

Contribution to the Theme Section 'Tracking fitness in marine vertebrates'

Heart rates of emperor penguins diving at sea: implications for oxygen store management

Alexandra K. Wright*, Katherine V. Ponganis, Birgitte I. McDonald, Paul J. Ponganis

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093-0204, USA

ABSTRACT: Heart rate (f_H) contributes to control of blood oxygen (O_2) depletion through regulation of the magnitude of pulmonary gas exchange and of peripheral blood flow in diving vertebrates such as penguins. Therefore, we measured f_H during foraging trip dives of emperor penguins *Aptenodytes forsteri* equipped with digital electrocardiogram (ECG) recorders and time depth recorders (TDRs). Median dive f_H (total heartbeats/duration, 64 beats min^{-1}) was higher than resting f_H (56 beats min^{-1}) and was negatively related to dive duration. Median dive f_H in dives greater than the 5.6 min aerobic dive limit (ADL; dive duration associated with the onset of a net accumulation of lactic acid above resting levels) was significantly less than the median dive f_H of dives less than the ADL (58 vs. 66 beats min^{-1}). f_H profile patterns differed between shallow (<50 m) and deep dives (>250 m), with values usually declining to levels near resting f_H in shallow, short-duration dives, and to levels as low as 10 beats min^{-1} during the deepest segments of deep dives. The total number of heartbeats in a dive was variable in shallow dives and consistently high in deep dives. A true bradycardia (f_H below resting levels) during segments of 31% of shallow and deep dives of emperor penguins is consistent with reliance on myoglobin-bound O_2 stores for aerobic muscle metabolism that is especially accentuated during the severe bradycardias of deep dives. Although f_H is low during the deepest segments of deep dives, the total number and distribution of heartbeats in deep, long dives suggest that pulmonary gas exchange and peripheral blood flow primarily occur at shallow depths.

KEY WORDS: Aerobic dive limit · Diving physiology · Electrocardiogram · ECG · Emperor penguin · Gas exchange · Heart rate · Oxygen store management · Peripheral perfusion

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Regulation of heart rate (f_H) underlies the oxygen store management and dive capacity of seabirds and marine mammals. The reduction in cardiac output associated with a decline in f_H during forced submersion results in: (1) decreased organ blood flow and perfusion-dependent O_2 consumption, (2) decreased blood flow to locomotory muscle and a decline in blood-to-muscle O_2 transfer, and (3) decreased pulmonary blood flow and blood oxygen uptake from the lung (Scholander 1940, Irving et al. 1941, Ponganis et al. 2011). The decrease in peripheral blood flow associated with a diving bradycardia conserves the blood O_2 store and maximizes breath-hold capacity

(Scholander 1940, Irving et al. 1941). A decrease in pulmonary flow similarly conserves the respiratory O_2 store and additionally preserves the respiratory O_2 fraction, which, in turn, optimizes oxygenation of any blood passing through the lung, thus maintaining arterial oxygen saturation longer and maximizing breath-hold capacity (Andersson et al. 2002).

Although severe bradycardia occurs in forced submersions (the classic dive response), the diving f_H response in free-ranging animals is often less intense and more variable. For example, f_H decreases from pre-dive levels during dives of king penguins *Aptenodytes patagonicus* and macaroni penguins *Eudyptes chrysolophus* at sea, but it does not decline below resting levels on land, nor does it approach levels

*Corresponding author: awright@ucsd.edu

observed in simulated dives (Kooyman et al. 1973, Ponganis et al. 1997, 1999a, Green et al. 2003, Froget et al. 2004). In terms of muscle blood flow and oxygen delivery, this f_H pattern during the penguin's dive has been considered a trade-off between the classic dive response of forced submersions and the exercise response of flighted birds and terrestrial mammals (Butler 1988, Green et al. 2003). Higher f_H in diving king and macaroni penguins than in forced submersions should also enhance pulmonary blood flow and lung-to-blood O_2 transfer, thus contributing to rapid utilization of the respiratory O_2 store.

In contrast, in emperor penguins *Aptenodytes forsteri* diving at an isolated dive hole, dive f_H often declined below levels of birds resting on ice and even reached levels recorded during simulated dives, especially in dives beyond the previously measured 5.6-min aerobic dive limit (ADL; dive duration associated with the onset of a net accumulation of lactic acid above resting levels) (Kooyman 1989, Ponganis et al. 1997, 1999a, Meir et al. 2008). These lower f_H values, especially in longer dives, imply a greater reliance on muscle O_2 stores in emperor penguins than in other penguin species. Indeed, myoglobin desaturation profiles in diving emperor penguins revealed that the large muscle O_2 store is utilized and often depleted, although at variable rates and in variable patterns (Williams et al. 2011). The low f_H values observed in emperor penguins diving at an isolated dive hole likely contributed to the slow venous O_2 depletion observed in dives as long as 22 min and to maintenance of arterial oxygen saturation during most of the dive, including dives as long as 10 min (Meir & Ponganis 2009).

Regarding O_2 store management of emperor penguins at sea, especially during their long, deep dives, the question remains as to whether a trade-off occurs, analogous to that of king and macaroni penguins, between elevated f_H values characteristic of the exercise response and depressed f_H values specific to the classic dive response. Or does a classic dive response with more extreme bradycardia predominate, as with emperor penguins at the isolated dive hole? Lower f_H values would conserve respiratory and blood O_2 at the potential expense of muscle O_2 depletion and the subsequent onset of glycolysis, while higher f_H values could lead to longer maintenance of aerobic muscle metabolism but more rapid depletion of respiratory and blood O_2 stores.

We investigated f_H responses during dives of emperor penguins making foraging trips to sea during the chick-rearing period. Specifically, we used an electrocardiogram (ECG) recorder to measure f_H and

a time depth recorder (TDR) to record the dive profile in order to: (1) examine the relationship between dive f_H (total heartbeats during the dive/dive duration) and dive duration, (2) investigate how f_H fluctuated throughout the course of dives of varying depths, and (3) examine the f_H profile of dives of different depths to evaluate the potential for variation in the number of heartbeats, an index of cumulative cardiac output, during different segments of these dives.

We suspected that, because both the diving air volume and the total number of wing strokes during a dive increased with maximum dive depth in emperor penguins (Sato et al. 2011), the total number of heartbeats during early descent and throughout the course of the dive would increase in deeper dives despite an overall lower dive f_H in order to accommodate greater pulmonary gas exchange. A greater number of heartbeats, especially during the gradual decline in f_H typical of descent, could also potentially increase muscle O_2 delivery during that segment of the dive. Therefore, we hypothesized that: (1) dive f_H would negatively correlate with dive duration, (2) dive f_H of dives >ADL would be less than resting f_H , and (3) the total number of heartbeats would be greater in deeper dives throughout the total dive and during the gradual decline in f_H during descent.

MATERIALS AND METHODS

In November 2010 and 2011, emperor penguins were captured at the sea-ice edge as they departed on foraging trips from the Cape Washington breeding colony (74° 40' S, 165° 28' E), equipped with ECG recorders, TDRs, and VHF transmitters, and then released. Upon return from foraging trips, penguins were recaptured to recover the devices. All procedures were approved under a University of California San Diego Animal Subjects Committee Protocol (S02153) and a US Antarctic Treaty Permit (2011–016).

Instrument deployments

Under 0.5% bupivacaine local anesthetic (3–5 ml per bird), 2 subcutaneous ECG electrodes were inserted dorsally, with one right of midline at the level of the axilla and the other left of midline above the pelvis in manually restrained, hooded emperor penguins. The electrodes were connected to a custom-built digital ECG recorder (3991 BioLog, UFI) in

an underwater cylindrical housing (215 g, 16 × 3 cm), secured to the feathers of the mid-back with 5 min epoxy glue (Loctite; Henkel Corp.) and steel cable ties. ECG signals were recorded for 48 h at a sampling rate of 50 Hz. ECG recording was programmed to start 4 d after deployment of the recorder in order to collect data during the mid-portion of the foraging trip. Additionally, all birds were equipped with an Mk9 TDR (Wildlife Computers; sensitive to 0.5 m, 30 g, 6.7 × 1.7 × 1.7 cm) to record depth at a sampling rate of 1 Hz, and a VHF transmitter (Model MM130, ATS) to facilitate recapture.

Data processing and statistics

The f_H and dive data were processed, graphed, and statistically analyzed using Origin (ver. 8.6, Origin-Lab), Microsoft Excel, R software (R Development Core Team 2012), MATLAB (The MathWorks), and JMP (ver. 10.0.2, SAS Institute). TDR data were analyzed in MATLAB using a custom-written dive analysis program (IKNOS; Y. Tremblay unpubl.) and Instrument Helper (Wildlife Computers), which calculated a zero offset correction at the surface and identified dives on the basis of a minimum depth and duration. Dives were defined as submergences of ≥ 5 m and ≥ 1 min. Dive depth categories were designated as shallow (<50 m), intermediate (50–250 m), and deep (>250 m). ECG and TDR data were synchronized and a custom peak detection program (K. Ponganis) was utilized to mark R-wave peaks from the digital ECG records and calculate R-R intervals in Origin. All peaks were visually confirmed in order to ensure marking accuracy. The number of dives analyzed for individual birds was dictated by the clarity of the ECG signal. Portions of the ECG record that were difficult to decipher were omitted ($n = 48$ dives with f_H data gaps; all gap durations were <5% of dive duration).

A custom R script was used to determine dive f_H and total dive heartbeats. Dive f_H for each dive was calculated from the total number of heartbeats for each dive divided by the dive duration. In dives with a gap in the ECG record (<5% of dive duration), the gap duration was subtracted from the dive duration in the calculation of the overall dive f_H . Pre- and post-dive f_H values were calculated from the total number of heartbeats during the final and initial minute prior to and following a dive, respectively. Lowest resting f_H values were determined for individual birds over a period of 1 h. Resting periods were selected during long surface intervals at least

1 h after or before a dive bout, when the birds were presumably at rest.

Total number of heartbeats during a dive and the number of heartbeats from the start of a dive to the time that instantaneous f_H was consistently below resting f_H were determined through visual inspection of f_H profiles for each dive. Dives with f_H data gaps resulting from brief periods of indecipherable ECG signals were omitted from this analysis.

Mean f_H values at 30 s intervals were also analyzed by dividing depth profiles of dives into 7 categories of dive depth (0–25, >25–50, >50–100, >100–150, >150–250, >250–400, and >400 m). Instantaneous f_H for 30 s periods was determined using a custom R script and calculated as the mean of all instantaneous f_H values within a 30 s period. Dives with f_H data gaps resulting from brief periods of indecipherable ECG signals were excluded from this analysis.

Linear mixed-effects models (JMP) were used to examine the relationships of dive duration with dive f_H , dive depth with total dive heartbeats, and dive duration with total dive heartbeats. One model was fitted with dive f_H as a response variable, dive duration as a fixed effect, and individual as a random effect to account for repeated measures. A second model was fitted with total dive heartbeats as a response variable, dive depth as a fixed effect, and individual as a random effect. A third model was fitted with total dive heartbeats as a response variable, dive duration as a fixed effect, and individual as a random effect. An additional model was constructed to assess whether dive f_H values for dives with durations less than or greater than ADL (5.6 min) (Ponganis et al. 1997) were significantly different, with dive f_H as a response variable, dive duration category (< or >ADL) as a fixed effect, and individual as a random effect. Corrected Akaike's information criterion (AIC_c) was used to select the most parsimonious model. All means and medians are listed as means \pm SE and median (range).

RESULTS

Data recovery

The ECG signal was indecipherable in 2 of 6 birds, due to obfuscation of the signal by muscle artifact and possibly movement of ECG electrodes or mechanical malfunction. Consequently, simultaneous measurements of instantaneous f_H and depth were recorded from 4 birds (24.6 ± 0.4 kg), resulting

Table 1. *Aptenodytes forsteri*. Individual and pooled heart rate (f_H) data of emperor penguins diving at Cape Washington. The number of dives for each bird was dictated by the clarity of the ECG signal. Maximum depth, dive duration and dive f_H are presented as means \pm SE and medians (range)

Penguin	Body mass (kg)	No. of dives	Resting f_H (beats min^{-1})	Maximum depth (m)	Dive duration (min)	Dive f_H (beats min^{-1})
1	25.0	87	55	127.0 \pm 8.2 126.0 (15.0–340.0)	5.10 \pm 0.17 5.18 (1.32–8.77)	67 \pm 1 68 (38–90)
2	24.0	54	71	96.0 \pm 7.3 100.3 (5.0–246.0)	5.04 \pm 0.20 5.23 (1.32–7.22)	68 \pm 1 67 (53–91)
5	25.5	247	50	44.1 \pm 4.3 19.0 (6.0–422.5)	2.88 \pm 0.11 2.23 (1.00–9.22)	61 \pm 1 62 (9–117)
7	24.0	4	64	299.8 \pm 89.9 364.5 (39.0–431.0)	6.96 \pm 1.52 7.82 (2.80–9.42)	64 \pm 6 64 (52–76)
Grand mean ^a	24.6 \pm 0.4		56 \pm 4 ^b	72.3 \pm 4.1	3.71 \pm 0.10	64 \pm 1
Grand median (range) ^a	24.5 (24–25.5)		55 (50–64) ^b	32.5 (5.0–431.0)	3.08 (1.00–9.42)	64 (9–117)

^aPooled data (n = 4 birds, 392 dives); ^bPenguin 2 was excluded from resting f_H grand mean and median

in f_H and depth records for 392 dives ≥ 1 min in duration.

Resting heart rate profiles

Resting f_H ranged from 50 to 71 beats min^{-1} (n = 4 birds; Table 1). However, it is unknown whether the lowest resting f_H calculated for Penguin 2 was the minimum resting f_H over a 1 h period because of a dearth of resting hours with high signal clarity. Excluding Penguin 2, resting f_H ranged from 50 to 64 beats min^{-1} , with a mean of 56 ± 4 beats min^{-1} (n = 3 birds; Table 1). For this study, 56 ± 4 beats min^{-1} was selected as a conservative estimate of resting f_H for free-ranging emperor penguins.

General description of dive behavior

Dive durations from all dives ranged from 1 to 9.42 min, with a grand median of 3.08 min (Table 1). Sixty-one percent of dives were shorter than 4 min (Fig. 1A). Twenty-one percent of dives in the study were greater in duration than the previously

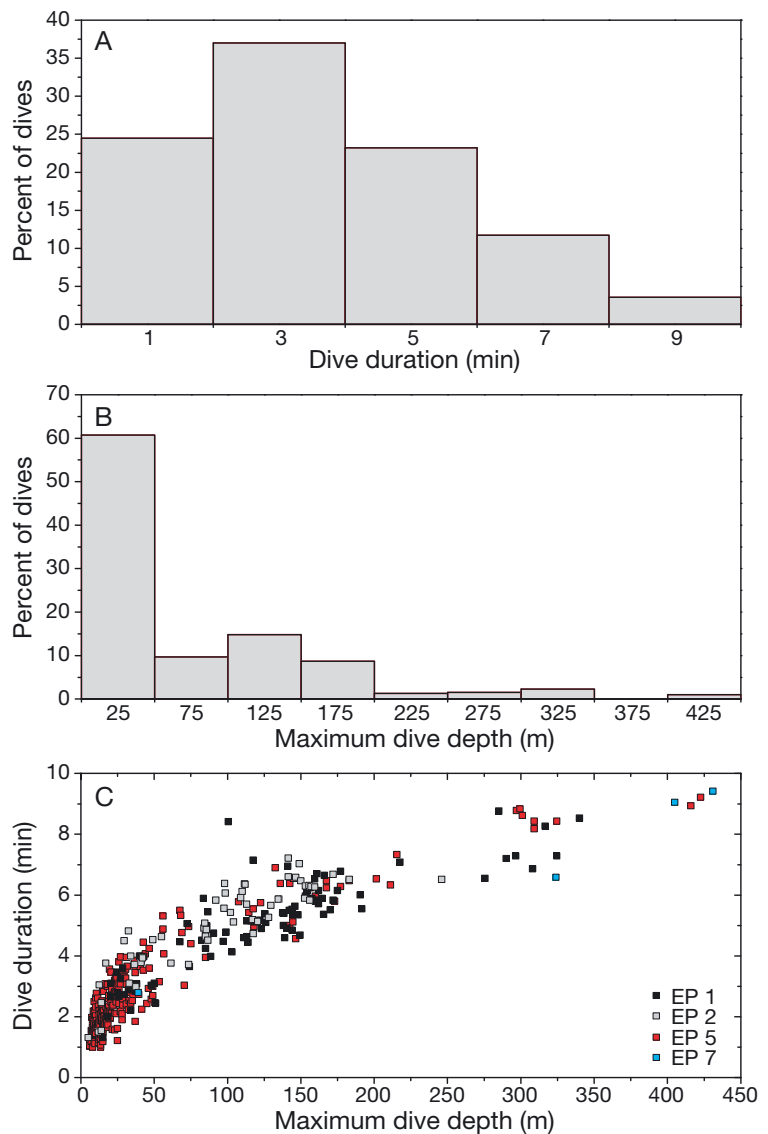


Fig. 1. *Aptenodytes forsteri*. Distributions of (A) dive duration and (B) maximum dive depth, and (C) dive duration versus maximum dive depth of dives from emperor penguins (EP) at sea. In (C), individual birds are denoted by color (n = 4 birds, 392 dives)

measured ADL of 5.6 min, and 4% of dives were greater than 8 min. Maximum depth of all dives ranged from 5 to 431 m, with a grand median of 32.5 m. The maximum dive depth for each bird ranged from 246 to 431 m (Table 1). Most dives were shallower than 100 m; however, 30% of dives were deeper than 100 m, and 5% of dives were deeper than 250 m (Fig. 1B).

Heart rate profiles during diving

All dives exhibited a characteristic pattern of f_H , with a pre- and post-dive tachycardia during surface intervals, reduced f_H upon submersion and throughout dives, and anticipatory tachycardia (increase in f_H coinciding with ascent) prior to surfacing. The pre-dive tachycardia (median = 202 beats min^{-1} , range = 109–231 beats min^{-1}) and post-dive tachycardia (median = 200 beats min^{-1} , range = 145–226 beats min^{-1}) were both $>f_H$ at rest (56 beats min^{-1}). Instantaneous f_H profiles in 3 dives of varying depth are shown in Fig. 2. The f_H response was characterized by: (1) pre- and post-dive tachycardia, (2) an abrupt partial decline from pre-dive levels (usually with a transient decrease to below resting levels), (3) a progressive, gradual decline in f_H during early descent, sometimes to below resting levels, (4) a continuation of lower f_H values during the bottom phase of the dive, and (5) a gradual increase in f_H during ascent (Fig. 2, 3). In 27% of dives, dive f_H was below the resting level of 56 beats min^{-1} (Table 2).

Diving heart rate and dive duration

The median dive f_H was 64 beats min^{-1} (range = 9–117 beats min^{-1}) for all dives (Table 1). The median dive f_H for dives shorter than the ADL (79% of the dives in this study; 66 beats min^{-1} , range = 9–117 beats min^{-1}) was significantly greater than the median dive f_H of 58 beats min^{-1} (range = 38–76 beats min^{-1}) for dives longer than the ADL (21% of the dives in this study; Tables 2, 3).

Eighteen percent of dives below the ADL had dive f_H values less than the resting level of 56 beats min^{-1} , while 45% of dives above the ADL had dive f_H values less than 56 beats min^{-1} (Table 2). For the previously reported f_H at rest of 73 beats min^{-1} (Meir et al. 2008) in emperor penguins at the isolated dive hole, dive f_H was less than resting f_H in 76% of dives shorter than the ADL and in 98% of dives longer than the ADL.

In dives ≥ 1 min in duration, there was a significant negative relationship between dive duration and dive f_H (Fig. 4, Table 3). However, dive f_H of dives

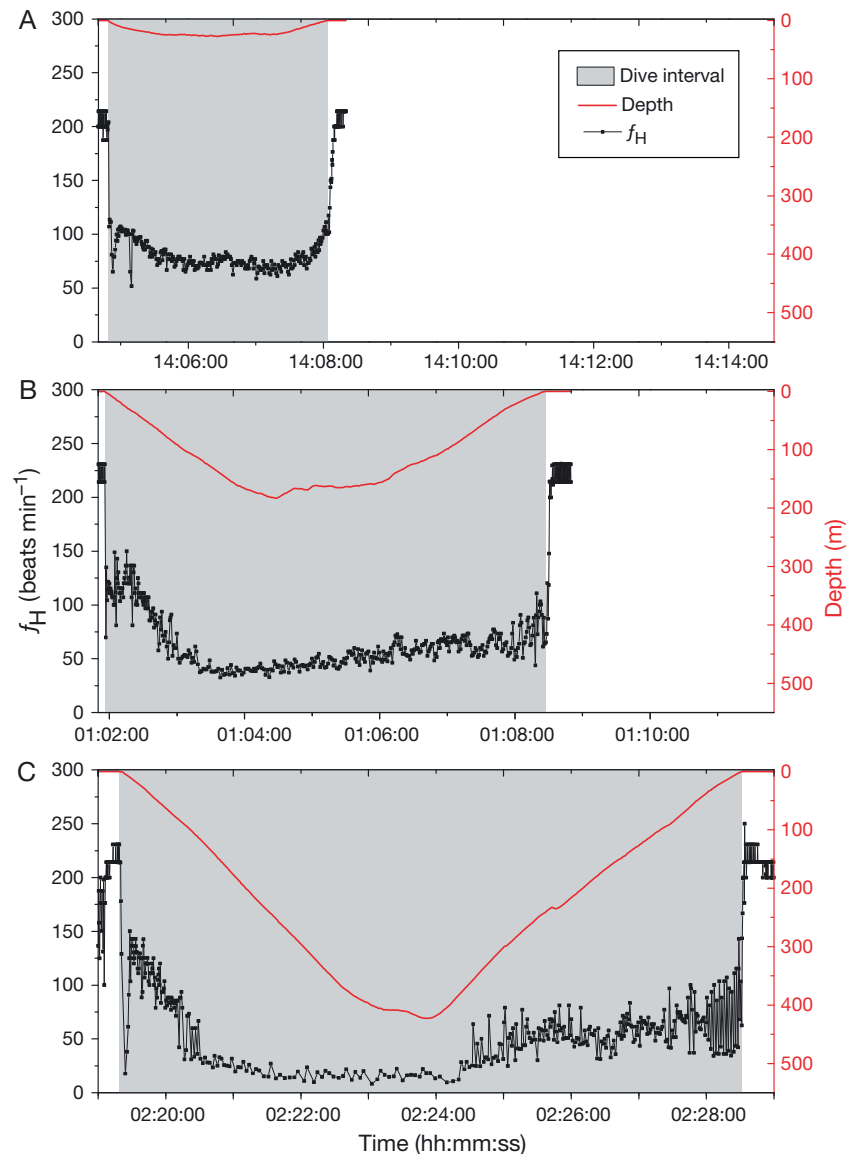


Fig. 2. *Aptenodytes forsteri*. Instantaneous heart rate (f_H) and dive depth profiles from (A) a shallow (27 m, <ADL) dive of Emperor Penguin 1, (B) an intermediate (183 m, >ADL) dive of Emperor Penguin 2, and (C) the deepest (423 m, >ADL) dive of Emperor Penguin 5. In (A), f_H reaches a minimum of 52 beats min^{-1} . In (B), f_H reaches a minimum of 33 beats min^{-1} . In (C), mean f_H is 17 beats min^{-1} for over 3 min, reaching a minimum of 8 beats min^{-1}

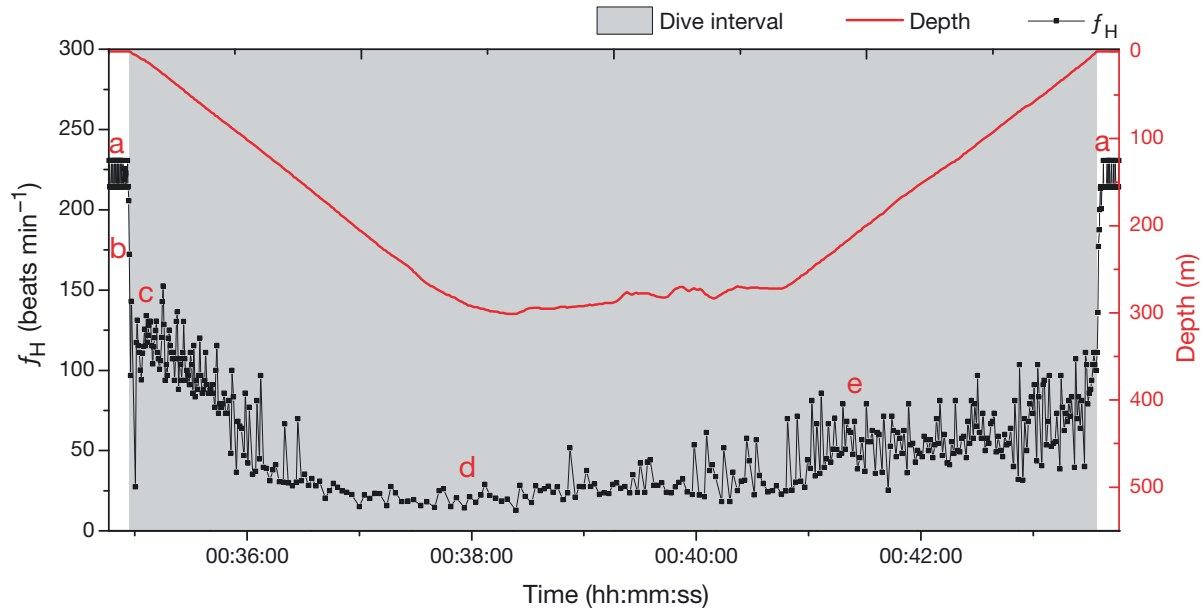


Fig. 3. *Aptenodytes forsteri*. Instantaneous heart rate (f_H) and dive depth profiles from a deep (301 m) dive of Emperor Penguin 5 with prominent features typical of the dive f_H profile: a, surface interval tachycardia (pre- and post-dive); b, initial sharp partial decline in f_H , immediately upon submersion, often with transient decrease to below resting levels; c, gradual decline in elevated f_H during early descent; d, prolonged severe bradycardia during latter descent and near maximum dive depth; e, slow increase in f_H during ascent

less than 2 min in duration or less than 50 m in depth varied considerably (Fig. 4).

In the analysis of dive f_H , 12% of dives had a data gap in the f_H profile. Eighty-one percent of gaps were ≤ 5 s and 75% of all gaps occurred at the start of the dive when wing movement, and thus the potential for muscle artifact, was greatest. Gaps of short duration should not significantly affect the results or interpretation of the data. For example, a dive with a 6 s gap and dive f_H of 100 beats min^{-1} would only have an increase in dive f_H by 2 beats min^{-1} for a 5 min dive.

Heart rate within dives

For all dives at sea with a complete ECG record (without a f_H data gap; 344 dives), total number of

dive heartbeats was in the same range as total heartbeats for dives of equivalent duration performed at the isolated dive hole (Meir et al. 2008) (Fig. 5). For dives near 5–7 min in duration, total dive heartbeats ranged between 250 and 400 heartbeats (Fig. 5).

In dives ≥ 1 min in duration, there was a significant relationship between dive depth and total number of dive heartbeats (Fig. 6A, Table 3). Total dive heartbeats was variable but increased until 150 m maximum depth, after which total dive heartbeats began to level off and remained high (Fig. 6A). A significant relationship was also observed between dive duration and total number of dive heartbeats (Fig. 6B, Table 3).

Instantaneous f_H reached values consistently below resting f_H in 31% of dives (dives without gaps in the f_H profile). The number of heartbeats prior to

Table 2. *Aptenodytes forsteri*. Heart rate (f_H) data for dives shorter and longer than the aerobic dive limit (ADL; 5.6 min). Maximum depth, dive duration and dive f_H are presented as means \pm SE and medians (range)

	No. dives	Maximum depth (m)	Dive duration (min)	Dive f_H (beats min^{-1})	% dives with dive f_H below resting f_H
All dives	392	72.3 \pm 4.1 32.5 (5.0–431.0)	3.71 \pm 0.10 3.08 (1.00–9.42)	64 \pm 1 64 (9–117)	27
Dives < ADL	310	40.4 \pm 2.3 23.5 (5.0–191.5)	2.91 \pm 0.07 2.58 (1.00–5.57)	65 \pm 1 66 (9–117)	18
Dives > ADL	82	192.9 \pm 9.3 160.5 (83.5–431.0)	6.74 \pm 0.11 6.43 (5.63–9.42)	57 \pm 1 58 (38–76)	45

Table 3. Data from mixed-effect models examining the relationships between dive duration and dive heart rate (f_H), aerobic dive limit (ADL) and dive f_H , dive depth and total dive heartbeats, and dive duration and total dive heartbeats. The corrected Akaike's information criteria (AIC_c) for all models are reported. The fixed effects and intraclass correlation coefficient (ICC) are presented for the superior model(s) (indicated in **bold**). Dive duration, dive duration category (<ADL, >ADL), and dive depth were fixed effects in corresponding models, and individual penguin was the random effect (with random intercept or random intercept and slope) in all models

Model	Model variables		AIC _c	Fixed effect				Random effect			
	Fixed effect	Random effect		Coefficient	Error	df	t	p	F-ratio	p	ICC (%)
Duration vs. dive f_H	Duration		3066.6								
	Duration	Penguin ID (intercept)	2959.3	-4.12307	0.31506	390	-13.09	<0.0001	171.2628	<0.0001	39.5
	Duration	Penguin ID (intercept + slope)	2959.3	-4.05915	0.73829	2	-5.5	0.0251	30.2284	0.0251	39.2
ADL vs. dive f_H	ADL	Penguin ID (intercept)	3081.2								
	ADL	Penguin ID (intercept)	3034.3	-6.37484	0.76622	390	-8.32	<0.0001	69.2198	<0.0001	17.5
	ADL	Penguin ID (intercept + slope)	3035.0	-6.11369	1.33275	2	-4.59	0.0390	21.0431	0.0390	16.4
Depth vs. total dive heartbeats	Depth		3834.2								
	Depth	Penguin ID (intercept)	3575.2	0.95637	0.15875	3	6.02	0.0108	36.2917	0.0108	62.4
	Depth	Penguin ID (intercept + slope)	3557.4								
Duration vs. total dive heartbeats	Duration		3539.4								
	Duration	Penguin ID (intercept)	3332.6	44.79090	4.53256	3	9.88	0.0019	97.6545	0.0019	59.8
	Duration	Penguin ID (intercept + slope)	3310.0								
		Penguin ID (intercept)	3949.4								

reaching resting f_H (56 beats min^{-1}) was variable (2–164 heartbeats), but as depth of dive increased, variability decreased, and values leveled off and remained between 100 and 120 heartbeats in most dives (Fig. 7A). A similar relationship was observed between the dive depth and the number of heartbeats prior to reaching the previously reported resting f_H (Meir et al. 2008) (Fig. 7B).

The profiles of mean instantaneous f_H at 30 s intervals of dives in 7 dive depth categories (Fig. 8) reflected instantaneous f_H profiles. Deeper dives had higher initial 30 s values, but then had lower f_H values throughout the middle portions of dives.

DISCUSSION

Resting heart rate

The resting f_H for free-ranging emperor penguins (56 ± 4 beats min^{-1}) was significantly less than the resting f_H determined for emperor penguins at the isolated dive hole (Meir et al. 2008). Differences in surrounding conditions can affect baseline f_H and may account for the large disparity of resting f_H values observed in free-ranging and captive emperor penguins (Halsey et al. 2008). Resting f_H for emperor penguins at the isolated dive hole may have been elevated due to stress associated with captivity, interactions with other birds, differences in dive and prey types, and longer diving recovery periods. In addition, the lower resting f_H values of emperor penguins at sea were the minimum 1 h resting f_H values found during prolonged surface intervals. These lower f_H values may occur during sleep and reflect a lower metabolic rate induced by sleep (Stahel et al. 1984, Dewasmes et al. 1989, Halsey et al. 2008). Thus, in our analyses of dive f_H , we consider the resting f_H of 73 and 56 beats min^{-1} to represent the upper and lower limits of resting f_H , respectively (Fig. 7), and, consequently, the upper and lower thresholds for f_H associated with a resting level of muscle blood flow.

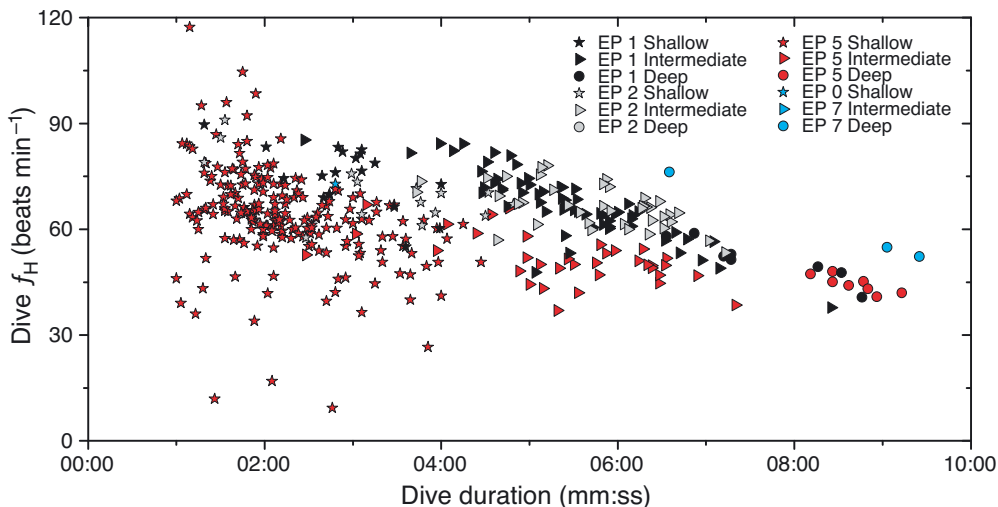


Fig. 4. *Aptenodytes forsteri*. Dive heart rate (f_H) (total number of heartbeats/dive duration) versus dive duration for emperor penguins (EP) at sea. Individual birds are denoted by color; dive depth categories (shallow: <50 m; intermediate: 50–250 m; deep: >250 m) are denoted by symbols (see key; $n = 4$ birds, 392 dives)

Dive behavior

Dive durations and maximum depths of dives of emperor penguins in this study (Fig. 1A,B) were typical of those reported in previous studies of free-ranging emperor penguins on foraging trips to sea during the chick-rearing period (Kooyman & Kooyman 1995, Kirkwood & Robertson 1997, Wienecke et al. 2007, Sato et al. 2011, Williams et al. 2012) and of dive durations of emperor penguins at the isolated dive hole (Ponganis et al. 2001, 2007, Meir et al. 2008, Sato et al. 2011, Williams et al. 2011). The tightly coupled positive relationship between dive duration and maximum dive depth (Fig. 1C) during foraging trips

to sea was similar to results from prior studies of free-ranging emperor penguins (Kooyman & Kooyman 1995, Sato et al. 2011). Despite the exclusion of dives less than 1 min in duration, the grand median dive duration was 3.08 min (range = 1.00–9.42 min; Table 1), below the ADL of 5.6 min.

Heart rate profiles during dives

Examination of individual f_H profile patterns revealed notable differences between shallow (<50 m) and deep (>250 m) dives. In shallow, short-duration dives, the overall f_H pattern was similar to that

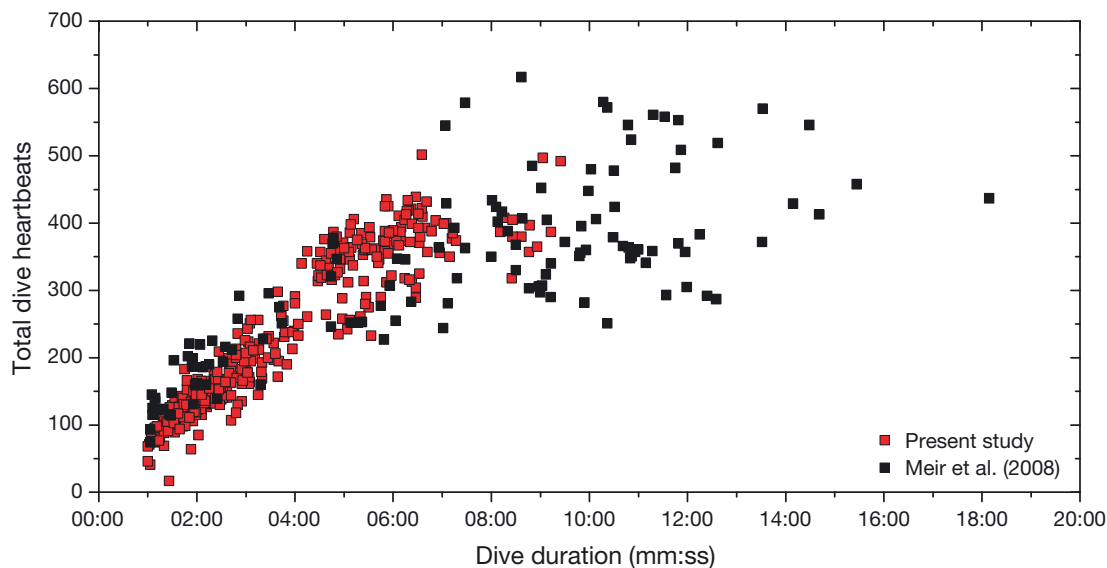


Fig. 5. *Aptenodytes forsteri*. Total dive heartbeats versus dive duration for the present study of emperor penguins diving at sea ($n = 4$ birds, 344 dives) and the Meir et al. (2008) study of emperor penguins diving at an isolated dive hole ($n = 9$ birds, 125 dives)

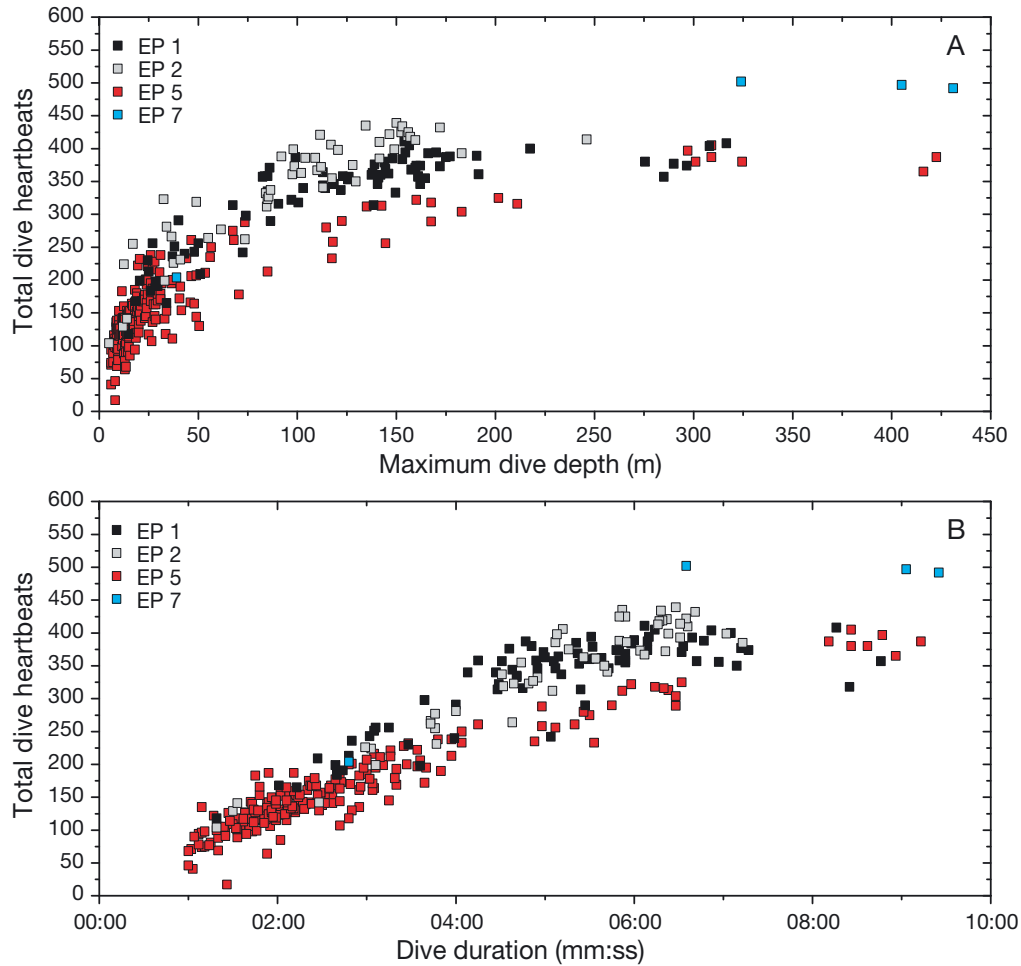


Fig. 6. *Aptenodytes forsteri*. Total dive heartbeats versus (A) maximum dive depth and (B) dive duration for emperor penguins (EP) at sea. Individual birds are denoted by color (see key; $n = 4$ birds, 344 dives)

observed in other free-ranging penguin species (Green et al. 2003, Froget et al. 2004) and in emperor penguins making short dives at an isolated dive hole (Meir et al. 2008). The f_H profile pattern of these dives was characterized by an initial rapid decrease in f_H from pre-dive values, followed by a gradual decline in f_H throughout the dive to a level sometimes below that at rest, and lastly, an increase in f_H during ascent (Fig. 2A). Although shallow, short-duration dives of emperor penguins had f_H profile patterns similar in shape to those of free-ranging birds of other penguin species, the f_H values during these shallow dives were much lower in emperor penguins than in the other species. Instantaneous f_H and overall dive f_H of these shallow dives were lower on both an absolute and a relative-to-resting basis than in the other 2 penguin species. Therefore, if there is a trade-off between the elevated f_H of the exercise response and the depressed f_H of the classic dive response in emperor penguins, the response of

the emperor penguin is much closer to the classic dive response than those of other penguins.

In contrast to shallow dive profiles of emperor penguins (both at sea and at an isolated dive hole) and all dive profiles of free-ranging king and macaroni penguins, the f_H profile pattern of emperor penguins in deep, long-duration dives differed in that the gradual decline in f_H during early descent culminated in a severe bradycardia to as low as $10 \text{ beats min}^{-1}$ during late descent and during the bottom phase of the dive (Figs. 2C, 3, 8). Dives to intermediate depths had less-intense bradycardias than the deep dives, but rates were lower than during shallow dives (Figs. 2B, 8).

The extremely low f_H during late descent or near the greatest depth of deep dives may serve to limit pulmonary gas exchange as well as peripheral perfusion during this segment of the dive. In addition to conserving respiratory O_2 for potential use later in the dive, the extreme bradycardia should also limit nitrogen absorption at maximal depths of deep dives,

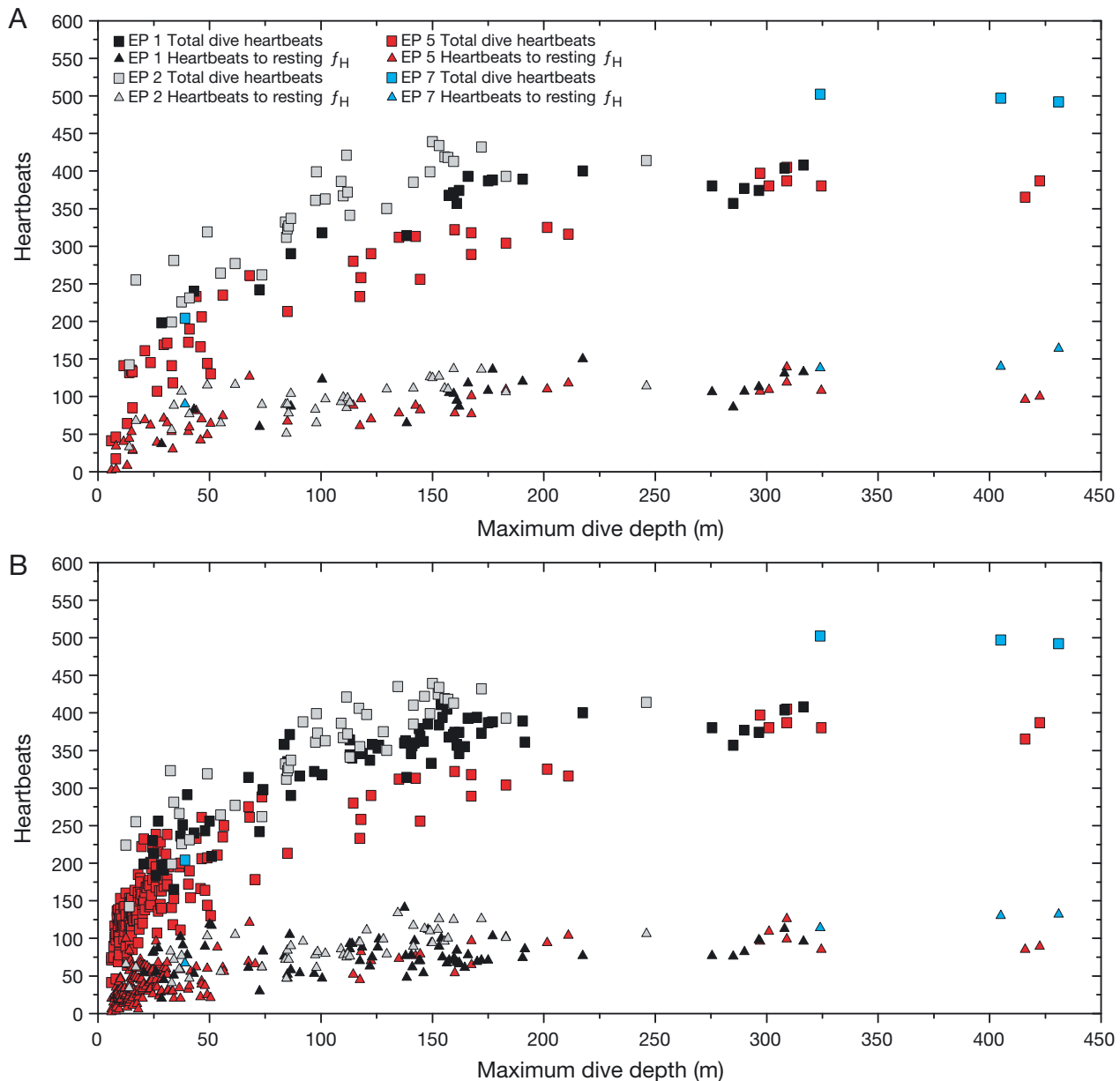


Fig. 7. *Aptenodytes forsteri*. Total dive heartbeats (squares) and heartbeats to reach resting heart rate (f_H ; triangles); (A) 56 beats min^{-1} (present study) and (B) 73 beats min^{-1} (Meir et al. 2008) versus maximum dive depth for emperor penguins (EP) at sea. Individual birds are denoted by color (see key; $n = 4$ birds, 105 dives in A, 301 dives in B)

a potential advantage in avoidance of decompression sickness. The decrease in cardiac output associated with a bradycardia of 10–20 beats min^{-1} would also limit perfusion of central organs and muscle, decreasing the rate at which blood O_2 is consumed and isolating muscle from the circulation. During this bottom phase of deep dives, stroke rates are highest (Williams et al. 2012), so the locomotory muscle, isolated from the circulation, is most probably dependent on myoglobin-bound O_2 for maintenance of aerobic metabolism.

Heart rate and the potential for muscle blood flow during dives

In 27% of all dives, the dive f_H was less than the lower limit of f_H at rest (56 beats min^{-1}). In 45% of dives greater than the ADL and 18% of dives shorter than the ADL, dive f_H demonstrated a true bradycardia across a range of dive depths at sea (Table 2). Additionally, the degree of bradycardia during dives increased with dive duration (Fig. 4). Such low f_H values (even relative to surface f_H or resting f_H) have not

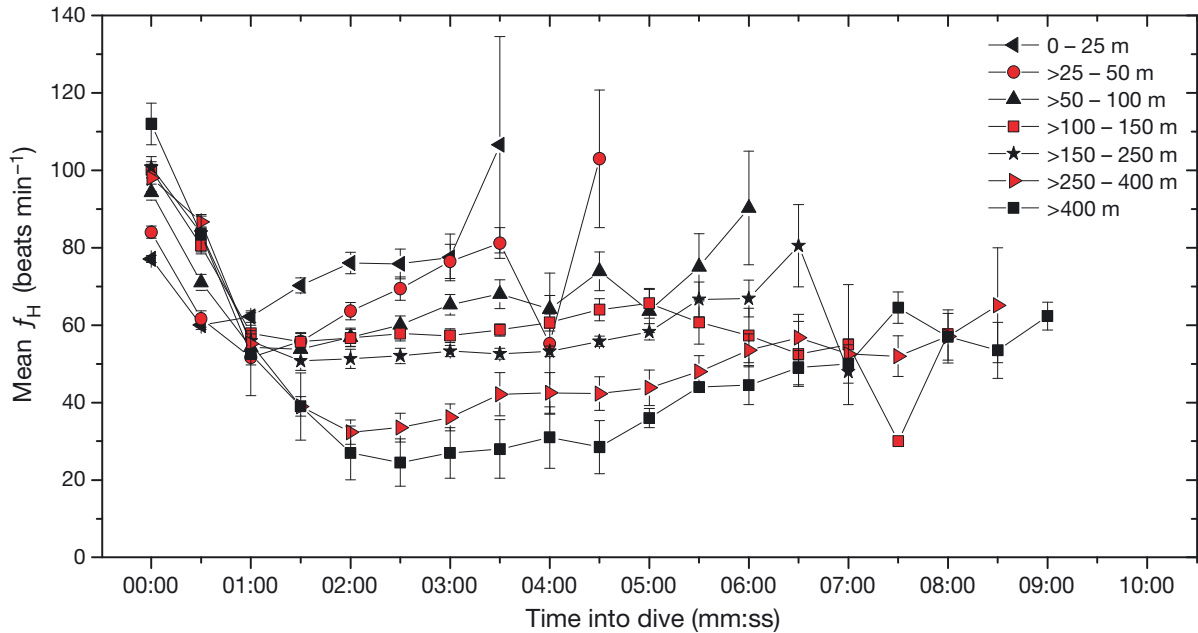


Fig. 8. *Aptenodytes forsteri*. Profiles of mean heart rate (f_H) at 30 s intervals of dives for 7 depth categories (0–25, >25–50, >50–100, >100–150, >150–250, >250–400, and >400 m). Standard error bars shown ($n = 4$ birds, 344 dives)

been reported in free-ranging macaroni and king penguins (Green et al. 2003, Froget et al. 2004). Lower dive f_H of emperor penguins at sea suggests relatively lower organ blood flow and lower muscle blood flow than in other penguin species, with slow depletion of blood O_2 and greater dependence of aerobic muscle metabolism on the higher O_2 content of muscle in emperor penguins (Ponganis et al. 2010, 2011). The myoglobin concentration of emperor penguin locomotory muscle is about 1.5 to 2 times greater than in other penguin species (Weber et al. 1974, Baldwin et al. 1984, Ponganis et al. 1999b). Thus, while the distribution of O_2 stores is similar in emperor and king penguins, emperor penguins have higher muscle myoglobin concentrations and greater muscle O_2 stores (Ponganis et al. 2010, 2011). Consequently, in regard to muscle metabolism, emperor penguins would be more tolerant of muscle ischemia and depressed f_H than other penguin species. In addition, due to their large size, the locomotory effort of the emperor penguin is potentially less than that of other penguin species, leading to a lower muscle metabolic rate and myoglobin desaturation rate (Sato et al. 2010). Thus, both larger muscle O_2 stores and less locomotory effort in emperor penguins probably make them more tolerant than other penguin species of lower f_H and less muscle perfusion during both shallow and deep dives.

It is also notable, in regard to potential restriction of muscle blood flow during dives at sea, that the total number of heartbeats in dives of equivalent duration

were similar in emperor penguins at sea and at the isolated dive hole (Fig. 5). Given that the number of wing strokes for dives of equivalent duration was greater at sea than at the isolated dive hole (Sato et al. 2011), the similarity between total heartbeats for dives of equivalent duration at sea and at the isolated dive hole suggests that the relationship of muscle work effort and f_H (i.e. muscle blood flow and O_2 delivery) is even more restricted at sea than at the isolated dive hole, resulting in greater dependence on myoglobin-bound O_2 . Alternatively, one might propose that selective dilatation of the locomotory muscle vascular bed as demonstrated in diving ducks may also occur in penguins (Bevan & Butler 1992). Such dilatation during dives at sea could then account for a greater distribution of cardiac output to muscle and, thus, allow more muscle blood flow per heartbeat. However, this has not been investigated in diving penguins. In addition, f_H of the diving duck is much higher, even on a relative basis (Bevan & Butler 1992), than that of diving emperor penguins.

Comparison of the relationships of total number of heartbeats and total number of wing strokes with dive duration offers additional support for a reduction in muscle blood flow in prolonged dives of free-ranging emperor penguins. The total number of heartbeats in a dive leveled off at approximately 6 min duration (Fig. 6B); in addition, for dives between 6 and 10 min of individual birds, the total number of heartbeats is within the same range. In contrast, the total number of

strokes during dives of emperor penguins at sea increased linearly with dive duration, with 10 min dives having nearly twice the number of strokes as 6 min dives (Sato et al. 2011). Despite a 2-fold increase in the total number of strokes between 6 min and 10 min dives, there is no increase in the total number of heartbeats, which again would suggest a greater reliance on muscle myoglobin and less muscle blood flow relative to stroke rate for longer dives.

Although myoglobin desaturation profiles of diving emperor penguins have demonstrated that muscle blood flow may occur during dives (Williams et al. 2011), the findings of the present study do not offer any evidence that there is a coupling of stroke rate (muscle workload) and f_H , as in a classic exercise f_H response or even as in the exercise-modified dive response suggested in other penguin species and, more recently, in dolphins and seals (Butler 1988, Green et al. 2003, Davis & Williams 2012). The lack of coupling of f_H and stroke rate is reflected, especially in deeper dives, by the fact that the lowest f_H values in the deepest dives of emperor penguins (Fig. 8) occurs at a time when previously measured stroke rates are near maximal levels (Williams et al. 2012). Similarly, although muscle blood flow may be enhanced by increasing f_H during ascent (Figs. 2, 3, 8), stroke rate is declining during this period (Williams et al. 2012). The same conclusion of a lack of coupling of stroke rate and f_H was also reached from f_H and stroke rate analyses during dives of emperor penguins at an isolated dive hole (Meir et al. 2008).

In contrast, because f_H and stroke rate during dives at sea are always highest during initial descent (Figs. 2 & 8) (Williams et al. 2012), it could be argued that muscle blood flow matches work effort at least during this period of the dive. However, during this time, at least in dives at the isolated dive hole, venous partial pressure of O_2 (P_{O_2}) and hemoglobin saturation are usually increasing, sometimes to arterial levels (Meir & Ponganis 2009). With increasing venous saturations it is unlikely that there is muscle blood flow and muscle O_2 extraction; if there were such flow to exercising muscle, venous saturation should decrease. Indeed, it has been suggested that arterialized venous blood O_2 levels early in the dive are consistent with flow-through peripheral arterio-venous shunts (Ponganis et al. 2009). Thus, the regulation of muscle blood flow during dives of emperor penguins at sea still requires further investigation.

It should be noted that in shorter, shallower dives, emperor penguins exhibit a range of f_H responses in relation to resting f_H limits, and therefore the muscle blood flow response may be variable during these

dives. For a large proportion of short, shallow dives, the f_H response of emperor penguins resembles that of other penguin species with a high potential for muscle blood flow and reduced dependence on myoglobin. However, when dive f_H exceeds resting f_H in shallow dives, it is still unknown whether diving emperor penguins perfuse muscle or possibly utilize peripheral arterio-venous shunts to enhance blood O_2 levels (Ponganis et al. 2009, Williams et al. 2011).

Heart rate and the potential for pulmonary blood flow during dives

The higher f_H values during short-duration, shallow dives imply more pulmonary blood flow, greater potential for gas exchange and more rapid utilization of the respiratory O_2 store than during deep dives. Although overall f_H is slower in longer dives (Fig. 4), the total number of heartbeats is maximal during these deeper dives secondary to (1) the duration of the dive, (2) the greater number of beats during early descent, and (3) the gradual increase in f_H during long ascents (Figs. 2, 3, 8). Thus, despite an overall low dive f_H and extreme bradycardia during the late descent and bottom phases of the deepest dives, the cumulative potential for pulmonary blood flow and gas exchange is maximized during these dives, consistent with the increased diving air volumes determined in deeper dives of emperor penguins (Sato et al. 2011). Overall utilization of the respiratory O_2 store should be slower under such circumstances, although gas exchange is probably greatest early in the dive, when f_H values are high (Figs. 2, 3, 6–8) and when air movement through the parabronchi of the lung is increased by high wing stroke rates (Boggs et al. 2001). Higher f_H values and a maximal number of strokes during this phase of deep dives (Figs. 6–8) support this suggestion. As already discussed, the severe bradycardias during the late descent and bottom phases of deep dives would limit gas exchange at great depths, a potential advantage for limiting nitrogen absorption as well as conserving respiratory O_2 for utilization during the increased f_H periods during ascent, as recently proposed for deep-diving sea lions (McDonald & Ponganis 2012).

Heart rate variability: a multi-factored response

Although the greater part of this discussion has been devoted to variations in f_H and implications for pulmonary gas exchange and muscle blood flow, individual dive f_H variability is also likely influenced

by an assortment of endogenous and exogenous factors. In short, shallow dives, physiological variability has been documented not only in f_H responses but also in levels and rates of blood O_2 depletion and in diving air volumes (Meir & Ponganis 2009, Sato et al. 2011). f_H and other physiological responses are clearly dependent on the nature and circumstances of individual dives (Furilla & Jones 1987, Jones et al. 1988, Noren et al. 2012). For example, extremely low dive f_H (range = 9–17 beats min^{-1}) in a few short-duration dives in this study corresponded to dives with very short surface intervals (descent following a limited post-dive surface interval of ≤ 5 s). After such short surface intervals, low dive f_H in the subsequent dive may function to conserve partially loaded O_2 reserves resulting from the abbreviated surface interval and may also reflect uncertainty about the timing of the next surface period. Future f_H studies of free-ranging emperor penguins should aid in the interpretation of dive responses in relation to specific behaviors and circumstances of individual dives (i.e. hunting, prey capture, travelling, and escape).

In summary, we have investigated the diving f_H response of emperor penguins during foraging trips at sea. We confirmed that: (1) dive f_H varies inversely with dive duration; (2) a significant proportion of dives have dive f_H values that are less than the value at rest, and dive f_H of dives greater than the ADL is lower than dive f_H of dives less than the ADL; (3) the total number of heartbeats in dives of equivalent duration at sea was similar to that of birds diving at an isolated dive hole, but dive profiles, especially in deeper dives, had different f_H patterns; (4) a profound bradycardia occurs during deep dives; and (5) the total number of heartbeats, and thus the cumulative cardiac output, is maximized in deep dives due to (a) the duration of the dive, (b) higher instantaneous f_H and a maximized number of heartbeats during early descent, and (c) the increase in f_H during the long ascent.

f_H values below resting rates during long-duration, deep dives at sea are consistent with conservation of blood and respiratory O_2 stores and with significant reliance on myoglobin-bound O_2 for aerobic muscle metabolism. Our findings suggest that adjustments to diving f_H preserve blood and pulmonary O_2 stores during deep dives by: (1) maintaining higher f_H to promote gas exchange during early descent and, perhaps, ascent periods, (2) lowering f_H to decrease gas exchange during deep portions of dives, and (3) limiting muscle blood flow. In contrast, f_H values during short-duration, shallow dives are usually higher, implying a greater potential for muscle perfusion, and perhaps also arterio-venous shunting.

Acknowledgements. We thank G. L. Kooyman, G. Marshall, J. Goldbogen, M. Tift, and M. Fowler for assistance in the field, and J. U. Meir for use of f_H data from the isolated dive hole study. We greatly appreciate the support of the United States Antarctic Program and McMurdo Station staff, and in particular, the efforts of the Berg Field Center, Fixed Wing Operations, and the pilots/crew of Ken Borek Air. This work was supported by National Science Foundation grant 09-44220. A.K.W. was supported by the NSF Graduate Research Fellowship Program.

LITERATURE CITED

- Andersson JP, Linér MH, Rünow E, Schagatay E (2002) Diving response and arterial oxygen saturation during apnea and exercise in breath-hold divers. *J Appl Physiol* 93:882–886
- Baldwin J, Jardel JP, Montague T, Tomkin R (1984) Energy metabolism in penguin swimming muscles. *Mol Physiol* 6:33–41
- Bevan R, Butler P (1992) Cardiac output and blood flow distribution during swimming and voluntary diving of the tufted duck (*Aythya fuligula*). *J Exp Biol* 168:199–217
- Boggs DF, Baudinette RV, Frappell PB, Butler P (2001) The influence of locomotion on air-sac pressures in little penguins. *J Exp Biol* 204:3581–3586
- Butler P (1988) The exercise response and the 'classical' diving response during natural submersion in birds and mammals. *Can J Zool* 66:29–39
- Davis RW, Williams TM (2012) The marine mammal dive response is exercise modulated to maximize aerobic dive duration. *J Comp Physiol A* 198:583–591
- Dewasmes G, Buchet C, Geloën A, Le Maho Y (1989) Sleep changes in emperor penguins during fasting. *Am J Physiol* 256:R476–R480
- Froget G, Butler P, Woakes A, Fahlman A, Kuntz G, Le Maho Y, Handrich Y (2004) Heart rate and energetics of free-ranging king penguins (*Aptenodytes patagonicus*). *J Exp Biol* 207:3917–3926
- Furilla R, Jones D (1987) The relationship between dive and pre-dive heart rates in restrained and free dives by diving ducks. *J Exp Biol* 127:333–348
- Green JA, Butler PJ, Woakes AJ, Boyd IL (2003) Energetics of diving in macaroni penguins. *J Exp Biol* 206:43–57
- Halsey LJ, Butler PJ, Fahlman A, Woakes AJ, Handrich Y (2008) Behavioral and physiological significance of minimum resting metabolic rate in king penguins. *Physiol Biochem Zool* 81:74–86
- Irving L, Scholander P, Grinnell S (1941) Significance of the heart rate to the diving ability of seals. *J Cell Comp Physiol* 18:283–297
- Jones D, Furilla R, Heieis M, Gabbott G, Smith F (1988) Forced and voluntary diving in ducks: cardiovascular adjustments and their control. *Can J Zool* 66:75–83
- Kirkwood R, Robertson G (1997) Seasonal change in the foraging ecology of emperor penguins on the Mawson Coast, Antarctica. *Mar Ecol Prog Ser* 156:205–223
- Kooyman GL (1989) Diverse divers: physiology and behavior. Springer-Verlag, Berlin
- Kooyman GL, Kooyman TG (1995) Diving behavior of emperor penguins nurturing chicks at Coulman Island, Antarctica. *Condor* 97:536–549
- Kooyman GL, Schroeder JP, Greene DG, Smith VA (1973) Gas exchange in penguins during simulated dives to 30 and 68 m. *Am J Physiol* 225:1467–1471

- McDonald BI, Ponganis PJ (2012) Lung collapse in the diving sea lion: hold the nitrogen and save the oxygen. *Biol Lett* 8:1047–1049
- Meir JU, Ponganis PJ (2009) High-affinity hemoglobin and blood oxygen saturation in diving emperor penguins. *J Exp Biol* 212:3330–3338
- Meir JU, Stockard TK, Williams CL, Ponganis KV, Ponganis PJ (2008) Heart rate regulation and extreme bradycardia in diving emperor penguins. *J Exp Biol* 211:1169–1179
- Noren SR, Kendall T, Cuccurullo V, Williams TM (2012) The dive response redefined: underwater behavior influences cardiac variability in freely diving dolphins. *J Exp Biol* 215:2735–2741
- Ponganis PJ, Kooyman GL, Starke LN, Kooyman CA, Kooyman TG (1997) Post-dive blood lactate concentrations in emperor penguins, *Aptenodytes forsteri*. *J Exp Biol* 200:1623–1626
- Ponganis PJ, Kooyman GL, Van Dam R, LeMaho Y (1999a) Physiological responses of king penguins during simulated diving to 136 m depth. *J Exp Biol* 202:2819–2822
- Ponganis PJ, Starke LN, Horning M, Kooyman GL (1999b) Development of diving capacity in emperor penguins. *J Exp Biol* 202:781–786
- Ponganis PJ, Van Dam RP, Knowler T, Levenson DH (2001) Temperature regulation in emperor penguins foraging under sea ice. *Comp Biochem Physiol A* 129:811–820
- Ponganis PJ, Stockard TK, Meir JU, Williams CL, Ponganis KV, Van Dam RP, Howard R (2007) Returning on empty: extreme blood O₂ depletion underlies dive capacity of emperor penguins. *J Exp Biol* 210:4279–4285
- Ponganis PJ, Stockard TK, Meir JU, Williams CL, Ponganis KV, Howard R (2009) O₂ store management in diving emperor penguins. *J Exp Biol* 212:217–224
- Ponganis PJ, Meir JU, Williams CL (2010) Oxygen store depletion and the aerobic dive limit in emperor penguins. *Aquat Biol* 8:237–245
- Ponganis PJ, Meir JU, Williams CL (2011) In pursuit of Irving and Scholander: a review of oxygen store management in seals and penguins. *J Exp Biol* 214:3325–3339
- R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Sato K, Shiomi K, Watanabe Y, Watanuki Y, Takahashi A, Ponganis PJ (2010) Scaling of swim speed and stroke frequency in geometrically similar penguins: They swim optimally to minimize cost of transport. *Proc R Soc B* 277:707–714
- Sato K, Shiomi K, Marshall G, Kooyman GL, Ponganis PJ (2011) Stroke rates and diving air volumes of emperor penguins: implications for dive performance. *J Exp Biol* 214:2854–2863
- Scholander PF (1940) Experimental investigations on the respiratory function in diving mammals and birds. *Hvalrad Skr* 22:1–131
- Stahel C, Megirian D, Nicol S (1984) Sleep and metabolic rate in the little penguin, *Eudyptula minor*. *J Comp Physiol B* 154:487–494
- Weber RE, Hemmingsen EA, Johansen K (1974) Functional and biochemical studies of penguin myoglobin. *Comp Biochem Physiol B* 49:197–214
- Wienecke B, Robertson G, Kirkwood R, Lawton K (2007) Extreme dives by free-ranging emperor penguins. *Polar Biol* 30:133–142
- Williams CL, Meir JU, Ponganis PJ (2011) What triggers the aerobic dive limit? Patterns of muscle oxygen depletion during dives of emperor penguins. *J Exp Biol* 214:1802–1812
- Williams CL, Sato K, Shiomi K, Ponganis PJ (2012) Muscle energy stores and stroke rates of emperor penguins: implications for muscle metabolism and dive performance. *Physiol Biochem Zool* 85:120–133

Submitted: February 15, 2013; Accepted: October 9, 2013

Proofs received from author(s): January 21, 2014