marine mammals: the harbor porpoise and gray seal. *Am. J. Physiol.* **281**, R490-R494.
- Butler, P. J. and Jones, D. R.** (1997). Physiology of diving of birds and mammals. *Physiol. Rev.* **77**, 837-899.
- Butler, P. J. and Woakes, A. J.** (1979). Changes in heart rate and respiratory frequency during natural behaviour of ducks, with particular reference to diving. *J. Exp. Biol.* **79**, 283-300.
- Butler, P. J. and Woakes, A. J.** (1984). Heart rate and aerobic metabolism in Humboldt penguins, *Spheniscus humboldti*, during voluntary dives. *J. Exp. Biol.* **108**, 419-428.
- Carbone, C. and Houston, A. I.** (1996). The optimum allocation of time over the dive cycle: an approach based on aerobic and anaerobic respiration. *Anim. Behav.* **51**, 1247-1255.
- Castellini, M. A., Kooyman, G. L. and Ponganis, P. J.** (1992). Metabolic rates of freely diving Weddell seals: Correlations with oxygen stores, swim velocity and diving duration. *J. Exp. Biol.* **165**, 181-194.
- Chu, K. C.** (1987). Dive times and ventilation patterns of singing humpback whales. *Can. J. Zool.* **66**, 1322-1327.
- Cohn, J. E. and Shannon, R.** (1968). Respiration in unanesthetized geese. *Resp. Physiol.* **5**, 259-268.
- Croxall, J. P., Naito, Y., Kato, A., Rothery, P. and Briggs, D. R.** (1991). Diving patterns and performance in the Antarctic blue-eyed shag *Phalacrocorax atriceps*. *J. Zool. Lond.* **225**, 177-199.
- Culik, B. M. and Wilson, R. P.** (1991). The energetics of underwater swimming in Adeline penguins (*Pygoscelis adeliae*). *J. Comp. Physiol. B* **161**, 285-291.
- Eckert, R.** (1993). *Tierphysiologie*. 2<sup>nd</sup> edition. Stuttgart: Georg Thieme Verlag.
- Fedde, M. R., Burger, R. E. and Kitchell, R. L.** (1964). Electromyographic studies of the effects of bilateral, cervical vagotomy on the action of the respiratory muscles of the domestic duck. *Poult. Sci.* **43**, 1177-1184.
- Furilla, R. A. and Bernstein, M. H.** (1995). Intrapulmonary rise time and ventilation in ducks. *J. Appl. Physiol.* **79**, 1397-1404.
- Gandini, P., Frere, E. and Holik, T.** (1992). Implicancias de las diferencias en el tamaño corporal entre colonias para el uso de medidas morfométricas como método de sexado en *Spheniscus magellanicus*. *Hornero* **13**, 211-213.
- Geist, N. R.** (2000). Nasal respiratory turbinate function in birds. *Physiol. Biochem. Zool.* **73**, 581-589.
- Kadono, H., Okada, T. and Ono, K.** (1963). Electromyographic studies on the respiratory muscles of the chicken. *Poult. Sci.* **42**, 121-128.
- Keijer, E. and Butler, P. J.** (1982). Volumes of the respiratory and circulatory systems in tufted and mallard ducks. *J. Exp. Biol.* **101**, 213-220.
- Kooyman, G. L.** (1989). *Diverse Divers*. Berlin, Springer-Verlag.
- Kramer, D. L.** (1988). The behavioral ecology of air breathing by aquatic animals. *Can. J. Zool.* **66**, 89-94.
- Lasiewski, R. C.** (1972). Respiratory function in birds. In *Avian Biology 2* (ed. D. S. Farner and J. R. King), pp 287-342. New York: Academic Press.
- Le Boeuf, B. J., Crocker, D. E., Grayson, J., Gedamke, J., Webb, P. M., Blackwell, S. B. and Costa, D. P.** (2000). Respiration and heart rate at the surface between dives in northern elephant seals. *J. Exp. Biol.* **203**, 3265-3274.
- Le Maho, Y., Karmann, H., Briot, D., Handrich, Y., Robin, J., Mioskowski, E., Cherel, Y. and Farni, J.** (1992). Stress in birds due to routine handling and a technique to avoid it. *Am. J. Physiol.* **263**, R775-R781.
- Luna-Jorquera, G. and Culik, B. M.** (2000). Metabolic rates of swimming Humboldt penguins. *Mar. Ecol. Progr. Ser.* **203**, 301-309.
- Maren, T. H.** (1967). Carbonic anhydrase: Chemistry, physiology and inhibition. *Physiol. Rev.* **47**, 595-781.
- McLelland, J.** (1989). Larynx and trachea. In *Form and Function in Birds* (ed. A. S. King and J. McLelland), pp. 69-104. London: Academic Press.
- Perry, G. and Pianka, E. R.** (1997). Animal foraging: past present and future. *Trends Evol. Ecol.* **12**, 360-364.
- Powell, F. L.** (2000). Respiration. In *Avian Physiology*, fifth edition (ed. G. C. Whitton), pp. 233-264. San Diego: Academic Press.
- Scheipers, G., Kawashiro, T. and Scheid, P.** (1975). Oxygen and carbon dioxide dissociation of duck blood. *Resp. Physiol.* **24**, 1-13.
- Schmidt-Nielsen, K., Kanwisher, R. C., Lasiewski, R. C., Cohn, J. E. and Bretz, W. L.** (1969). Temperature regulation and respiration in the ostrich. *Condor* **71**, 341-352.
- Schmidt-Nielsen, K.** (1993). *Animal Physiology: Adaptation and Environment*. Cambridge: Cambridge University Press.
- Schoener, T. W.** (1971). Theory of feeding strategies. *Annu. Rev. Ecol. Syst.* **2**, 369-404.
- Schoener, T. W.** (1986). A brief history of optimal foraging theory. In *Foraging behavior* (ed. A. C. Kamil, J. R. Krebs and H. R. Pulliam), pp. 5-67. New York: Plenum Press.
- Seifert, E.** (1896). Über die Atmung der Reptilien und Vögel. *Pflügers Arch. Ges. Physiol.* **64**, 321-506.
- Smith, R. M.** (1972). Circulation, respiratory volumes and temperature regulation of the pigeon in dry and humid heat. *Comp. Biochem. Physiol.* **43A**, 477-490.
- Stahel, C. D. and Nicol, S. C.** (1988). Ventilation and oxygen extraction in the little penguin (*Eudyptula minor*), at different temperatures in air and water. *Resp. Physiol.* **71**, 387-398.
- Taylor, S. S., Boness, D. J. and Majluf, P.** (2001). Foraging trip duration increases for Humboldt Penguins tagged with recording devices. *J. Avian Biol.* **32**, 369-372.
- Wanless, S., Corfield, T., Harris, M. P., Buckland, S. T. and Morris, J. A.** (1993). Diving behaviour of the shag *Phalacrocorax aristotelis* (Aves: Pelecaniformes) in relation to water depth and prey size. *J. Zool. Lond.* **231**, 11-25.
- Williams, T.** (1995). *The Penguins*. Oxford: Oxford University Press.
- Williams, T. D., Friedl, W. A., Fong, M. L., Yamada, R. M., Sedivy, P. and Haun, J. E.** (1992). Travel at low energetic cost by swimming and wave-riding bottlenose dolphins. *Nature* **355**, 821-823.
- Williams, T. D., Haun, J. E. and Friedl, W. A.** (1999). The diving physiology of bottlenose dolphins (*Tursiops truncatus*). I. Balancing the demands of exercise for energy conservation at depth. *J. Exp. Biol.* **202**, 2739-2748.
- Wilson, R. P. and Duffy, D. C.** (1986). Prey seizing in African Penguins *Spheniscus demersus*. *Ardea* **74**, 211-214.
- Wilson, R. P., Pütz, K., Peters, G., Culik, B., Scolaro, J. A., Charrassin, J.-B. and Ropert-Coudert, Y.** (1997). Long term attachment of transmitting and recording devices to penguins and other seabirds. *Wildl. Soc. Bull.* **25**, 101-106.
- Wilson, R. P., Steinfurth, A., Ropert-Coudert, Y., Kato, A. and Kurita, M.** (2002). Lip-reading in remote subjects: An attempt to quantify and separate ingestion, breathing and vocalisation in free-living animals. *Mar. Biol.* **140**, 17-27.
- Zusi, R. L.** (1975). An interpretation of skull structure in penguins. In *The Biology of Penguins* (ed. B. Stonehouse), pp. 59-84. London: Macmillan.