

## RESEARCH ARTICLE

# Cervical air sac oxygen profiles in diving emperor penguins: parabronchial ventilation and the respiratory oxygen store

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## ABSTRACT

Some marine birds and mammals can perform dives of extraordinary duration and depth. Such dive performance is dependent on many factors, including total body oxygen ( $O_2$ ) stores. For diving penguins, the respiratory system (air sacs and lungs) constitutes 30–50% of the total body  $O_2$  store. To better understand the role and mechanism of parabronchial ventilation and  $O_2$  utilization in penguins both on the surface and during the dive, we examined air sac partial pressures of  $O_2$  ( $P_{O_2}$ ) in emperor penguins (*Aptenodytes forsteri*) equipped with backpack  $P_{O_2}$  recorders. Cervical air sac  $P_{O_2}$  values at rest were lower than in other birds, while the cervical air sac to posterior thoracic air sac  $P_{O_2}$  difference was larger. Pre-dive cervical air sac  $P_{O_2}$  values were often greater than those at rest, but had a wide range and were not significantly different from those at rest. The maximum respiratory  $O_2$  store and total body  $O_2$  stores calculated with representative anterior and posterior air sac  $P_{O_2}$  data did not differ from prior estimates. The mean calculated anterior air sac  $O_2$  depletion rate for dives up to 11 min was approximately one-tenth that of the posterior air sacs. Low cervical air sac  $P_{O_2}$  values at rest may be secondary to a low ratio of parabronchial ventilation to parabronchial blood  $O_2$  extraction. During dives, overlap of simultaneously recorded cervical and posterior thoracic air sac  $P_{O_2}$  profiles supported the concept of maintenance of parabronchial ventilation during a dive by air movement through the lungs.

**KEY WORDS:** Antarctica, Anterior air sacs, Avian lung, *Aptenodytes forsteri*, Parabronchus, Partial pressure of oxygen, Posterior air sacs

## INTRODUCTION

Many marine endotherms are able to perform dives of extraordinary duration and depth. Such dive performance is dependent on many factors, including increased oxygen ( $O_2$ ) stores, the decreased heart rate and redistribution of blood flow associated with the cardiovascular dive response, hypoxemic and pressure tolerance of tissues, body size, hydrodynamics and efficient swim patterns (Butler and Jones, 1997; Kooyman and Ponganis, 1998; Ponganis,

2015). Increased  $O_2$  stores and management of those stores, located in the respiratory system, blood, and muscle, are especially critical to extended dive durations (Ponganis et al., 2011). Although the respiratory  $O_2$  store fraction in pinnipeds and cetaceans is usually <20% and often <10% of the total  $O_2$  store (Burns et al., 2007; Noren et al., 2012; Ponganis, 2015; Weise and Costa, 2007), in marine birds such as murres, auklets, tufted ducks (*Aythya fuligula*) and Adélie penguins (*Pygoscelis adeliae*), the air sacs and lungs contain approximately 50% of the total body  $O_2$  store (Chappell et al., 1993; Croll et al., 1992; Elliott et al., 2010; Keijer and Butler, 1982; Yamamoto et al., 2011). Similarly, in king (*Aptenodytes patagonicus*) and emperor penguins (*Aptenodytes forsteri*), the respiratory  $O_2$  store is approximately one-third the total body  $O_2$  store (Ponganis et al., 2015; Sato et al., 2002, 2011). Despite the importance of the respiratory system in diving birds, we only have a limited understanding of the air volumes inhaled prior to dives, the transfer of  $O_2$  from air sacs and lungs to blood during dives, and the movement of air between the air sacs and lungs for gas exchange during dives.

There are three important considerations necessary to understand the role of the air sacs and lungs during dives: (1) the anatomy of the avian respiratory system and unidirectional air flow; (2) the diving air volume (DAV) and distribution of air between air sacs and lungs; and (3) how gas exchange might occur during dives.

## Unidirectional air flow

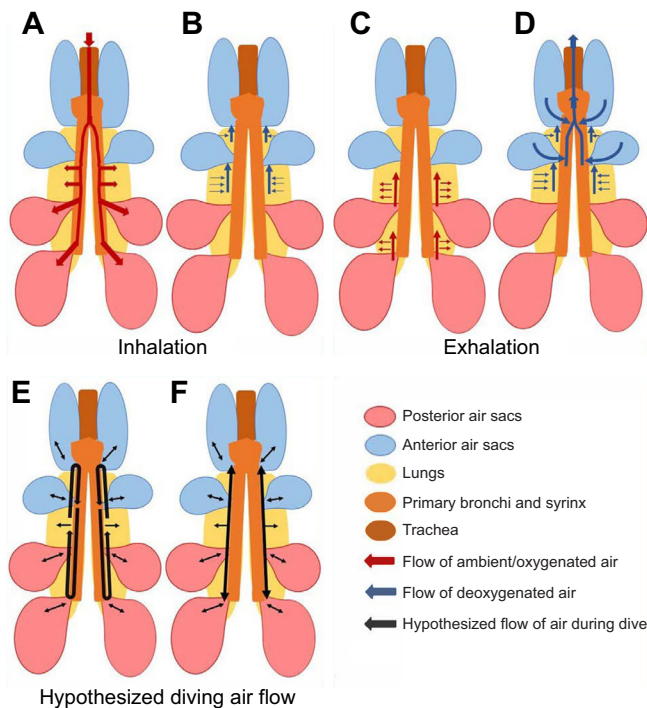
The avian respiratory system includes both lungs and air sacs. The volumes of the lungs of birds, including marine birds, scale allometrically and are small relative to those of similarly sized mammals (Lasiewski and Calder, 1971; Maina et al., 1989; Ponganis, 2015). The air sacs comprise a much larger portion of the respiratory system. In hens and ducks, the volume of the air sacs is approximately 10× larger than lung volume (Scheid, 1979; Scheid et al., 1974). Gas exchange occurs only in the lungs, specifically, in the air capillaries, which extend from the parabronchi, while the air sacs do not participate in gas exchange (Duncker, 1972; Fedde, 1998; Maina, 2006). Typically, respiratory air movement in eupneic birds occurs through unidirectional air flow. During inhalation, ambient air enters the lungs and posterior air sacs, while air already in the lungs moves into the anterior air sacs (Fig. 1A,B). During exhalation, air moves from the posterior air sacs into the lungs, while air already in the lungs and anterior air sacs exits through the trachea (Fig. 1C,D). However, the exact distribution and magnitude of air movement into and out of the lungs, air sacs and interconnecting bronchi during inspiration and expiration are still not completely documented (Maina, 2006; Scheid, 1979).

As a result of unidirectional air flow,  $O_2$  levels in the anterior and posterior air sacs will differ. Because air in the anterior air sacs has already undergone gas exchange in the lung, it reflects gas concentrations in the distal parabronchus. Consequently, the

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**Fig. 1. Unidirectional air flow in birds and hypothesized flow of air during dives.** (A) Ambient air enters the lungs and posterior air sacs during inhalation. (B) Simultaneously, deoxygenated air moves out of the lungs and into the anterior air sacs. (C) During exhalation, air moves from the posterior air sacs to the lungs. (D) Deoxygenated air exits from the lungs and anterior air sacs through the trachea during the same exhalation. (E) Hypothesized unidirectional air flow in the lungs with air moving back and forth between the anterior and posterior air sacs via the bronchial system. (F) Hypothesized bi-directional air flow in the lungs between the anterior and posterior air sacs. Branches of bronchus and interclavicular air sac not shown.

partial pressure of  $O_2$  ( $P_{O_2}$ ) is uniform among the different anterior air sacs, as confirmed in laboratory studies of domestic fowl and geese (Brackenbury et al., 1981; Piiper et al., 1970; Scheid et al., 1989). In these studies,  $P_{O_2}$  in the anterior air sacs was 20–30 mmHg less than posterior air sac  $P_{O_2}$ , which was closer to inspired  $P_{O_2}$  (Brackenbury et al., 1981; Piiper et al., 1970; Scheid et al., 1989). During the hyperventilation of exercise, anterior air sac  $P_{O_2}$  increased relative to values at rest (Brackenbury et al., 1981). This increase in anterior air sac  $P_{O_2}$  is considered secondary to a higher  $P_{O_2}$  exiting the distal parabronchus owing to greater parabronchial ventilation relative to parabronchial  $O_2$  extraction by pulmonary blood (Brackenbury et al., 1981; Fedde, 1998; Scheid, 1979; Scheid and Piiper, 1970; Shams and Scheid, 1987). In other words, because the blood  $O_2$  extraction through pulmonary capillaries along the parabronchus is not matched to the increased ventilation through the parabronchus during exercise, air exiting the lungs into the anterior air sacs has a higher  $P_{O_2}$  than at rest.

### Diving air volume

While air volumes of the lungs and air sacs of freely diving birds have not been directly measured, they have been estimated with end-of-dive buoyancy calculations (Sato et al., 2002, 2011). In several penguin species, estimated DAVs increased with maximum depth in apparent anticipation of deep dives (Sato et al., 2002, 2011; Williams et al., 2012). These values were greater than volumes measured in simulated dives in pressure chambers (Kooyman et al., 1973; Ponganis et al., 1999), but were much less than maximum

inspiratory volumes measured with computerized tomographic (CT) scans during anesthesia (Ponganis et al., 2015). In the CT study, regardless of inflation pressure, the volume of air sacs in emperor penguins was distributed with 40% in the anterior air sacs and 60% in the posterior air sacs and the volume of the penguin lung remained fairly constant at 18 ml  $kg^{-1}$  (Ponganis et al., 2015).

### Gas exchange during dives

In simulated dives in pressure chambers, gas exchange continued at the deepest depths of the studies, 68 m in Adélie penguins and 136 m in king penguins (Kooyman et al., 1973; Ponganis et al., 1999). To make use of the entire respiratory  $O_2$  store, birds would have to move air from the air sacs to the lungs for gas exchange during dives. During flight and terrestrial exercise, increased ventilation rates provide adequate movement of the air within the lungs and air sacs (Boggs, 1997, 2002; Butler, 1991). However, during dives, neither the path of air movement nor the mechanism to move air within the respiratory system is understood (Boggs et al., 2001). There are two possible paths for air movement from the air sacs through the parabronchi and into the gas-exchanging air capillaries (Fig. 1). This could be achieved by a circular pathway with unidirectional flow from the posterior air sacs to the anterior air sacs, and then from the anterior air sacs to the posterior air sacs via the bronchial system (Fig. 1E). Bi-directional air flow in the lungs back and forth between the anterior and posterior air sacs is the other potential path (Fig. 1F). One hypothesized mechanism to facilitate such air movement during breath holds is high frequency differential pressure oscillations between the anterior and posterior air sacs (generated through limb movements) (Boggs et al., 2001). Other possible mechanisms of air movement between air sacs during dives include underwater exhalation of air from the anterior air sacs, and mixing of air in the lungs and air sacs owing to depth-related compression/re-expansion of air sacs (Ponganis, 2015).

To investigate this question and the role and mechanism of parabronchial ventilation and  $O_2$  utilization both on the surface and during dives, we measured air sac  $P_{O_2}$  of emperor penguins. These birds are capable of exploiting the water column to depths over 500 m and dive durations as long as 27.6 min (Sato et al., 2011; Wienecke et al., 2006). Although 95% of dives during foraging trips are <6 min and <200 m, dives of 8 to 10 min to >400 m regularly occur in the Ross Sea (Kooyman et al., 2020; Kooyman and Kooyman, 1995; Sato et al., 2011; Wienecke et al., 2006). Such dive capacity is dependent on many factors, including increased  $O_2$  storage and efficient  $O_2$  management. The respiratory  $O_2$  store is particularly important for birds. Previous studies have demonstrated the contributions of the air sacs and lungs during both surface intervals and dives of emperor penguins, including: (1) increasing the  $P_{O_2}$  in both arterial and venous blood prior to longer dives; (2) maintenance of arterial hemoglobin (Hb) saturation above 90% for most of the duration of even 10-min-long dives; (3) end-of-dive arterial  $P_{O_2}$  values >75 mmHg for dives at the 5.6 min aerobic dive limit (ADL, the dive duration associated with the onset of post-dive blood lactate accumulation); and (4) post-dive recovery times of approximately 2 min for Hb and myoglobin (Mb) saturations (Meir and Ponganis, 2009; Ponganis et al., 2009, 2007; Williams et al., 2011).

Research on diving emperor penguins with backpack  $P_{O_2}$  recorders demonstrated that posterior air sac  $P_{O_2}$  at the start of dives was close to that of ambient air (Stockard et al., 2005). Air sac  $P_{O_2}$  initially underwent a compressive hyperoxia during descent, and then progressively declined throughout the dive sometimes to near-zero values by the end of some dives. Anterior air sac  $P_{O_2}$  measurements were not feasible at the time of the study. Recent

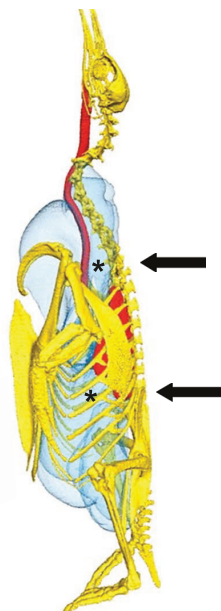
three-dimensional CT scan reconstructions of emperor penguins (Ponganis et al., 2015) have delineated the anatomy of the respiratory system and have better defined the location of anterior and posterior air sacs within the body (Fig. 2). Therefore, we attempted to monitor the  $P_{O_2}$  profiles of a representative anterior air sac (cervical air sac) in diving emperor penguins for documentation and comparison with  $P_{O_2}$  profiles from a representative posterior air sac (posterior thoracic air sac) (Stockard et al., 2005).

We had several hypotheses. First, anterior air sac (measured via the cervical air sac)  $P_{O_2}$  at rest would be much lower than the anterior air sac values reported in other birds. This is based on a previously measured slow respiratory rate of 4 breaths  $\text{min}^{-1}$  and a relatively low arterial  $P_{O_2}$  at rest (Meir et al., 2008; Ponganis et al., 2007). Second, cervical air sac  $P_{O_2}$  prior to a dive would be greater than that at rest. This is consistent with the hyperventilation of penguins and increases in arterial  $P_{O_2}$  observed prior to dives of emperor penguins (Ponganis et al., 2007; Wilson et al., 2003). Third, the size of the respiratory  $O_2$  store would be less than earlier estimates because of a lower pre-dive cervical air sac  $P_{O_2}$  than previously assumed. Fourth, the change between start-of-dive and end-of-dive  $O_2$  fractions in the cervical and posterior thoracic air sacs would result in a more accurate assessment of the depletion of the respiratory  $O_2$  store and its contribution to  $O_2$  consumption during a dive. Fifth, cervical air sac  $P_{O_2}$  would eventually reach levels previously observed in the posterior thoracic air sac owing to mixing of air between the anterior and posterior air sacs during diving. Simultaneous monitoring of both cervical and posterior thoracic air sac  $P_{O_2}$  profiles would be attempted to evaluate such mixing and its relationship to stroke rate and dive duration.

## MATERIALS AND METHODS

### Field camp

In early November 2019, five non-breeding emperor penguins (*Aptenodytes forsteri* Gray 1844; 20.5–25.7 kg) were collected near



**Fig. 2.** Three-dimensional reconstructions from CT scans of emperor penguins illustrate the respiratory and skeletal systems (Ponganis et al., 2015). The trachea (red), lungs (red), air sacs (blue) and skeleton (yellow) are highlighted in this lateral view of a penguin inflated to 40 cm  $H_2O$  airway pressure. Arrows and asterisks indicate  $P_{O_2}$  electrode probe sites.

the Cape Washington colony (74°40'S, 164°28'E) and transported by Twin Otter plane to a research camp (77°41'S, 165°59'E) on the sea ice of McMurdo Sound, Antarctica. As in past studies (Meir and Ponganis, 2009; Ponganis et al., 2007; Williams et al., 2011), the birds were maintained at the corralled, isolated dive hole of the camp for 5 weeks, after which they were released out onto the sea ice of McMurdo Sound. The birds were allowed to dive spontaneously to forage primarily on the sub-ice fish *Pagothenia borchgrevinki* (Ponganis et al., 2000).

### Experimental studies

Penguins were equipped with backpack recorders for periods of 2 to 3 days (Ponganis et al., 2007). As in prior studies, instrumentation occurred under general anesthesia (isoflurane –  $O_2$ ) in the evening, followed by overnight recovery (Ponganis et al., 2007; Stockard et al., 2005). Penguins were then allowed to dive and forage spontaneously for 2 days, after which they were re-anesthetized for removal of instrumentation. After anesthetic induction by mask, birds were intubated for instrumentation and their vital signs were monitored, including heart rate (electrocardiogram), respiratory rate and temperature (Ponganis et al., 2007). All procedures were approved by the University of California San Diego Institutional Animal Care and Use Committee (S16071) and under Antarctic Conservation Act permit number 2020-002.

### Instrumentation

$O_2$  electrodes (Licox C1.1 Revoxode, Integra LifeSciences, Plainsboro, NJ, USA) were percutaneously inserted into the cervical and posterior thoracic air sacs (Fig. 2) with sterile technique through a 4.0 or 4.5 Fr peel-away introducer (Cook Medical, Bloomington, IN, USA), and secured to a 2.0 surgical suture in the skin with Tesa® tape (Beiersdorf AG, Hamburg, Germany). Prior to insertion, each  $P_{O_2}$  electrode was heparin coated with an aseptic 1-min immersion in 7% TDMAC heparin solution (Polysciences, Warrington, PA, USA). Both  $P_{O_2}$  electrodes were connected with waterproof cables (SeaCon, El Cajon, CA, USA) to a custom-built microprocessor (UFI, Morro Bay, CA, USA) inside a waterproof housing (300 g with recorder, 3.5×11 cm, Marine Science Development Shop, Scripps Institution of Oceanography, La Jolla, CA, USA) that was mounted mid-line to feathers on the back with Loctite® epoxy glue (Henkel Corp., Westlake, OH, USA) and metal cable ties. The UFI recorder logged  $P_{O_2}$  (two channels) and depth at 1 Hz. A Mk10-X tri-axial accelerometer–time depth recorder (Wildlife Computers, Redmond, WA, USA; 70 g, 3.7×5.7×2.3 cm; sensitive to 0.5 m depth, depth at 1 Hz, tri-axial acceleration at 16 Hz) was also attached with epoxy glue and cable ties to feathers on the lower back.

### Electrode calibration

As in the previous air sac  $O_2$  study (Stockard et al., 2005), the  $P_{O_2}$  electrodes were calibrated in sterile fashion in 0.9% saline-filled 10 ml vacutainer tubes (Becton Dickinson Co., Rutherford, NJ, USA) 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 and room air for a 21%  $O_2$  value. We calibrated  $P_{O_2}$  electrodes, as well as report  $P_{O_2}$  data, at 38°C for several reasons. First, air sac temperatures during dives are primarily between 38 and 39°C and are unrelated to dive duration (Stockard et al., 2005). Second, inserting thermistors into the air sacs in addition to  $P_{O_2}$  electrodes was considered

infeasible. And third, although electrode output does change with temperature, a  $\leq 1^\circ\text{C}$  temperature change would result in a minimal change in  $P_{\text{O}_2}$  ( $<5\%$   $^\circ\text{C}^{-1}$  per manufacturer specifications), which would not alter data interpretation.

### Data analysis

After removal of the recorders, the data were downloaded to personal computers. Depth data were processed with a dive analysis program (WC-DAP, Wildlife Computers). Dives were defined as submergences deeper than 10 m and exceeding 2 min to focus on dives where  $\text{O}_2$  use would likely be greater. Start-of-dive and end-of-dive times were recorded to the nearest second when the bird crossed a depth threshold of 2 m. Stroke rates were calculated from the accelerometry data measured on the  $x$ - or  $z$ -axis at 16 Hz with use of a custom-written algorithm in MATLAB (The MathWorks, Natick, MA, USA) (Tift et al., 2017). In brief, a 0.2 Hz high-pass Butterworth filter was used to filter out low frequency static acceleration resulting from gravitational acceleration and momentum. A power spectral density analysis was performed on the accelerometry data to identify the dominant stroke frequency for each animal (approximately 0.8–1.0 strokes  $\text{s}^{-1}$ ) and a peak detection algorithm was used to identify individual strokes (Jeanniard-du-Dot et al., 2016; Sato et al., 2003) with a peak magnitude greater than or equal to  $1.0 \text{ m s}^{-2}$ . We used  $1.0 \text{ m s}^{-2}$  to define individual strokes because the peak amplitude of strokes was typically at or above  $1.5 \text{ m s}^{-2}$  and signal noise was rarely higher than  $0.5 \text{ m s}^{-2}$ .

### $P_{\text{O}_2}$ data analysis

A prior evaluation of this type of  $P_{\text{O}_2}$  electrode demonstrated that the 90% response time from 0 to 148 mmHg is approximately 48 s (Stockard et al., 2005). During dives, air sac  $P_{\text{O}_2}$  will change as a result of  $\text{O}_2$  consumption, and it will also increase and decrease as a direct result of changing hydrostatic pressure. The lag time of the electrode limits how quickly the electrode responds to the actual instantaneous air sac  $P_{\text{O}_2}$  during rapid changes in depth. In addition,  $P_{\text{O}_2}$  values at depth may be beyond the calibration range of the electrode (0–21%  $\text{O}_2$ ) owing to increased hydrostatic pressure. In the original evaluation of these electrodes (Stockard et al., 2005), low  $P_{\text{O}_2}$  values were more accurately recorded with a 0 to 21%  $\text{O}_2$  calibration than with a 0 to 100%  $\text{O}_2$  calibration. Consequently, the analysis and interpretation of  $P_{\text{O}_2}$  profiles focus primarily on surface data and start- and end-of-dive data.

The  $P_{\text{O}_2}$  electrode output was converted to  $P_{\text{O}_2}$  with the electrode's calibration curve in Origin (version 2018, OriginLab Corp., Northampton, MA, USA). The  $P_{\text{O}_2}$  data were synchronized with the depth and stroke rate data from the Mk10-X recorder by matching the depth profiles from the two recorders. Data from individual dives were extracted with a custom-written program in R (<https://www.r-project.org/>) using the cowplot (<https://CRAN.R-project.org/package=cowplot>), lubridate (Grolemund and Wickham, 2011) and RcppRoll (<https://CRAN.R-project.org/package=RcppRoll>) packages. Graphs were plotted in Origin (version 8.6). Results are expressed as means $\pm$ s.d.  $P_{\text{O}_2}$  data are expressed as measured in mmHg (7.5 mmHg=1 kPa). Start-of-dive  $P_{\text{O}_2}$  values were the first  $P_{\text{O}_2}$  value after the penguin reached or passed the 2 m depth threshold. Because  $P_{\text{O}_2}$  values were typically flat prior to a dive, the pre-dive value was the mean  $P_{\text{O}_2}$  over 30 s prior to start-of-dive. End-of-dive  $P_{\text{O}_2}$  values were the last  $P_{\text{O}_2}$  value before the penguin reached the 2 m threshold during ascent.

### $\text{O}_2$ fraction

$P_{\text{O}_2}$  values were converted to  $\text{O}_2$  fraction ( $F_{\text{O}_2}$ ) as follows:

$$F_{\text{O}_2} = P_{\text{O}_2} / (P_{\text{atm}} + (760 \times (\text{depth}/10)) - P_{\text{H}_2\text{O}}), \quad (1)$$

where  $P_{\text{atm}}$  is the barometric pressure (mmHg) closest to the time of dive from 3-h interval data obtained from the McMurdo Weather Office,  $P_{\text{H}_2\text{O}}$  (mmHg) is water vapor pressure and depth (m) is the dive depth at which the  $P_{\text{O}_2}$  value (mmHg) was obtained. For surface (depth=0), Eqn 1 simplifies to  $P_{\text{O}_2} / (P_{\text{atm}} - P_{\text{H}_2\text{O}})$ .

For anterior air sac  $P_{\text{O}_2}$  values, we used start-of-dive, pre-dive and end-of-dive results of the present study on the cervical anterior air sac. For the posterior air sac values, we used  $P_{\text{O}_2}$  data from a previous study (Stockard et al., 2005). Anterior air sacs include the two cervical air sacs, the two anterior thoracic air sacs and the interclavicular air sac. Posterior air sacs include the two posterior thoracic air sacs and the two abdominal air sacs. We assumed that  $P_{\text{O}_2}$  data from the cervical air sacs were representative of all the anterior air sacs based on prior studies in domestic fowl and geese (Brackenbury et al., 1981; Piiper et al., 1970; Scheid et al., 1989). A similar assumption was made for the posterior air sac data (Stockard et al., 2005). We also assumed the air in the lungs had  $P_{\text{O}_2}$  values similar to the posterior air sacs at the beginning and end of dives.

### Available respiratory $\text{O}_2$ store

The volumes of the anterior air sacs  $\text{O}_2$  store and the posterior air sacs+lungs  $\text{O}_2$  store were calculated using Eqns 2 and 3:

$$\text{Anterior air sacs } \text{O}_2 \text{ store} = (F_{\text{O}_2\text{B}} - F_{\text{O}_2\text{F}}) \times \text{DAV}_{\text{Ant}}, \quad (2)$$

$$\text{Posterior air sacs + lungs } \text{O}_2 \text{ store} = (F_{\text{O}_2\text{B}} - F_{\text{O}_2\text{F}}) \times \text{DAV}_{\text{Post+lungs}}. \quad (3)$$

$\text{O}_2$  fractions for start-of-dive ( $F_{\text{O}_2\text{B}}$ ) and end-of-dive ( $F_{\text{O}_2\text{F}}$ ) were determined for each dive as described above. For the three components of the DAV, we used values of  $31.2 \text{ ml kg}^{-1}$  for the posterior air sacs,  $20.8 \text{ ml kg}^{-1}$  for the anterior air sacs and  $18 \text{ ml kg}^{-1}$  for the lungs, determined as follows. The total air volume of the air sacs and lungs was based on an estimated DAV of  $70 \text{ ml kg}^{-1}$ , which is in the range of air volumes measured in shallow dives (similar to dive depths in the present study) (Sato et al., 2011; Stockard et al., 2005). The three components were divided based on volumes determined in a CT scan study (Ponganis et al., 2015). Because air volume within the parabronchi and air capillaries has not been measured in the emperor penguin lung, and because the lungs are rigid and undergo only minor changes with increased inflation pressure, lung volume was fixed at the CT-measured value of  $18 \text{ ml kg}^{-1}$  (Ponganis et al., 2015). The remaining  $52 \text{ ml kg}^{-1}$  volume was divided between the two types of air sac, with 40% of  $52 \text{ ml kg}^{-1}$  attributed to the anterior air sac volume ( $20.8 \text{ ml kg}^{-1}$ ) and 60% ( $31.2 \text{ ml kg}^{-1}$ ) attributed to posterior air sac volume (Ponganis et al., 2015). The combined posterior air sacs+lung volume was  $49.2 \text{ ml kg}^{-1}$ .

### $\text{O}_2$ depletion rate

$\text{O}_2$  depletion rate is the available respiratory  $\text{O}_2$  store (Eqns 2, 3) divided by dive duration. An  $\text{O}_2$  depletion rate was calculated for the anterior air sacs for dives in the present study and for posterior air sacs+lungs for dives from a previous study (Stockard et al., 2005).

### Statistics

Descriptive statistics were completed for individual penguins' dives for  $P_{\text{O}_2}$  at rest, dive duration and depth, and pre-dive, start-of-dive and end-of-dive  $P_{\text{O}_2}$ . Distribution of dive durations were also

**Table 1. Anterior air sac  $P_{O_2}$  data and dive characteristics of emperor penguins diving at an experimental isolated dive hole**

| Penguin ID | Mass (kg) | Number of dives ( $N$ ) | $P_{O_2}$ at rest (mmHg) | Dive duration (min)        | Dive depth (m)         | Pre-dive $P_{O_2}$ (mmHg) | Start-of-dive $P_{O_2}$ (mmHg) | End-of-dive $P_{O_2}$ (mmHg) |
|------------|-----------|-------------------------|--------------------------|----------------------------|------------------------|---------------------------|--------------------------------|------------------------------|
| EP1        | 23.3      | 43 (10,801)             | 55±3.5<br>(54, 49–86)    | 3.7±0.7<br>(3.6, 2.6–5.2)  | 32±11.1<br>(29, 13–63) | 79±14.0<br>(79, 52–112)   | 82±14.1<br>(83, 54–114)        | 58±25.2<br>(52, 24–128)      |
| EP3        | 20.5      | 16 (10,801)             | 33±13.3<br>(33, 16–87)   | 5.2±2.5<br>(4.1, 3.5–10.7) | 35±7.7<br>(37, 22–46)  | 63±9.9<br>(62, 51–89)     | 65±10.6<br>(64, 53–91)         | 35±16.7<br>(35, 3–65)        |
| EP4        | 23.5      | 41 (10,801)             | 62±5.7<br>(61, 47–93)    | 3.9±1.2<br>(3.8, 2.1–7.1)  | 32±9.8<br>(31, 14–51)  | 70±10.9<br>(69, 49–94)    | 77±15.0<br>(76, 45–118)        | 44±29.6<br>(36, 9–167)       |
| EP5        | 25.7      | 20 (7201)               | 52±4.4<br>(52, 35–84)    | 4.0±1.0<br>(3.8, 2.0–5.9)  | 30±12.6<br>(27, 12–52) | 58±11.4<br>(56, 42–87)    | 63±12.7<br>(58, 43–90)         | 40±29.9<br>(33, 4–105)       |

$P_{O_2}$ , dive duration and dive depth values are expressed as means±s.d. (values in parentheses are median, range).  $P_{O_2}$  data were collected at rest and during dives at 1-s intervals with a backpack recorder.  $N$  represents the number of 1-s samples collected during periods of minimum activity. Pre-dive  $P_{O_2}$  was the mean of  $P_{O_2}$  values 30 s prior to start-of-dive. Start- and end-of-dive  $P_{O_2}$  values were the initial and final values recorded during the dive. 1 mmHg=0.133 kPa.

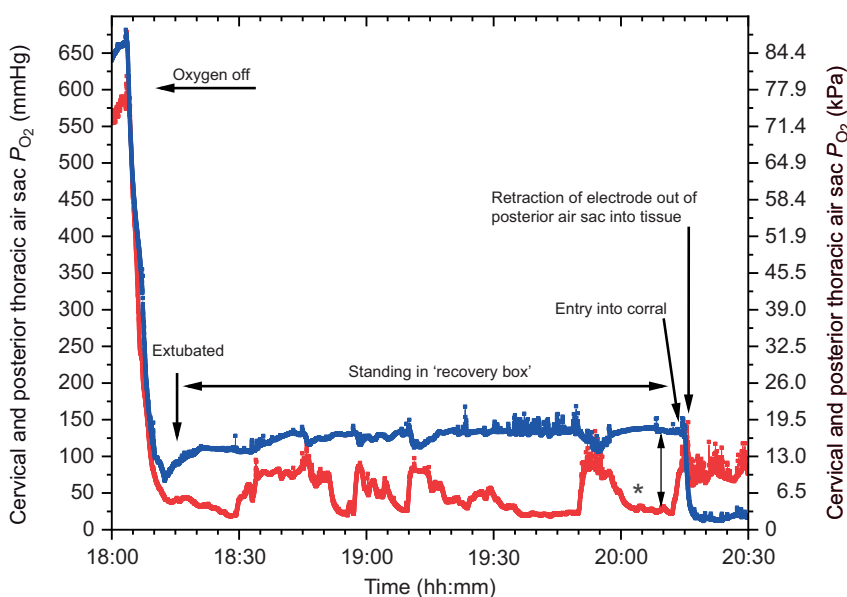
plotted. For each dive, end-of-dive  $O_2$  and the resulting calculated anterior air sac  $O_2$  depletion rates were plotted against dive duration. Because of the small sample size ( $n=4$  birds) and the heterogeneous distribution of dive durations among birds, particularly the three longest dives by one bird (~3 min longer than any other dive), no statistical analyses of dive parameters were performed. However, a paired  $t$ -test was performed, after confirming assumptions were met, to assess whether pre-dive  $P_{O_2}$  was higher than  $P_{O_2}$  at rest. For the analysis of the simultaneous  $P_{O_2}$  recorded from the anterior and posterior air sac of individual EP5, results are plotted; however, statistical analysis of data from a single bird was not undertaken. To further explore the potential relationship between stroke rates and overlapping profiles, the stroke rate data of five dives from EP5 that had overlapping anterior and posterior air sac  $P_{O_2}$  profiles occurring at the end of descent and the five dives with no overlapping  $P_{O_2}$  profiles were divided into serial 10-s intervals. The mean stroke rate of each 10-s period of both sets of five dives was calculated and plotted. Although no statistics were performed on these data from one penguin, the stroke rate periods where the overlap occurred were noted on the plot.

## RESULTS

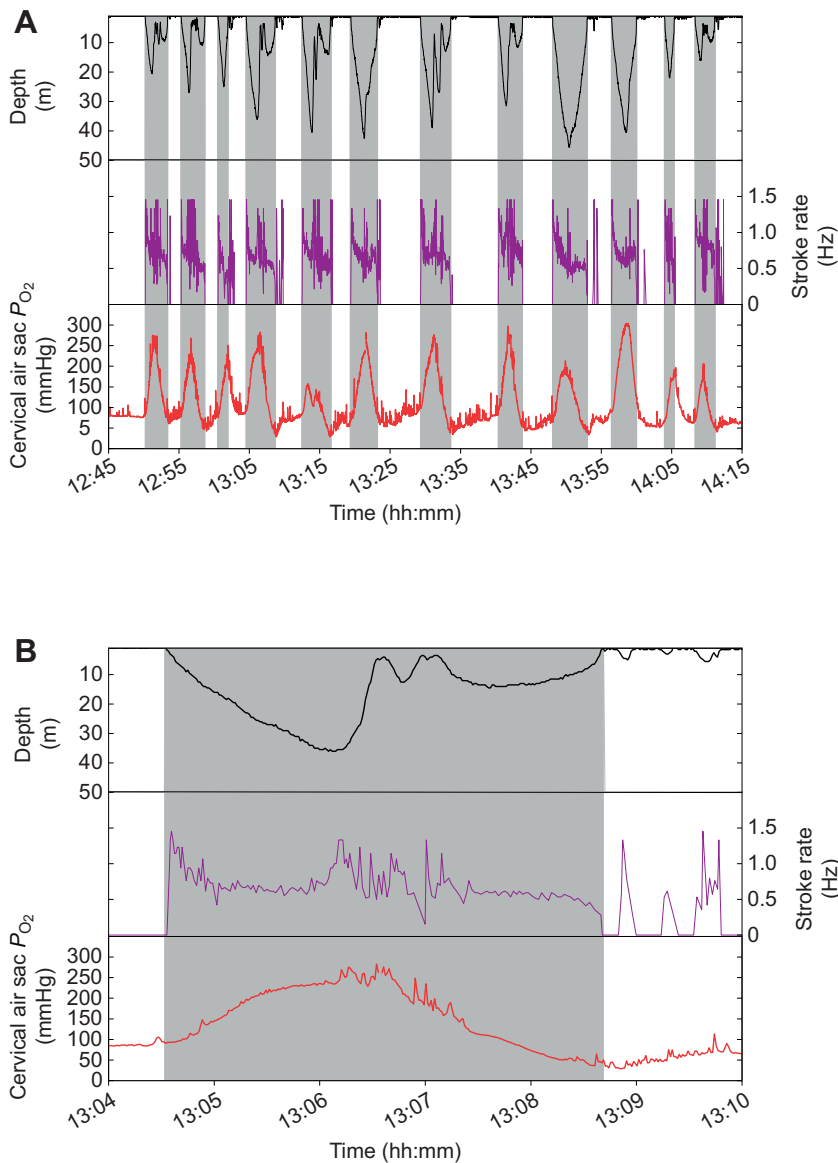
Cervical air sac data (Table 1) were successfully collected at rest and during dives from four emperor penguins. Simultaneous posterior thoracic air sac data were collected during dives in only one of the

four penguins (EP5) owing to migration of the  $P_{O_2}$  electrode out of the posterior thoracic air sac in three of the birds when they awakened from anesthesia and walked into the corral. Although the electrode remained inside the body, we suspect that the tip was retracted out of the air sac cavity by muscle movement. In the fifth penguin (EP2, 21.9 kg), simultaneous cervical and posterior thoracic air sac  $P_{O_2}$  data were collected only during the first 2 h after emergence from anesthesia because the posterior thoracic electrode migrated out of the air sac as the penguin entered the corral and the other electrode failed before diving began (Fig. 3). Notably, the only available  $P_{O_2}$  electrodes were 15 cm long in comparison to the 30 cm length in the prior air sac study (Stockard et al., 2005).

The number of dives ranged from a low of 16 to a high of 41. Bird behavior, weather and continued function of the recorders and electrodes determined the number of dives performed by each bird (Table 1). Dive depth, duration and stroke rate profiles (Table 1, Figs 4 and 5) were typical in range and pattern of those previously recorded at the isolated dive hole (Meir and Ponganis, 2009; Meir et al., 2008; Ponganis et al., 2007, 2000; van Dam et al., 2002; Williams et al., 2011). Dive durations varied among individuals (Table 1), ranging from 2 to 10.7 min (Fig. S1). Maximum depths reached by individual penguins were between 41 and 63 m. Mean values, median values and ranges for dive durations and depths are shown in Table 1.



**Fig. 3. Variation in cervical air sac  $P_{O_2}$  in an inactive bird (EP2) during the post-anesthesia period.** When the anesthetic carrier gas was switched from 100%  $O_2$  to ambient air,  $P_{O_2}$  dropped rapidly in both air sacs. From the end of anesthesia (extubated) to entry into the corral, large fluctuations in cervical air sac  $P_{O_2}$  occurred despite a relatively constant posterior thoracic  $P_{O_2}$ . Although potentially affected by residual effects of anesthesia on ventilation, cervical air sac  $P_{O_2}$  fluctuated markedly over a 2-h period. The difference in  $P_{O_2}$  between the two air sacs varied from <10 to 120 mmHg (\*). Cervical air sac  $P_{O_2}$  ranged from ~20 to 125 mmHg as the bird stood, essentially motionless, in its recovery box. On release from the box and entry into the corral,  $P_{O_2}$  rapidly decreased to 10 mmHg and did not change thereafter. This is consistent with retraction of the  $P_{O_2}$  electrode out of the air sac and into surrounding tissue owing to muscle/body wall movements.



**Fig. 4. Cervical air sac  $P_{O_2}$  profiles in a diving emperor penguin (EP1).** (A) A 90-min record of  $P_{O_2}$  (red), depth (black) and stroke rate (purple) profiles from emperor penguins during shallow dives from the isolated dive hole. Emperor penguins commonly capture the sub-ice fish *Pagothenia borchgrevinki* on the underside of the ice (Ponganis et al., 2000). Cervical air sac  $P_{O_2}$  initially increased during these dives, and then declined to variable levels by the end of the dive. (B) A 6-min extract from A at 13:05 h showing  $P_{O_2}$ , depth and stroke rate profiles before, during and after a 4.2-min dive to 35 m. Gray shading indicates dive.

### Cervical air sac $P_{O_2}$ during rest

During 2- to 3-h rest periods of the four birds (while free in the corral and with minimum activity as indicated by tri-axial acceleration), cervical air sac  $P_{O_2}$  was variable (Table 1) with a grand mean of  $51 \pm 9.5$  mm Hg. Cervical air sac  $P_{O_2}$  could also vary between rest periods in the same bird. As an example, in EP3, early in the morning during a 3-h period of presumed sleep,  $P_{O_2}$  was 33 mmHg (Table 1), while later in the morning, when the bird was similarly inactive over an 18-min period,  $P_{O_2}$  was  $45 \pm 0.7$  mmHg (Fig. S2). The range and pattern of variability of air sac  $P_{O_2}$  was also demonstrated in the simultaneous cervical and posterior thoracic  $P_{O_2}$  profiles during a period of inactivity as EP2 recovered from anesthesia (Fig. 3). Over a 2-h rest period at night in EP5, both the posterior thoracic and cervical air sacs were fairly steady, such that the  $P_{O_2}$  difference was consistently near 60 mmHg (Table 2).

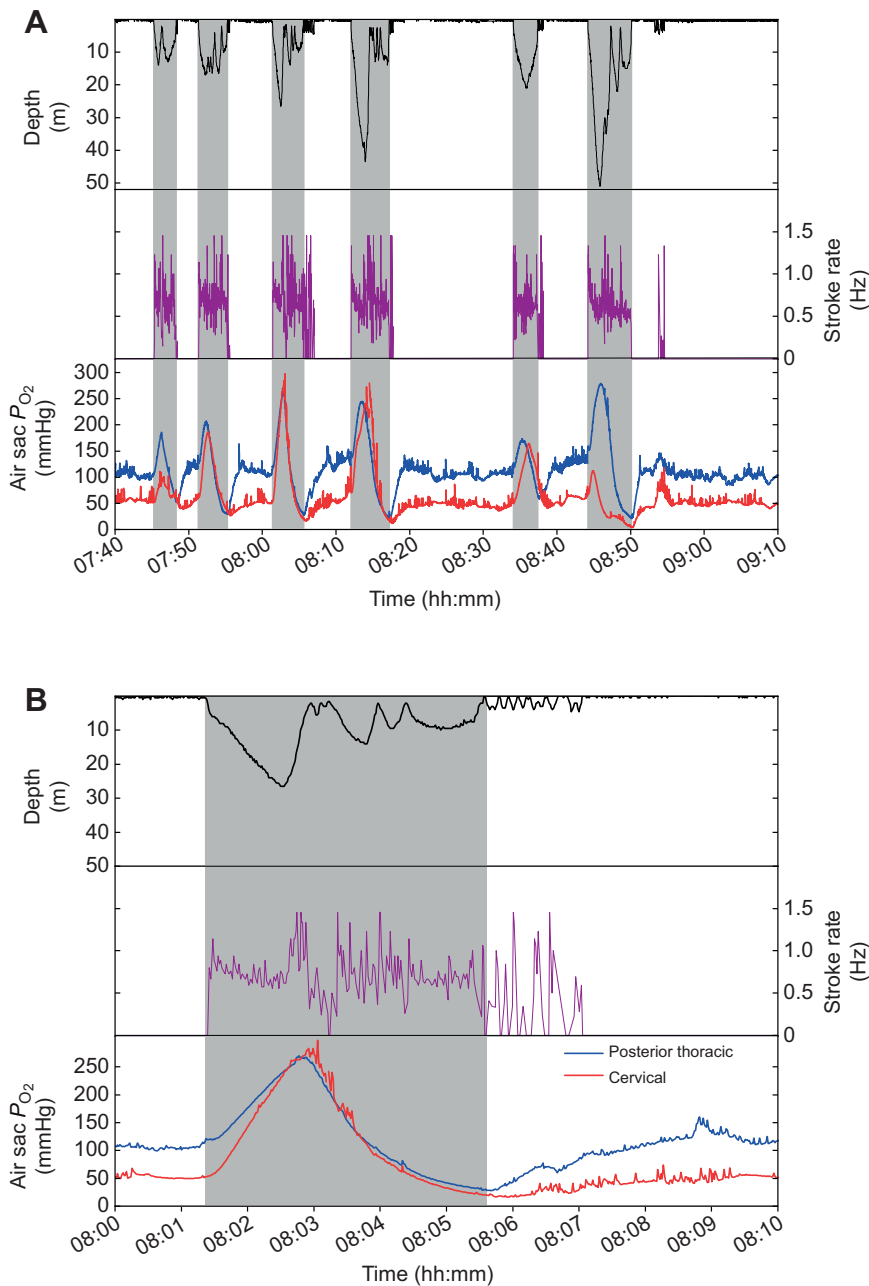
### Diving cervical air sac $P_{O_2}$ and $O_2$ fractions

Mean pre-dive  $P_{O_2}$  values in the cervical air sac of each bird ranged from 58 to 79 mmHg (Table 1) and were not significantly higher

than  $P_{O_2}$  at rest ( $t_3 = -3.04$ ,  $P = 0.56$ ). Start-of-dive cervical air sac  $P_{O_2}$  values, which could be recorded at a depth of several meters because the threshold for start-of-dive was 2 m, were equivalent or slightly higher than pre-dive  $P_{O_2}$  values (Table 1). Pre-dive and start-of-dive  $O_2$  fractions in the cervical air sac varied widely at almost all dive durations. During dives, although  $P_{O_2}$  started at lower values, the cervical air sac  $P_{O_2}$  profile was similar in shape to that previously observed in the posterior thoracic air sac (Stockard et al., 2005). Cervical air sac  $P_{O_2}$  typically increased during descent, and eventually began to decrease, especially during ascent (Figs 4 and 5). Mean end-of-dive  $P_{O_2}$  in the anterior air sac for each bird ranged from 35 to 58 mmHg (Table 1). End-of-dive  $O_2$  fraction varied considerably in dives less than the ADL, from 0.04 to 0.19, but was more uniformly low in dives over 6 min, ranging from 0.003 to 0.03 (Fig. 6).

### Size of the available respiratory $O_2$ store

Air sac  $P_{O_2}$  values of emperor penguins were highly variable. As evident in Table 1 and Figs 4, 5 and 6, and in a previous publication (Stockard et al., 2005), pre-dive and end-of-dive  $P_{O_2}$  values in the



**Fig. 5. Simultaneous cervical and posterior thoracic air sac  $P_{O_2}$  profiles in a diving emperor penguin (EP5).** (A) A 90-min record of cervical air sac  $P_{O_2}$  (red), posterior thoracic air sac  $P_{O_2}$  (blue), depth (black) and stroke rate (purple) profiles in a diving emperor penguin. Both air sac  $P_{O_2}$  profiles were variable, but, at times, overlapped during dives, and frequently reached similar end-of-dive values. (B) A 10-min record of cervical air sac  $P_{O_2}$ , posterior thoracic air sac  $P_{O_2}$ , depth and stroke rate profiles before, during and after a 4.3-min dive to 30 m. The two air sac  $P_{O_2}$  profiles approached similar values during descent and overlapped during most of the dive. During surface intervals, differences between the cervical and posterior thoracic air sac  $P_{O_2}$  levels were distinct. Gray shading indicates dive.

air sacs have a wide range, particularly during short dives. In the cervical air sacs (Table 1), although mean pre-dive  $P_{O_2}$  values of each bird were 58 to 79 mmHg, the  $P_{O_2}$  values prior to individual dives were as high as 87 to 112 mmHg. Pre-dive  $P_{O_2}$  in the posterior air sacs for individual dives (Stockard et al., 2005) was usually in the 120–140 mmHg range (Fig. 5). End-of-dive  $P_{O_2}$  could be as low as 4 mmHg in either air sac, although individual values for most dives were much higher (Table 1, Figs 4, 5 and 6). Therefore, to calculate the maximum respiratory  $O_2$  store available, we used pre-dive  $P_{O_2}$  values of 100 and 140 mmHg for the anterior and posterior air sacs, respectively, and end-of-dive  $P_{O_2}$  values of 4 mmHg for both air sacs. The chosen pre-dive values were representative of the range of peak pre-dive values in the cervical (Table 1) and posterior thoracic air sacs (Stockard et al., 2005). The end-of-dive values used in the calculation were the lowest values found for each air sac. Using these values and Eqns 2 and 3, the maximum

respiratory  $O_2$  store available during shallow dives at the isolated dive hole is  $9.4 \text{ ml } O_2 \text{ kg}^{-1}$  for the posterior air sacs and lung combined and  $2.8 \text{ ml } O_2 \text{ kg}^{-1}$  for anterior air sacs, for a total of  $12.2 \text{ ml } O_2 \text{ kg}^{-1}$ .

#### Respiratory $O_2$ store depletion

The  $O_2$  depletion rate of the anterior air sacs was slow, typically  $<0.5 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$  for dives of up to almost 11 min duration (Fig. 7A). For dives less than 5 min,  $O_2$  depletion rates varied between  $-1.1$  and  $0.5 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$ , with the negative anterior air sac  $O_2$  depletion rates reflecting end-of-dive  $P_{O_2}$  values greater than those at the start (Fig. 7A). However, for dives beyond the ADL (5.6 min), the anterior air sac  $O_2$  depletion rate remained fairly constant (between  $0.13$  and  $0.3 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$ ) (Fig. 7A). The mean calculated anterior air sac  $O_2$  depletion rate was  $0.16 \pm 0.22 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$  (Fig. 7A).

**Table 2. Simultaneous cervical and posterior thoracic air sac  $P_{O_2}$  data from one emperor penguin (EP5)**

|                                | Posterior thoracic air sac | Cervical air sac    | Posterior thoracic–cervical air sac difference |
|--------------------------------|----------------------------|---------------------|--|
| $P_{O_2}$ at rest (mmHg)       | 111±8.7                    | 52±4.4              | 59±10.8  |
| Pre-dive $P_{O_2}$ (mmHg)      | 114±16.2 (116, 72–136)     | 58±11.4 (56, 42–87) | 56±14.1 (60, 18–75)                            |
| Start-of-dive $P_{O_2}$ (mmHg) | 127±21.8 (133, 80–162)     | 63±12.7 (58, 43–90) | 65±15.8 (32.6, 27–101)                         |
| End-of-dive $P_{O_2}$ (mmHg)   | 39±24.5 (29, 14–105)       | 40±29.9 (33, 4–105) | –1±14.0 (1, –27–21)                            |

$P_{O_2}$  values are expressed as means±s.d. (values in parentheses are median, range).  $P_{O_2}$  at rest values are based on a 2-h rest period ( $n=7201$  1 Hz samples). For all other parameters,  $n=20$  dives. Pre-dive  $P_{O_2}$  was the mean of the 30 s prior to start-of-dive. Start- and end-of-dive  $P_{O_2}$  values were the initial and final values recorded during the dive. 1 mmHg=0.133 kPa.

### Simultaneous air sac $P_{O_2}$ profiles

In EP5, the only bird with simultaneous cervical and posterior thoracic air sac  $P_{O_2}$  measured during dives, pre-dive, start-of-dive and end-of-dive posterior thoracic air sac  $P_{O_2}$  values (Table 2) were similar in range to those in the prior study (Stockard et al., 2005). Pre-dive and start-of-dive posterior thoracic air sac  $P_{O_2}$  values were 50 to 60 mmHg greater than the corresponding simultaneous cervical air sac values in this bird (Table 2). The differences between start-of-dive thoracic posterior and cervical air sacs in EP5 were between 25 and 105 mmHg (Fig. 8). By the end of dives, there was less difference between the air sacs (–27 to 20 mmHg). Of the 20 dives, five had air sac end-of-dive  $P_{O_2}$  differences <5 mmHg, another six had differences between 5 to 10 mmHg, and four had differences >20 mmHg. In eight of 20 dives, end-of-dive posterior thoracic air sac  $P_{O_2}$  was less than that in the cervical air sac by 3 to 27 mmHg (Fig. 8). The difference in end-of-dive  $P_{O_2}$  between the cervical and posterior thoracic air sacs did not appear to vary with total number of strokes in the dive (Fig. 8).

The two  $P_{O_2}$  profiles during dives followed typical depletion patterns for cervical and posterior air sacs (Fig. 5). As illustrated in some of the dives in Fig. 5, there was extensive overlap of the cervical and posterior thoracic air sac  $P_{O_2}$  profiles with cervical air sac  $P_{O_2}$  sometimes greater than posterior thoracic air sac  $P_{O_2}$ . Such overlap occurred in 15 of the 20 dives of EP5. Dive durations and maximum depths of those 15 dives (2.0–5.2 min, 12–47 m) were in the same range as those of the five dives with non-overlapping  $P_{O_2}$  profiles (3.7–5.9 min, 15–52 m). In the dives with overlapping

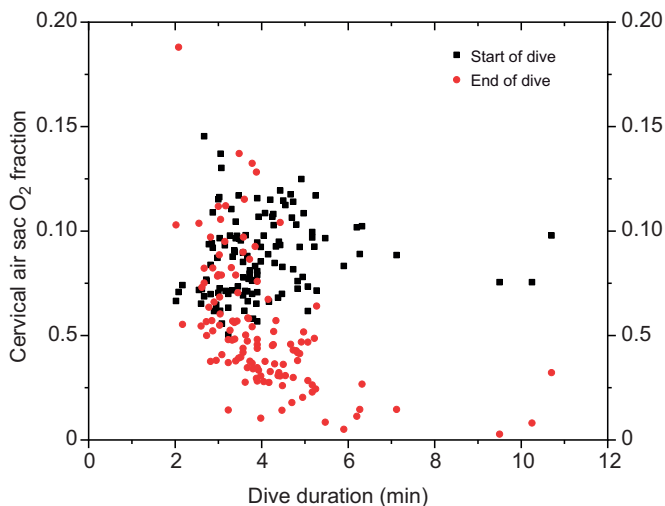
$P_{O_2}$  profiles, initial overlap of the two profiles occurred during descent or at the bottom in eight dives, during feeding ascents to the sub-ice surface in three dives, and during the ascent to exit the hole in four dives. The initial overlap times occurred at a wide range of depths (7–38 m), elapsed time into dive (0.3–4.9 min), fraction of time into dive (0.08–0.96) and number of strokes into the dive (15–159 strokes). The stroke rates at or before overlapping of anterior and posterior air sac  $P_{O_2}$  profiles were in the same range as stroke rates of dives with no overlap in  $P_{O_2}$  profiles (Fig. S3).

### DISCUSSION

There were four important findings in this study. First, the cervical air sac  $P_{O_2}$  measured in emperor penguins at rest was lower than the previously reported arterial  $P_{O_2}$  (Ponganis et al., 2007). This narrows the possible mechanisms responsible for low arterial  $P_{O_2}$  values in resting emperor penguins. Second, the difference between the highly variable pre-dive anterior air sac  $P_{O_2}$  values and  $P_{O_2}$  values at rest were not statistically significant. Third, when representative anterior air sac  $P_{O_2}$  and volume data are included in  $O_2$  store calculations, the estimated body  $O_2$  store of emperor penguins does not change, but the overall dive  $O_2$  consumption rate is lower. Finally, our results support the premise that air moves between air sacs and through the lungs during dives of emperor penguins.

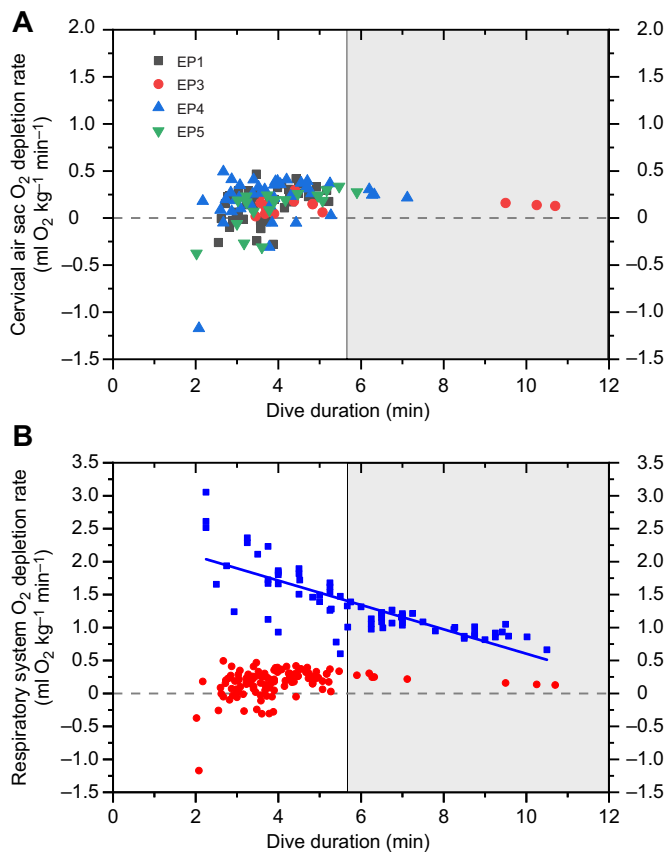
### Cervical air sac $P_{O_2}$ at rest

Although resting cervical air sac  $P_{O_2}$  values were often variable within rest periods and between individuals (Table 1, Fig. 3, Fig. S2), values were low in comparison with those of other birds. The anterior air sac  $P_{O_2}$  values of resting geese (*Anser anser*) and domestic fowl (80–100 mmHg) are nearly twice that of the emperor penguin (51 mmHg) (Brackenbury et al., 1981; Piiper et al., 1970; Scheid et al., 1989). The resting cervical air sac  $P_{O_2}$  values were also approximately 60 mmHg lower than posterior thoracic air sac  $P_{O_2}$  values previously measured in this species (Stockard et al., 2005), a difference confirmed in the simultaneously measured  $P_{O_2}$  in the air sacs of EP5, which differed by 59 mmHg (Table 2). This difference between anterior and posterior air sac  $P_{O_2}$  values is two to three times larger than the difference observed in other birds, a consequence of the emperor penguin's low anterior air sac  $P_{O_2}$ , as its posterior air sac  $P_{O_2}$  values are similar to those of domestic fowl and ducks (Piiper et al., 1970; Scheid et al., 1989). These anterior air sac values are consistent with the emperor penguin's arterial  $P_{O_2}$  at rest (68 mmHg), which is low compared with those of ducks, geese, chickens and emus (Black and Tenney, 1980; Kawashiro and Scheid, 1975; Ponganis et al., 2007; Scheid et al., 1989; Schmitt et al., 2002), and its low respiratory rate at rest (4 breaths  $\text{min}^{-1}$ ), which is approximately two-thirds of allometrically predicted rates (Calder, 1968; Frappell et al., 2001). Although Adélie and little penguins (*Eudyptula minor*) also have lower than allometrically predicted respiratory rates, it is unknown whether other diving birds



**Fig. 6. Start-of-dive and end-of-dive  $O_2$  fraction versus dive duration.** Start-of-dive  $O_2$  fractions were higher than end-of-dive  $O_2$  fractions in dives longer than the 5.6-min ADL. End-of-dive  $O_2$  fractions sometimes reached near-zero values. Both start-of-dive and end-of-dive  $O_2$  fractions were considerably variable over the range of dive durations.





**Fig. 7. Anterior air sac and respiratory system  $O_2$  depletion rates during dives of emperor penguins at an isolated dive hole.** (A) The  $O_2$  depletion rate of the anterior air sacs (measured via the cervical air sac) during dives ( $n=120$ ) to almost 11-min duration. Depletion rates for short dives were often negative because  $P_{O_2}$  at the end of the dive was higher than at the start. Although there was considerable variation in depletion rates of dives below the 5.6-min ADL, the rates were low with an upper limit of 0.3 to 0.5 ml  $O_2$   $kg^{-1}$   $min^{-1}$ . In the few dives beyond the ADL (gray background), the anterior air sac depletion rates remained below that limit and showed little variation. (B) Anterior air sac values (red) are from A and posterior air sac+lung values (blue) are based on the start- and end-of-dive  $P_{O_2}$  and  $O_2$  fraction data for the posterior air sac (Stockard et al., 2005).  $O_2$  depletion in the posterior air sacs and lungs declined as dive duration increased, and were markedly higher than anterior air sac depletion rates, only approaching the anterior air sac rates in the longest dives.

have similarly low anterior air sac  $P_{O_2}$  values at rest and large differences in  $P_{O_2}$  between anterior and posterior air sacs (Chappell and Souza, 1988; Stahel and Nicol, 1988).

The relatively low cervical air sac and arterial  $P_{O_2}$  values in combination with the relatively large difference in  $P_{O_2}$  between the cervical and posterior thoracic air sacs suggest a low ratio of parabronchial ventilation to parabronchial blood  $O_2$  extraction. That ratio is determined by the respiratory rate, tidal volume, heart rate (cardiac output) and the  $O_2$  content of mixed venous blood entering the lung. Because resting heart rates in emperor penguins are comparable to the allometrically predicted rate and venous  $P_{O_2}$  is unremarkable (Calder, 1968; Meir et al., 2008; Ponganis et al., 2007), reduced parabronchial ventilation may contribute more to the low ratio during rest. A diffusion limitation or large post-pulmonary shunt could also cause low arterial  $P_{O_2}$ . Although simultaneous cervical air sac and arterial  $P_{O_2}$  data would be valuable, the  $-17$  mmHg difference between the mean cervical air sac and arterial  $P_{O_2}$  at rest ( $51-68=-17$ ) is not consistent with either of these

mechanisms. In a diffusion limitation or a large post-pulmonary shunt, the difference in  $P_{O_2}$  would decrease toward 0 or even become positive (Powell and Hopkins, 2004; Schmitt et al., 2002). However, some contribution from a ventilation–perfusion inhomogeneity cannot be ruled out for the low arterial  $P_{O_2}$  at rest. The difference between anterior air sac and arterial  $P_{O_2}$  can be negative with ventilation–perfusion inhomogeneities (Powell and Hopkins, 2004). However, even with low resting respiratory rates and a low ratio of parabronchial ventilation to parabronchial blood  $O_2$  extraction, the emperor penguin still maintains adequate, parabronchial gas exchange with hemoglobin saturation at rest over 90% and normal blood pH and carbon dioxide levels (Meir and Ponganis, 2009; Ponganis et al., 2007).

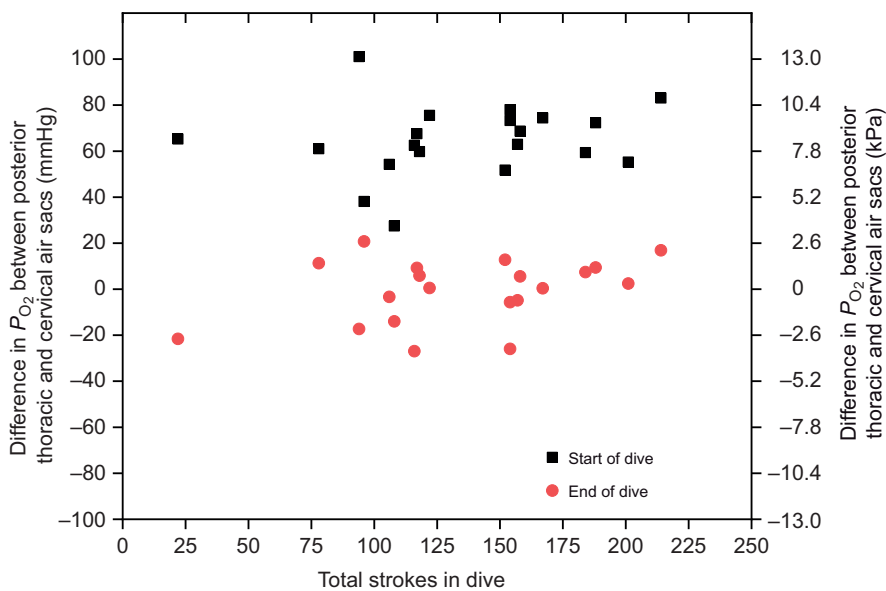
#### Pre-dive cervical air sac $P_{O_2}$ and hyperventilation

Pre-dive anterior air sac  $P_{O_2}$  values are likely driven by the ratio of parabronchial ventilation to parabronchial  $O_2$  extraction. During hyperventilation and tachycardia before diving, anterior air sac  $P_{O_2}$  values should increase owing to increased parabronchial ventilation, as observed in exercising domestic fowl (Brackenbury et al., 1981). However, in the present study, pre-dive  $P_{O_2}$  values varied between and within birds, ranging from values equivalent to  $P_{O_2}$  at rest to values as high as 112 mmHg (Table 1), suggesting variation in the level of hyperventilation and/or tachycardia before dives. Our hypothesis that pre-dive cervical air sac  $P_{O_2}$  would be higher than resting values was not supported. However, despite the overlap in values, the upper range of pre-dive cervical air sac  $P_{O_2}$  appeared higher than that in the resting state, suggesting there might be a significant difference with a larger sample size.

We doubt the ratio of parabronchial ventilation to parabronchial  $O_2$  extraction prior to individual dives will always be the same because of probable variation in pre-dive hyperventilation, pre-dive heart rate (cardiac output and pulmonary blood flow) and venous hemoglobin saturation. As illustrated in Fig. 3, even in an inactive bird, there can be remarkable changes in cervical air sac  $P_{O_2}$ . In addition, high pre-dive venous  $P_{O_2}$  and hemoglobin saturation can occur prior to some dives (Meir and Ponganis, 2009; Ponganis et al., 2007). Higher venous values would decrease the rate of parabronchial blood  $O_2$  extraction and contribute to a higher cervical air sac  $P_{O_2}$ .

Although we were not able to conduct a statistical analysis, our results did not appear to suggest a relationship between anterior air sac start-of-dive  $O_2$  fractions and dive durations (Fig. 6). The lack of such a relationship was also found for the posterior air sac in a previous study (Stockard et al., 2005). However, when diving at sea, birds appear to inhale greater air volumes prior to deeper dives at sea and may have higher pre-dive heart rates (Meir et al., 2008; Sato et al., 2011; Wright et al., 2014). Thus, for penguins diving at sea, we cannot rule out a relationship between dive duration and either start-of-dive  $O_2$  fractions or elevated pre-dive cervical air sac  $P_{O_2}$  values.

During the surface intervals between dives of emperor penguins, hyperventilation and tachycardia contribute to an increase in arterial and venous  $P_{O_2}$ , with venous blood sometimes even becoming arterialized (Kooyman et al., 1971; Meir and Ponganis, 2009; Meir et al., 2008). We hypothesize that, during surface intervals, as in Figs 4 and 5, the rates and patterns of replenishment of  $O_2$  in both the cervical and posterior thoracic air sacs are due to increased ventilation and cardiac output. As an example, in Fig. 5A, we suspect that the further increase in  $P_{O_2}$  in both air sacs at 07:51 h (prior to a dive during the latter part of a surface interval) was probably secondary to hyperventilation contributing to a higher ratio of parabronchial ventilation to parabronchial  $O_2$  extraction.



**Fig. 8. Start-of-dive and end-of dive  $P_{O_2}$  values in simultaneously recorded cervical and posterior thoracic air sacs of EP5.** Differences in  $P_{O_2}$  between the posterior thoracic and cervical air sacs at the start of dives were consistently above 20 mmHg, while those at the end of dives were consistently below 20 mmHg. In eight dives, this difference was negative because cervical air sac end-of-dive  $P_{O_2}$  was higher than in the posterior thoracic air sacs. Differences were calculated by subtracting cervical air sac  $P_{O_2}$  from posterior thoracic air sac  $P_{O_2}$ .

### Magnitude of the respiratory $O_2$ store: $12.2 \text{ ml } O_2 \text{ kg}^{-1}$

Contrary to our hypothesis, the maximum respiratory  $O_2$  store of  $12.2 \text{ ml } O_2 \text{ kg}^{-1}$  was not lower than previous respiratory  $O_2$  store calculations for shallow dives (Ponganis et al., 2010). This value, combined with blood and muscle  $O_2$  stores of 21.1 and  $24.4 \text{ ml } O_2 \text{ kg}^{-1}$ , respectively (Sato et al., 2011), results in a total body  $O_2$  store of  $57.7 \text{ ml } O_2 \text{ kg}^{-1}$ , with 21%, 37% and 42% in the respiratory system, blood and muscle, respectively.

We emphasize that this calculation of the respiratory  $O_2$  store represents a maximum value for these shallow dives. With lower individual pre-dive  $P_{O_2}$  values in anterior or posterior air sacs, and higher individual end-of-dive  $P_{O_2}$  values in anterior or posterior air sacs, the actual amount of respiratory  $O_2$  available or consumed during a given dive will vary. Further, the calculation of the maximum respiratory  $O_2$  store depends on an accurate DAV. The maximum total body  $O_2$  store calculated with a DAV of  $70 \text{ ml } O_2 \text{ kg}^{-1}$  is in accord with the  $58 \text{ ml } O_2 \text{ kg}^{-1}$  total body  $O_2$  store previously calculated for shallow dives, but less than the  $68 \text{ ml } O_2 \text{ kg}^{-1}$  estimated with a larger diving air volume for deep dives (Sato et al., 2011). However, as recently hypothesized in diving mammals and supported by earlier research in human breath-hold divers (Fahlman et al., 2020; Lanphier and Rahn, 1963), these DAVs, measured at the end of dives, may underestimate the air volumes at the start of dives owing to gas exchange and gas absorption throughout the dive. Consequently, for better estimation of the respiratory  $O_2$  store and better understanding of pulmonary function, start-of-dive air volumes in the respiratory systems of penguins and other birds still require further documentation.

### Air sac and respiratory system $O_2$ depletion rates

The varied and low depletion rates of the anterior air sac contrasted with the calculated  $O_2$  depletion rates from posterior air sacs and lungs, which steadily declined as dive duration increased (Fig. 7B) (Stockard et al., 2005). The combined results from the present study and the Stockard et al. (2005) study demonstrate the relative magnitude of the anterior air sac contribution in dives. For 4-min dives, the  $O_2$  depletion rates are approximately 1.9 and  $0.25 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$  for the posterior and anterior air sacs, respectively (Fig. 7B). For a dive of this duration by a 25 kg penguin, the posterior air sacs would contribute 143 ml  $O_2$  to the

blood and the anterior air sacs would contribute 25 ml  $O_2$ , or 15% of the total respiratory contribution. For a 10-min dive by the same penguin, contributions to the blood would be 150 ml  $O_2$  by the posterior air sacs and 38 ml  $O_2$  or 20% of total respiratory contribution by the anterior air sacs.

The mean anterior air sac  $O_2$  depletion rate ( $0.16 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$ ) was approximately one-eighth of the posterior air sac+lung rate ( $1.32 \pm 0.52 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$ ), making the overall depletion rate of the respiratory  $O_2$  store approximately  $1.5 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$ . This value is lower than the previously calculated value of  $2.1 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$  that was based only on changes in  $P_{O_2}$  in the posterior air sacs (Stockard et al., 2005). Applying this new respiratory  $O_2$  depletion rate results in an ~9% reduction in the overall dive  $O_2$  consumption rate, from the previously calculated value of 6.8 to  $6.2 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$  (Williams et al., 2011). The revised rate is at the lowest end of resting metabolic rates ( $6.2$  to  $6.7 \text{ ml } O_2 \text{ kg}^{-1} \text{ min}^{-1}$ ) of emperor penguins floating in a flume (Kooyman and Ponganis, 1994). These results further support the suggestion that diving in emperor penguins is extremely efficient (Nagy et al., 2001).

### Cervical and posterior thoracic air sac $P_{O_2}$ profiles during dives

There were several findings in our study that supported the hypothesis that air moves from the air sacs through the parabronchi of the lung during a dive. First, the changes in cervical air sac  $P_{O_2}$  profiles and the calculated air sac  $O_2$  depletion rates (Fig. 7B) suggest use of the cervical air sac  $O_2$  store during dives. Second, although the  $P_{O_2}$  profiles in the cervical and posterior thoracic air sacs generally paralleled each other, there was significant overlap of the profiles in 15 of 20 dives, with near equivalence of  $P_{O_2}$  values during ascent in the cervical and posterior thoracic air sacs in nine of those 15 dives (four such dives are shown in Fig. 5). Finally, in 40% of dives, end-of-dive cervical air sac  $P_{O_2}$  was greater than that in the posterior thoracic air sac (Fig. 8). All of these findings offer support for the mixing of air between the anterior and posterior air sacs during the dive.

However, we were unable to identify any specific characteristics of dives in which  $P_{O_2}$  profiles overlapped. Dives associated with overlap of the cervical and posterior thoracic air sac profiles were not distinguished by maximum depth or dive duration (Fig. 5). Further, the point during the dive when profiles overlapped was not

consistent in depth, time into dive or number of strokes. Although differential pressure oscillations induced in the air sacs by strokes (wingbeats) remain the most probable mechanism for such air movement through the lungs (Boggs et al., 2001), we did not find support for or against this mechanism. However, differences in the end-of-dive  $P_{O_2}$  between the posterior thoracic and cervical air sacs did not diminish with total number of strokes (Fig. 8), which we would expect if mixing were facilitated by stroking. Further, stroke rates at or before overlapping of anterior and posterior air sac  $P_{O_2}$  profiles did not appear different from stroke rates of dives with no overlap in  $P_{O_2}$  profiles (Fig. S3).

The differential pressure oscillations hypothesis was based on a study in little blue penguins (Boggs et al., 2001), in which the frequency of pressure oscillations and wing beats was 3 Hz. However, stroke rates of emperor penguins are much less, frequently <1.5 Hz (van Dam et al., 2002; Williams et al., 2012). It is possible that lower stroke rates may not be effective in air movement. Other factors such as inspired air volume or stroke amplitude may also affect mixing patterns and the observed  $P_{O_2}$  profiles. Thus, although it is apparent from these data that air movement within the respiratory system of the penguin occurs during a dive, the mechanism and pattern of air movement have still not been fully resolved and require further investigation.

From these findings in EP5, the dual  $P_{O_2}$  electrode biologging technique appears to be a promising approach for investigation of  $O_2$  transfer and utilization in penguins. The technique is applicable to further investigations of air sac  $O_2$  distribution, as well as to examinations of air sac-to-arterial  $P_{O_2}$  gradients, and arterial-venous  $P_{O_2}$  differences. In addition, this technique may prove valuable in the study of air sac  $P_{O_2}$  and parabronchial ventilation in other birds, such as the bar-headed goose (*Anser indicus*) exercising under hypoxic conditions (Hawkes et al., 2014; Meir et al., 2019).

## Conclusions

Air sac  $P_{O_2}$  profiles in emperor penguins at rest have revealed that, relative to other birds, cervical air sac  $P_{O_2}$  is low while the difference in  $P_{O_2}$  between cervical and posterior thoracic air sacs is high. This is consistent with the low arterial  $P_{O_2}$  and respiratory rate in these penguins at rest, and suggests that the ratio of parabronchial ventilation to parabronchial blood  $O_2$  extraction is low. Although pre-dive cervical air sac  $P_{O_2}$  was elevated prior to some dives, overall, there was a wide range of values and it was not significantly different from  $P_{O_2}$  values at rest. Incorporation of cervical air sac  $P_{O_2}$  data into calculation of the estimated maximum respiratory  $O_2$  store did not change that value in comparison with previous estimations. The calculated  $O_2$  depletion rates of the anterior air sacs during dives were low relative to those of the posterior air sacs. The overall respiratory  $O_2$  depletion rate was less than that previously calculated with posterior air sac  $P_{O_2}$  data alone; this lower value resulted in a 9% reduction of the previously estimated dive  $O_2$  consumption rate for emperor penguins. Near overlap of  $P_{O_2}$  profiles in the cervical and posterior thoracic air sacs, and occasional elevations of cervical air sac  $P_{O_2}$  above posterior air sac  $P_{O_2}$  supported the hypothesis of movement of air through the lung and between the air sacs during dives. Such air movement accounts for continued gas exchange and the decreases in air sac  $P_{O_2}$  during dives.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: C.L.W., P.J.P.; Methodology: C.L.W., M.F.C., J.S.J., J.S., P.J.P.; Software: C.L.W., M.F.C., J.S.J., P.J.P.; Validation: C.L.W., M.F.C.; Formal analysis: C.L.W., M.F.C., P.J.P.; Investigation: C.L.W., M.F.C., J.S.J., J.S., P.J.P.; Resources: M.S., P.J.P.; Writing - original draft: C.L.W., P.J.P.; Writing - review & editing: C.L.W., M.F.C., J.S.J., J.S., M.S., P.J.P.; Visualization: M.S.; Supervision: C.L.W., P.J.P.; Project administration: P.J.P.; Funding acquisition: P.J.P.

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## Data availability

Data are available from Dryad (Ponganis et al., 2020): <https://doi.org/10.6076/D1H01Z>.

## Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.230219.supplemental>

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