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Heart rate regulation and extreme bradycardia in diving emperor penguins

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SUMMARY

To investigate the diving heart rate (f_H) response of the emperor penguin (*Aptenodytes forsteri*), the consummate avian diver, birds diving at an isolated dive hole in McMurdo Sound, Antarctica were outfitted with digital electrocardiogram recorders, two-axis accelerometers and time depth recorders (TDRs). In contrast to any other freely diving bird, a true bradycardia (f_H significantly <f_H at rest) occurred during diving [dive f_H (total beats/duration)=57±2 beats min^{-1}, f_H at rest=73±2 beats min^{-1} (mean ± s.e.m.)]. For dives less than the aerobic dive limit (ADL; duration beyond which [blood lactate] increases above resting levels), dive f_H=85±3 beats min^{-1}, whereas f_H in dives greater than the ADL was significantly lower (41±1 beats min^{-1}). In dives greater than the ADL, f_H reached extremely low values: f_H during the last 5 mins of an 18 min dive was 6 beats min^{-1}. Dive f_H and minimum instantaneous f_H during dives declined significantly with increasing dive duration. Dive f_H was independent of swim stroke frequency. This suggests that progressive bradycardia and peripheral vasoconstriction (including isolation of muscle) are primary determinants of blood oxygen depletion in diving emperor penguins. Maximum instantaneous surface interval f_H in this study is the highest ever recorded for emperor penguins (256 beats min^{-1}), equivalent to f_H at $\dot{V}_{O_2}$ max., presumably facilitating oxygen loading and post-dive metabolism. The classic Scholander–Irving dive response in these emperor penguins contrasts with the absence of true bradycardia in diving ducks, cormorants, and other penguin species.

Key words: diving, electrocardiogram (ECG), emperor penguin, heart rate, respiratory sinus arrhythmia, stroke frequency.

INTRODUCTION

The emperor penguin, *Aptenodytes forsteri* Gray, is the consummate avian diver, reaching depths greater than 500 m (Kooyman and Kooyman, 1995) with durations longer than 23 min (Ponganis et al., 2007). This exceptional diving capacity has stimulated much interest in the physiology which underlies such behavior in this species (Butler and Jones, 1997; Kooyman and Ponganis, 1998; Nagy et al., 2001; Ponganis et al., 2003; Stockard et al., 2005). The duration of any given dive for an air-breathing animal is dependent on available oxygen stores, the rate of utilization of these stores, and the lowest tolerable level of oxygen (O_2). Based upon Scholander's and Irving's classic findings during forced submersion (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942), it is hypothesized that a reduction in heart rate (f_H) and peripheral perfusion are the principal determinants of the depletion rate of the respiratory and blood O_2 stores. This is because the metabolic rate of the kidneys, splanchnic organs and heart, which account for about half of O_2 consumption at rest (Butler and Jones, 1997; Schmidt-Nielsen, 1983), are either perfusion dependent or directly related to f_H (Davis and Kanatous, 1999). Adjustments in f_H can influence dive duration by providing both optimal loading of O_2 stores before the dive and a reduction and redistribution of blood flow to conserve O_2 during the dive (Butler and Jones, 1997).

In comparison to pre-dive tachycardic levels, most diving animals undergo a decrease in f_H upon submersion, with the magnitude of response varying between species and with dive duration (Butler and Jones, 1997; Irving et al., 1941; Scholander, 1940). Heart rate has also been used as a measure of field metabolic rate in several studies, based on the premise that O_2 consumption is directly proportional to f_H (Bevan et al., 1994; Boyd et al., 1999; Butler, 1993; Froget et al., 2004; Green et al., 2003). A relationship between mean f_H during the dive and dive duration has been documented in several diving species, including elephant seals, grey seals and cormorants (Andrews et al., 1997; Enstipp et al., 2001; Thompson and Fedak, 1993).

The objective of this experiment was to evaluate the cardiac responses of emperor penguins on a beat-to-beat basis using a digital electrocardiogram (ECG) recorder, specifically to assess diving f_H in relationship to: (1) f_H at the surface and at rest, (2) dive duration (3) the aerobic dive limit [ADL; duration beyond which blood lactate concentration increases above resting levels (Kooyman, 1989); also termed the diving lactate threshold (DLT) (Butler and Jones, 1997)] and (4) stroke frequency. An additional goal was to investigate variations in f_H associated with respiration while the birds were at rest and at the surface, and to consider how these variations might parallel the diving f_H response. The isolated dive hole is a useful model for this study, since field metabolic rate, the ADL and respiratory and blood O_2 store depletion rates have been determined for this species under these conditions (Nagy et al., 2001; Ponganis et al., 1997; Stockard et al., 2005; Ponganis et al., 2007).

We hypothesized that: (1) dive f_H would correlate negatively with dive duration, (2) a true bradycardia (f_H significantly <f_H at rest) would occur during diving, and (3) dive f_H would be independent of swim stroke frequency. Such findings would be consistent with the classic Scholander and Irving dive response (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942), with isolation of muscle and dependence of its metabolism on its large myoglobin-bound O_2 store. In addition, since f_H profiles at rest revealed a possible respiratory sinus arrhythmia (RSA), the naturally occurring respiratory-induced variation of f_H, we investigated the coupling of this potential RSA pattern and respirations with use of a chest impedance meter. Respiratory-induced variations in f_H were also compared to dive f_H values to assess any similarity in the physiological response and possible cardiorespiratory control mechanisms between these two conditions.

MATERIALS AND METHODS

Non-breeding emperor penguins (~15 birds/season, N=9 for this study, 25.1±0.6 kg) were captured near the McMurdo Sound ice edge or at Terra Nova Bay in October of 2001, 2004, and 2005 and were maintained for 2 months at an isolated dive hole enclosed within a corral at the Penguin Ranch (Kooyman et al., 1992) on the McMurdo Sound sea ice (77°41′, 165°59′). The birds dived and foraged under the ice daily, as evidenced by guano deposition and weight gain. Birds at the isolated dive hole typically exit the water after a dive and stand on the ice until initiating the next dive (Kooyman et al., 1992; Ponganis et al., 1997). All procedures were approved under a UCSD Animal Subjects Committee protocol and US Antarctic Treaty Permit. All animals were returned to the ice edge and released upon completion of the study.

Heart rate profiles

Investigation of respiratory sinus arrhythmia

In four emperor penguins under general isoflurane anesthesia (Ponganis et al., 2001), three subcutaneous electrodes were inserted dorsally (one just left of midline at the level of the axilla, the second just right of midline at hip level, and the reference electrode approximately 10 cm to the left of midline and 5 cm above the left hip). After recovery overnight, the electrodes were connected *via* a cable to an ECG/Impedance system (UFI, Morro Bay, CA, USA), and a BIOPAC A-D converter (BIOPAC Systems, Goleta, CA, USA) interfaced with a laptop personal computer (PC). Impedance and ECG signals were recorded at 62.5 and 250 Hz, respectively. Penguins were standing calmly in a penguin transport box (45×45×120 cm) located outside (ambient Antarctic temperature, –10 to –20°C) during the experiment, with the necessary wires and cords running back into the experimental hut where the hardware was located. Changes in the baseline impedance signal result from chest-volume-associated differences in skin impedance between inspiration and expiration and allow for the calculation of respiration rate. ECG and impedance signals were recorded simultaneously to accommodate analysis of corresponding changes between the two channels. The inspiratory–expiratory changes in the chest impedance signal were visually verified by an observer peering into the box for a portion of the recording cycle.

Heart rate at rest, during surface intervals, and during diving

Two-lead subcutaneous electrodes were inserted along the dorsal midline of nine emperor penguins (conducted separately from above protocol), roughly at the level of the axilla and just above the pelvis while birds were under general isoflurane anesthesia (Ponganis et al., 2001). The electrodes were connected to a custom-built digital ECG recorder (UFI) in an underwater housing (283 g, 16×6×2.5 cm), secured to the feathers of the mid-back with 5 min epoxy glue (Devcon; Danvers, MA, USA), a Velcro^{TM} patch, and plastic cable ties. ECG was recorded at a sampling rate of 50 Hz. An Mk9 time depth recorder (Wildlife Computers, Redmond, WA, USA; sensitive to 0.5 m, 30 g, 6.7×1.7×1.7 cm) was also attached to record the time/depth dive profile at 1 Hz. Transducer calibration from the TDR was verified in a pressure chamber at the Scripps Institution of Oceanography. After overnight recovery, the birds were provided access to the dive hole for 1–2 days. At the end of the data collection period, the electrodes and recorders were removed under general anesthesia.

Stroke frequency and heart rate

Five of the nine birds with the ECG recorder described above were simultaneously outfitted with a two axis acceleration data logger (M190-D2GT, Little Leonardo Ltd, Tokyo, Japan; 1.5 cm diameter, 6 cm length, 16 g). The accelerometer measures depth and acceleration in the y (vertical) and x (horizontal) axes. Depth was sampled at 1 Hz (±1 m accuracy and 0.05 m resolution) and acceleration at 16 Hz *via* an accelerometer sensor (model ADXL202E; Analog Device, Inc., Norwood, MA, USA). The measuring range of the accelerometer is ±29.4 $m\ s^{-2}$ with a resolution of 0.0196 $m\ s^{-2}$. The accelerometer was oriented so that the y-axis (head-to-tail) or 'surge' acceleration was in line with the spine (longitudinal axis of the animal), placed approximately midline between the apexes of the wings and secured with plastic cable ties and waterproof Tesa^{TM} tape. In this configuration, high frequency fluctuations in the y-axis acceleration signal correspond to 'surge', or the acceleration caused by the bird's stroking wing movements (Sato et al., 2004; Watanuki et al., 2003). The unit was calibrated in seawater against the earth's gravitational acceleration at the end of each experimental run.

Data analysis and statistics

All recorders were synchronized to the same PC clock, which was automatically synchronized to an Internet time server. Data were graphed and analyzed using Excel, Origin, SPSS, Acknowledge, and R software. Using a customized peak detection program, R-wave peaks from the digital ECG records were marked and R–R intervals calculated. All peaks were visually verified in order to ensure marking accuracy. In birds with the accelerometer, the same peak detection program was used to mark the high frequency fluctuation of surge acceleration (strokes), with stroke frequency calculated from the intervals between peaks. Instantaneous f_H was plotted with dive depth from the TDR to construct dive profiles, including stroke frequency for dives with the accelerometer. These instantaneous values are also reported throughout the text. For RSA data, f_H was plotted with chest impedance signal. Heart rates for different periods (at rest, dives and surface intervals) were calculated by dividing the total number of beats during the period by the period duration. This period consisted of 1 h for birds at rest, over the entire dive for dive f_H, and over 1 min immediately prior to and immediately following a dive for the surface interval f_H (pre- and post-dive). For RSA analysis, surface interval f_H values were also analyzed until the RSA pattern emerged after the dive (up to 5.2 min).

Heart rates from the different periods and within periods were log transformed and compared by independent samples t-tests. Log-transformed dive f_H and minimum f_H were also used for linear regression to assess the relationship of these variables to dive duration and stroke frequency. Dives were also divided into two categories of duration, those that were shorter or longer in duration than the previously measured ADL (5.6 min) (Ponganis et al., 1997). This value represents the transition point of a two-phase regression analysis of post-dive lactate accumulation and dive duration in that study. Although the ADL is probably a range of values, which shifts in accordance with factors such as activity and variable oxygen consumption during the dive (Ponganis et al., 1997), the value of 5.6 min was used for this analysis to provide a fixed point by which to categorize dives of different durations.

Table 1. Individual and pooled heart rate data for emperor penguin dives at the isolated dive hole

Bird	Number of dives	f_{H} (beats min^{-1}) At rest	Pre-dive	Dive	Post-dive	Duration (min:s)	Max. depth (m)
19	20	84	204±6	73±6	201±6	5.23±0.85	25.4±3.0
22	12	72	193±9	56±8	189±8	7.28±1.40	54.3±11.6
43	17	72	161±6	53±5	162±5	6.75±0.63	86.4±13.3
32	17	78	182±6	57±5	191±3	7.85±1.03	39.9±2.9
39	13	68	158±7	46±5	161±7	7.90±1.10	39.6±3.9
31	10	76	143±12	45±6	156±9	8.03±1.60	48.9±6.0
35	16	73	179±7	59±9	191±4	7.15±1.18	41.0±4.0
36	6	71	112±5	36±8	143±16	8.70±1.60	45.2±6.3
49	14	63	194±3	65±7	169±5	8.83±0.90	132.9±19.7
Grand mean* (range)		73±2 (63–84)	174±3 (96–256)	57±2 (23–134)	177±3 (97–256)	7.32±0.37 (1.05–18.15)	56.8±4.3 (5–225)

f_{H}, heart rate; values are mean ± s.e.m., *pooled data (N=9 birds, 125 dives).

Resting data are calculated as the total number of beats divided by the 1 h duration (resting on sea ice), and thus do not represent a mean. Pre- and post-dive data are calculated from the 1 min immediately prior to and immediately post-dive.

Individual variation among birds was addressed with ANOVA and Kruskal–Wallis tests. Testing for inter-individual effects specific to the f_{H} *vs* dive duration relationship was addressed with a resampling approach (R custom script). In this script, the number of dives specific to an individual bird was randomly removed from the pooled data and the regression equation (linear fit of log-transformed f_{H} *vs* dive duration) parameters from the resulting distribution recorded, with 1000 iterations. A penguin-specific regression equation was generated from another distribution with the specific dives of the individual bird removed from the pooled data. The parameters of the penguin-specific run were then compared to the distribution of parameters from the 1000 random iterations. If the penguin-specific parameter was not within the 2.5% tails of the random distribution, it was considered to fit within the distribution of pooled data. Otherwise, the data from this penguin were considered to significantly deviate from the pooled data. This procedure was repeated for each individual bird. Unless stated otherwise, statistical significance was assumed at $P<0.05$ and the significance level is quoted in the text. Values are expressed as means ± s.e.m.

RESULTS

ECG data were analyzed for nine birds over the three field seasons, resulting in f_{H} records for 125 dives. Accelerometer records for stroke frequency analysis were obtained from five of these birds, for a total of 62 of the 125 dives. Diving behavior in this study was typical of that at the isolated dive hole, with durations ranging from 1.1–18.2 min and a mean of 7.3±0.4 min. Sixty-five percent of dives in the study were greater in duration than the previously measured ADL. Depth ranged from 5–225 m, with a mean of 56.8±4.3 m. Birds instrumented with ECG recorders/accelerometers dived to similar depths and durations as those without these recorders. Heart rate in each bird at rest, measured over a 1-h period at night when access to the dive-holes was blocked, ranged from 63-84 beats min^{-1} with a mean of 73±2 beats min^{-1} (Table 1).

Heart rate profiles: electrocardiogram signal and heart rate patterns

ECG records demonstrated high signal integrity in all dives and surface intervals included in the analysis (Fig. 1). A characteristic pattern of f_{H} during the dives was evident, with high f_{H} before and after the dive (pre- and post-dive tachycardia), and a significant reduction in f_{H} during the dives. The dive f_{H} response consisted of an immediate decline upon submersion followed by a readjustment in which f_{H} increased briefly and then declined once again, with a further progressive decline in longer dives (Fig. 2). In 93% of the dives, f_{H} reached the level of f_{H} at rest within 1 min after submersion. In 71% of the dives, this level was reached within the first 20 s of the dive. An increase in f_{H} during ascent (prior to surfacing) occurred in most dives. Surface interval f_{H} peaked immediately pre- and post-dive, with a maximum instantaneous f_{H} of 256 beats min^{-1}.

In dives longer than the ADL there were typically extended periods of very low heart rates in the latter portions of the dive, whereas in short dives there was often a gradual increase in heart rate after a nadir about halfway through the dive (Fig. 2). Consistently low levels of f_{H} were found only in dives longer than the ADL, with the exception of one dive. In dives longer than the ADL, f_{H} remained below 30 beats min^{-1} for over 1 min in 59% of dives, below 20 beats min^{-1} for over 1 min in 35% of dives, and below 10 beats min^{-1} for over 1 min in 15% of dives. The mean of minimum instantaneous f_{H} values in dives shorter than the ADL was 44.8±2.7 beats min^{-1} (N=44), compared with 14.3±0.9 beats min^{-1} in dives longer than the ADL (N=81). The minimum instantaneous f_{H} was less than 20 beats min^{-1} in 72% of dives longer than the ADL, and less than 10 beats min^{-1} in 42% of those dives. Minimum f_{H} was less than or equal to 5 beats min^{-1} in 10% of dives longer than the ADL, with all of these dives being over 10.5 min in duration (Fig. 3). In the longest dive included in this study (18.2 min), the minimum f_{H} was 3 beats min^{-1}, with a rate of 6 beats min^{-1} for over 5 min (Fig. 2C). Instantaneous heart rate did not reach such extremely low values in short dives (Fig. 2A, Fig. 3A).

Heart rate profiles: comparisons of heart rate at rest, during dives and during surface intervals

In order to assess f_{H} over as wide a range of dive durations as possible, data from all birds were pooled. This was reasonable because the number of dives for each bird was relatively small, and because these data were meant as descriptive for the group, particularly in relation to a wide range of dive durations. According to ANOVA and Kruskal–Wallis tests, dive f_{H} and dive depth (max. depth recorded during dive) were significantly different between individual birds, while dive duration was not (Kruskal–Wallis: for f_{H}, depth, duration respectively, χ^2=24.265, 35.556, 10.527; P=0.002, 0.000, 0.230). The significant difference for dive f_{H}, however, appears only for bird 36. The resampling approach and comparison of regression equations generated from the f_{H} *vs* dive duration relationship also revealed differences among individuals,

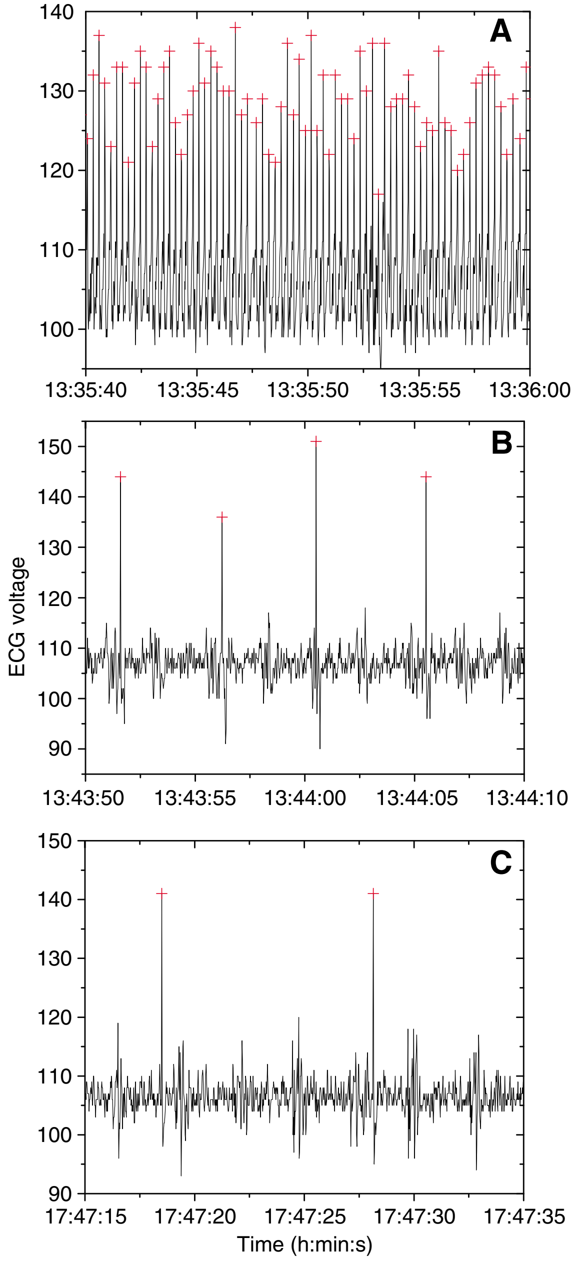

Fig. 1. Examples of ECG records of emperor penguin 35 over 20 s intervals, (A) at the surface and (B,C) during diving. From this ECG record, heart rate (f_H) was calculated as 214 beats min^{-1} (A) 12 beats min^{-1} (B) and 6 beats min^{-1} (C). Plus signs (red) represent detected peaks. The artifacts present in B and C correspond to wing strokes (as revealed by simultaneous review of stroke frequency and ECG profiles in this individual).

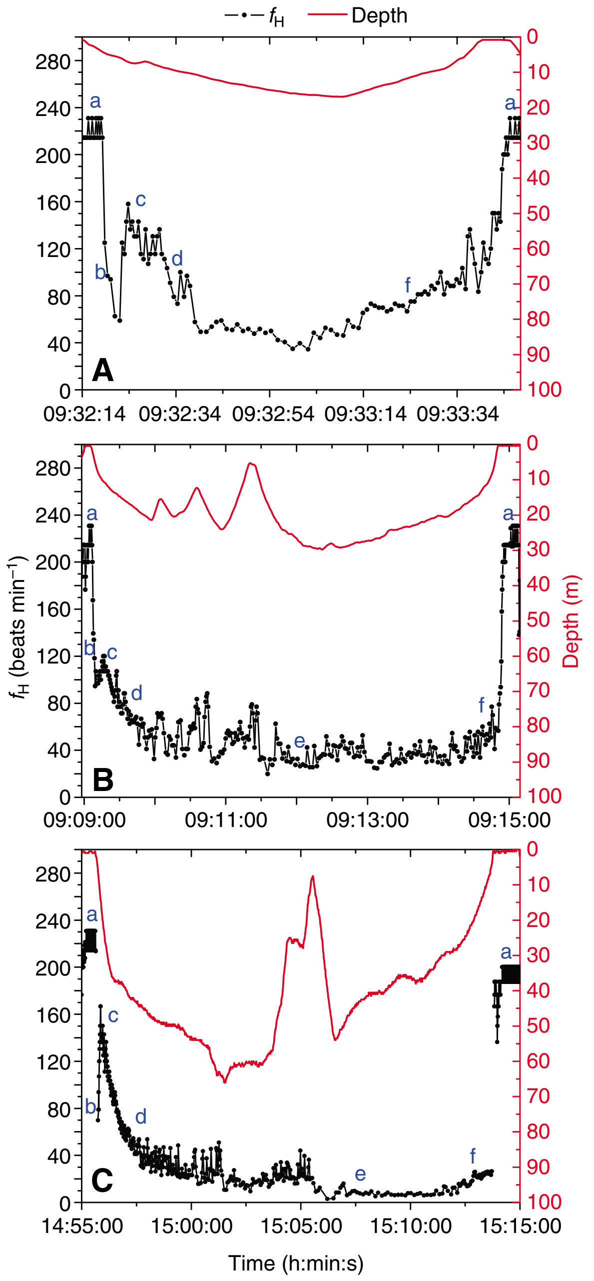

Fig. 2. Instantaneous heart rate (f_H) and dive depth profiles from (A) a typical short (~1.5 min) dive of emperor penguin 35, (B) a dive just longer than the ADL of emperor penguin 32, and (C) the longest dive in this study (18.2 min, emperor penguin 22). The f_H data gap at the end of the dive in C resulted from a brief period of unusable ECG signal. f_H in short dives does not approach extremely low values as in long dives. In C, f_H is 6 beats min^{-1} for over 5 min, reaching a minimum of 3 beats min^{-1}. Prominent features typical of dives are labeled as follows: a, surface interval tachycardia (pre- and post-dive); b, initial f_H decline, immediately upon submersion; c, readjustment of f_H (transient increase); d, secondary decline in f_H; e, progressive bradycardia in long dives; f, increase in f_H during ascent (prior to surfacing).

but when applying a Bonferonni correction due to multiple sampling ($P<0.006$), this difference was once again present for only one individual. Notably, if this one statistically deviating individual were removed, the results presented above did not change appreciably. Since inclusion of an outlier can only serve to weaken any resulting relationship, this analysis further reinforces the robustness of conclusions of the pooled data. Taking this into account, all nine individuals were included in the pooled dataset in all analyses in the study. Mean data from each of the f_H periods is shown for each bird in Table 1 (N=9, 125 dives).

The mean of dive f_H for pooled data (determined as the total number of beats divided by the dive duration) was 33% of the pre-dive f_H, and 78% of the mean f_H at rest (Table 2). This is similar to

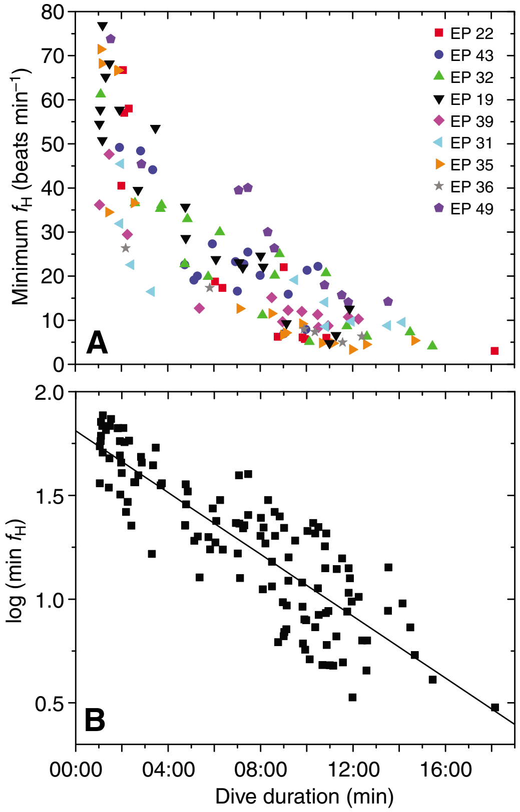

Fig. 3. (A) Minimum instantaneous heart rate (f_H) during the dive *vs* dive duration. Individual birds (EP) are denoted by colors and symbols (see legend), N=9 birds, 125 dives. (B) Log transformation of minimum f_H *vs* dive duration with regression equation for pooled dives: y=–107.27233x+1.81157, r^2=0.738, P<0.0001.

that reported in a previous study of emperor penguins, in which diving f_H was about 85% of the resting value (Kooyman et al., 1992). Although dive f_H was significantly lower than both pre-dive and at rest values for pooled data, for individual birds, the mean dive f_H was not significantly lower than rates at rest when considering dives of all duration collectively. To address f_H differences in relation to the previously measured ADL, dives from all birds were pooled and divided into those shorter than the ADL (35.2% of the dives in the study) and those longer than the ADL (64.8% of the dives in the study). For pooled data, f_H in dives longer than the ADL was significantly less than: (1) f_H in dives shorter than the ADL, (2) f_H at rest, and (3) f_H during surface intervals (Table 2). This was also true for each individual bird, with the exception of bird 49, in which f_H in dives longer than the ADL was not significantly lower than f_H at rest, but like other birds, was significantly lower when compared to f_H in dives shorter than the ADL.

Heart rate data of all birds were log transformed for regression analysis (Figs 3, 4, 7). Log transformation resulted in homoscedasticity and a linear fit for the f_H and dive duration relationship (Fig. 4B). Minimum instantaneous f_H recorded during the dive also decreased with dive duration, with a shape similar to that of the dive f_H *vs* dive duration relationship and a similarly negative linear relationship between the log of the minimum f_H *vs* dive duration (Fig. 3). There were no significant relationships between dive duration and max. depth of the dive, or between f_H and max. depth.

Table 2. Heart rate data

(A) Mean heart rate data divided between dives less than the aerobic dive limit and greater than the aerobic dive limit (5.6 min)

Period	f_H (beats min^{-1})	% of pre-dive f_H	% of f_H at rest
All dives	57±2	33	78
Dives<ADL	85±3	49	116
Dives>ADL	41±1	24	56

f_H, heart rate (mean ± s.e.m.); ADL, aerobic dive limit.

(B) Statistical analysis (*t*-test) of log-transformed data (analyzed with SPSS and R)

Dataset	Significant?	*P*	*t*
All *vs* rest	Yes	0.021	–2.337
<ADL *vs* rest	No	0.173	1.384
>ADL*vs* rest	Yes	0.000	–6.607
<ADL *vs* pre-dive	Yes	0.000	–17.837
>ADL *vs* pre-dive	Yes	0.000	–42.122
<ADL *vs* >ADL	Yes	0.000	14.380

ADL, aerobic dive limit.

A distinct respiratory sinus arrhythmia (RSA) was documented in emperor penguins at rest (Fig. 5). The change in the baseline chest impedance signal during respiration was visually verified as corresponding to actual breaths. The inspiration tachycardia is associated with the upstroke in the impedance signal. These patterns were present in all four of the penguins studied. Mean f_H at rest, peak instantaneous f_H (corresponding to inspiration), minimum instantaneous f_H (corresponding to expiration), the sinus arrhythmia index (SAI, difference between inspiratory and expiratory f_H), and the inferred respiration rate for a 10 min period are given for each bird in Table 3. The grand mean of minimum instantaneous f_H in the RSA (all minima during the 10 min period for all four birds) was 45.0±0.9 beats min^{-1} (N=181). During surface intervals, the RSA pattern was not evident until 107±10 s (N=43) after the dive.

Stroke frequency and heart rate

Emperor penguins diving at the isolated dive hole stroked continuously throughout the dive, with no prolonged periods of gliding. The highest stroke frequencies (~1.5–2.2 Hz) occurred during the initial descent (Fig. 6). Excluding descents, stroke frequency during the dive was very consistent, with typical values around 0.45–0.70 Hz, depending on the individual bird. This steady stroking frequency was usually achieved after the first 20–30 s of the dive. Dive stroke frequency (the calculated mean of all values during the dive) in relatively short dives was greater than that of long dives because of the high stroke frequencies associated with the initial descent and a relatively short post-descent period, during which stroke frequency was low and stable. Longer dives had inherently longer post-descent periods (Fig. 6). Because of this bias, dives in which the initial descent period constituted 20% or more of dive duration (generally dives shorter than 2.5 min, 15 out of the 62 dives analyzed) were not considered when assessing the relationship between dive stroke frequency and dive duration or to dive f_H. This was further justified by the fact that stroke frequency patterns showed no differences in short and long dives (Fig. 6). With the exclusion of these dives, there is no significant correlation between stroke frequency and the log of f_H during the dive (y=0.508x+1.27, r^2=0.082, P=0.051; Fig. 7A). Stroke frequency had only a weak negative relationship with dive duration

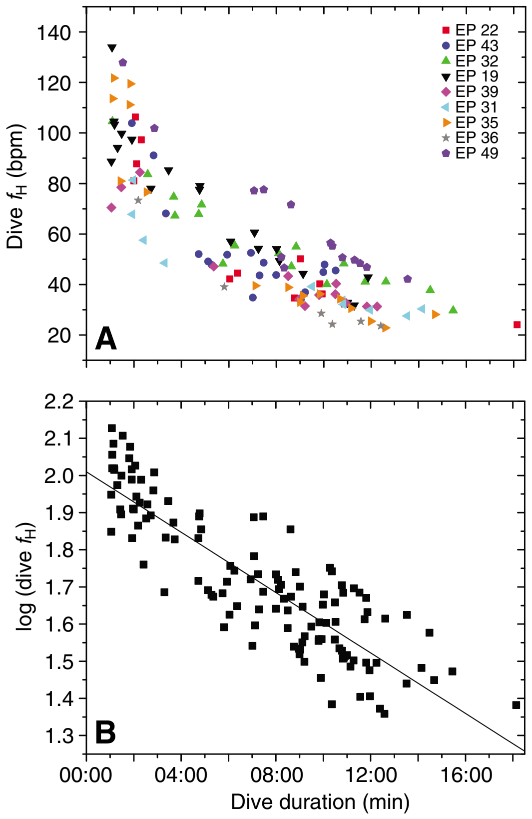

Fig. 4. (A) Dive heart rate (f_H) (total number of beats/dive duration) *vs* dive duration for emperor penguins diving at the isolated dive hole. Individual birds (EP) are denoted by colors and symbols (see legend), N=9 birds, 125 dives. (B) Log transformation of dive f_H *vs* dive duration with regression equation for pooled dives: y=–58.5361x+2.00996, r^2=0.755, P<0.0001.

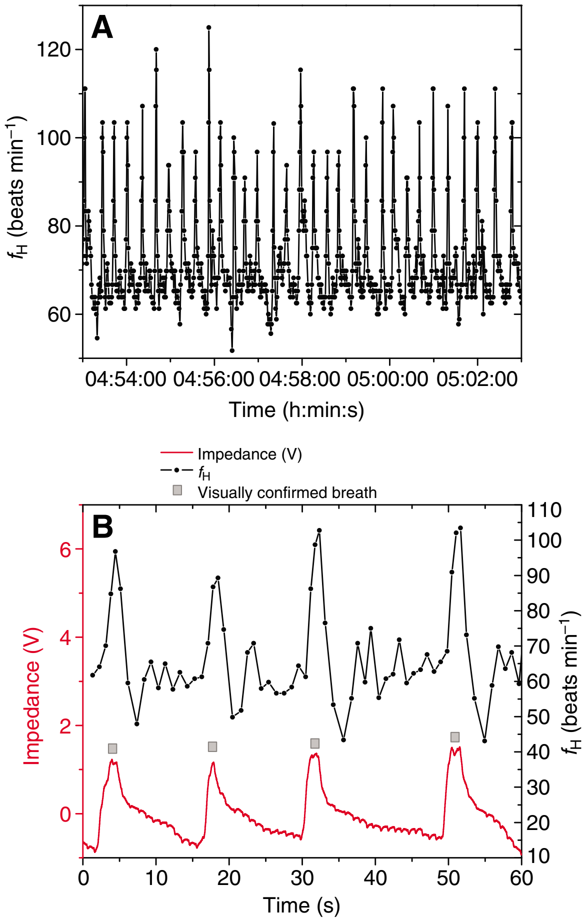

Fig. 5. (A) Heart rate (f_H) over a 10 min period in emperor penguin 22 resting on the sea ice. Note the regular, momentary tachycardias. (B) Heart rate (black) and impedance signal (red) from emperor penguin 32 during 1 min at rest. The maxima in the impedance signal were visually verified as corresponding to inspiration (grey squares). The tachycardia associated with each visually confirmed inspiration is associated with the upstroke in the impedance signal. Respiratory rate in this case is counted as 4 breaths min^{-1}.

(y=–17.70x+0.72, r^2=0.267, P=0.0002; Fig. 7B). The lack of coupling of stroke frequency and f_H is most apparent when considering individual dive profiles (Fig. 6). Simultaneous profiles of depth, stroke frequency, and instantaneous f_H during a dive revealed that changes in stroke frequency are not consistently associated with changes in f_H.

DISCUSSION

Heart rate profiles: at rest

Heart rates during rest periods in this study were equivalent to those of both previous studies and to allometric predictions (Calder, 1968; Grubb, 1983; Kooyman et al., 1992). Respiratory sinus arrhythmias (RSAs) have been documented in a variety of birds and diving species (Andrews et al., 1997; Bartholomew, 1954; Butler and Taylor, 1983; Casson and Ronald, 1975; Castellini et al., 1994a; Castellini et al., 1994b; Enstipp et al., 1999; Irving, 1939) and are more pronounced in some species than others [for example, in dogs (Hamlin et al., 1966)]. Previous work on elephant seals has demonstrated that RSAs can be used to estimate respiratory frequency in diving animals, even while at sea (Andrews et al., 2000). Apparent RSA has recently been used to determine probable breathing frequency in resting king penguins (Halsey et al., 2008). The complete ECG record afforded by the digital recorders in this study reveal an RSA pattern in emperor penguins and validate the inference of respiration rate from the regular, momentary tachycardias present in f_H data of birds at rest (Fig. 5). Analysis of these tachycardias in ECG records of emperor penguins at rest on the sea ice in McMurdo

Table 3. Mean heart rate at rest, during inspiration (peak instantaneous heart rate) and expiration (minimum instantaneous heart rate), sinus arrythmia index and the inferred number of breaths min^{-1} for each bird

	f_H (beats min^{-1})				No. breaths min^{-1}
	Mean at rest	Peak (inspiration)	Minimum (expiration)	SAI	(10 min period)
Bird 31	68.6	93.3±1.7	56.3±0.8	37.0±1.6	4.4
Bird 32	67.2	98.6±1.8	48.9±1.3	49.7±2.0	4.3
Bird 33	42.9	80.0±3.6	31.7±0.6	48.4±3.4	3.5
Bird 34	50.3	83.5±2.3	37.8±1.0	45.7±2.2	4.3

f_H, heart rate; SAI, sinus arrythmia index (difference between inspiratory and expiratory heart rates). The f_H value at rest is calculated as the total number of beats divided by the duration, and thus does not represent a mean.

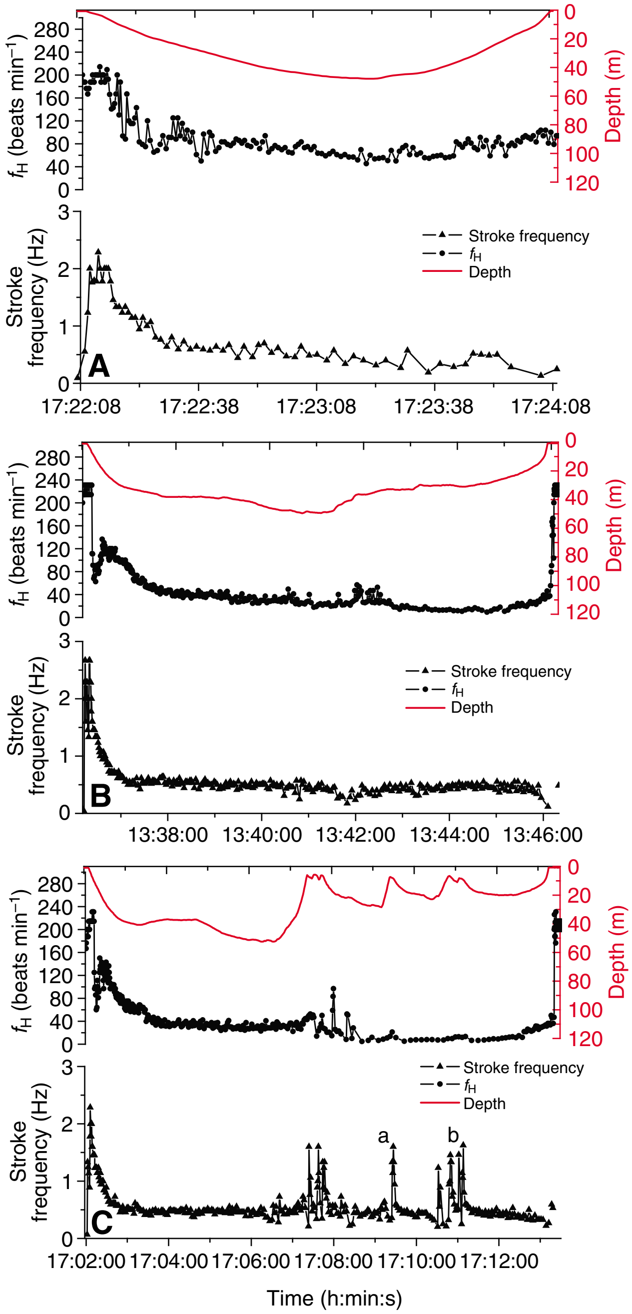

Fig. 6. Heart rate (f_H), stroke frequency, and dive profiles of: (A) a relatively short (<2 min) dive of emperor penguin 31; (B) a ~10 min dive of emperor penguin 35; (C) a ~12 min, variable depth dive of emperor penguin 35. In B f_H drops by a factor of 2 by the latter half of the dive, while stroke frequency remains constant. An increase in f_H is also apparent prior to surfacing, again with no corresponding increase in stroke frequency. In C stroke frequency is always highest during descents (labeled a and b), but f_H does not increase during descents. f_H remained near 5 beats min^{-1} for much of the second half of the dive compared to around 35 beats min^{-1} in the first half. Stroke frequency, however, remains near 0.45 Hz, increasing only during descents and while near the surface.

Sound reveals a mean respiration rate of 4.1±0.3 breaths min^{-1} (mean ± s.e.m., N=9; 1 h period/bird). Respiration rates of the birds standing calmly in the carrier box for this portion of the study (Table 3) were equivalent. These data are comparable to observed emperor penguin respiratory rates from a previous study (Kooyman et al., 1971) and

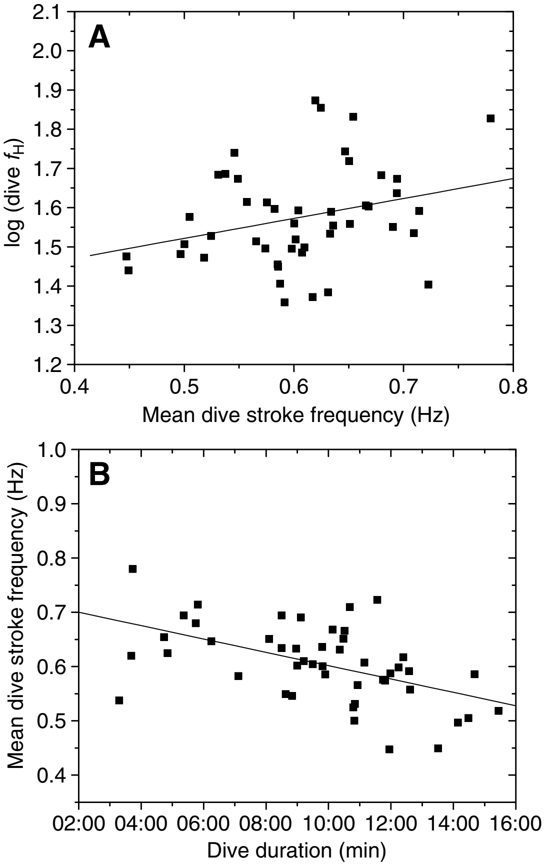

Fig. 7. Log transformation of dive heart rate (f_H) *vs* mean dive stroke frequency (A) and mean dive stroke frequency *vs* dive duration (B), N=5 birds, 47 dives. As seen in the linear fits in A the log of dive f_H does not have a significant relationship to stroke frequency (y=0.508x+1.27, r^2=0.082, P=0.051), and in B, dive stroke frequency has a significant, but weak negative relationship to dive duration (y=–17.70x+0.72, r^2=0.267, P=0.0002).

are significantly lower than predicted respiratory rates based on allometry (Calder, 1968) (t-test, P=0.000).

The grand mean of minimum instantaneous f_H in the RSA (all minima during the 10 min period for all four birds; mean= 45.0±0.9 beats min^{-1}, N=181) is strikingly similar to the minimum f_H in dives shorter than the ADL (mean=44.8±2.7 beats min^{-1}, N=44). That is, f_H during dives shorter than the ADL falls to a level only equivalent to that reached during routine expiration at rest, as opposed to a much more extreme drop in dives longer than the ADL (mean=14.3±0.9 beats min^{-1}, N=81). This finding is similar to the f_H response during sleep apnea of elephant seals, in which the apneic f_H was comparable to the minimum value during normal respiratory sinus arrhythmia (Castellini et al., 1994a; Castellini et al., 1994b). Similar to what was conjectured for elephant seals, perhaps the reduction in f_H during dives shorter than the ADL in emperor penguins is controlled by a similar level or mechanism of cardiorespiratory control to that governing the respiratory sinus arrhythmia, with a further reduction in f_H (and true bradycardia as defined in this work) occurring only in dives longer than the ADL.

Heart rate profiles: diving

Heart rates of emperor penguins in dives shorter than their ADL (<5.6 min) in this study are in agreement with f_H of other diving birds. For example, cormorants and several penguin species show a significant decrease in diving f_H as compared to pre-dive values, with f_H maintained throughout the dive at a level similar to that at rest (Butler and Woakes, 1984; Enstipp et al., 2001; Froget et al., 2004; Millard et al., 1973). Mean f_H in the emperor penguin in pooled dives, and, more notably, in dives longer than the ADL, is significantly lower than both surface interval f_H and f_H at rest (Table 2), consistent with findings from the previous study of f_H in diving emperor penguins (Kooyman et al., 1992). This demonstrates a true bradycardia (f_H significantly $<f_H$ at rest) for the diving emperor penguin at the isolated dive hole. Such a response has never been documented in any other diving bird, even in dives greater in length than their calculated aerobic dive limit (cADL; though this cannot be considered a direct comparison to the measured ADL as reported here) (Butler and Woakes, 1984; Enstipp et al., 2001; Froget et al., 2004; Millard et al., 1973).

An immediate decline in f_H upon submersion was characteristic of all dives in the study. The intensity of bradycardia during the dive increased with dive duration. This applies to individual dives (lower f_H as the dive progresses) and to the dive f_H and dive duration relationship (Figs 2, 4). Because f_H is indicative of the rate of blood O_2 transport and delivery to the tissues, the bradycardia documented in this study is probably associated with a reduction in blood flow to muscle and other organs. Although it occurs immediately upon submersion, the initial decline in f_H in the emperor penguin is gradual, as described in Scholander's forced submersion studies with penguins (Scholander, 1940). This initial decline occurs more gradually than that seen in some diving seals (Andrews et al., 1997; Thompson and Fedak, 1993). Emperor penguins have 19% of their O_2 stores in the respiratory system (Kooyman and Ponganis, 1998) and dive upon inspiration (Kooyman et al., 1971). Thus, the gradual decrease in f_H in the beginning of dives may reflect the continued uptake from respiratory O_2 stores during this period. Deep diving seals, however, with <5% of O_2 stores in the lungs, would not benefit from maintenance of f_H (i.e. continued O_2 extraction) in this respect, consistent with the immediate drop to low f_H in these species.

Heart rate decreased to extremely low values in long dives, in some cases remaining below 5–6 beats min^{-1} for several minutes (Fig. 2C, Fig. 6C). Consistently low levels of f_H were frequent in dives longer than the ADL. These data demonstrate that such extreme responses are a common component of emperor penguin diving physiology. The minimum instantaneous f_H during the dive decreased significantly with increasing dive duration (Fig. 3). This distribution emphasizes that extremely low values are only present in longer dives, with the degree of bradycardia reflecting dive duration. Such extremely low values of f_H have been demonstrated in free diving seals (Andrews et al., 1997; Thompson and Fedak, 1993), but in birds, have only been documented in forced submersion or exclusion studies (Butler, 1982; Butler and Jones, 1968; Folkow et al., 1967; Jones, 1969; Jones et al., 1988; Jones and Holeton, 1972; Ponganis and Kooyman, 2000; Stephenson et al., 1986). Low f_H, present in later portions of long dives, may reflect a reinforcement of the initial bradycardia by low O_2 partial pressure (P_{O2}), through a possible chemoreceptor response (Butler and Stephenson, 1988; Enstipp et al., 2001). It is also interesting to note that some extremely low f_Hs correspond to sudden reversals (descent immediately following an ascent) in the depth profile (Fig. 2C, Fig. 6C), as has been documented in other diving species (Andrews et al., 1997). These reversals in the depth profile in emperor penguins diving at the isolated dive hole have been associated with the ascents and descents of foraging events (Ponganis et al., 2000). In addition, in some cases, such reversals might indicate an additional descent after a return toward a hole in which exit was blocked by other birds or an occasional Weddell seal.

Assuming stroke volume is constant (Butler and Jones, 1997), the ratio of dive f_H to f_H at rest is equal to that of the respective cardiac outputs. Considering the mean of dive f_H for all dives longer than the ADL, this indicates a mean 44% reduction in cardiac output during these dives. For the longest dive in the study (18.2 min), mean cardiac output would be decreased by 77%. During the periods of very low f_H toward the end of longer dives (Fig. 2C, Fig. 6B,C), cardiac output may be reduced by as much as 93% for five or more minutes. This is consistent with a low blood O_2 depletion rate while diving, and demonstrates an extreme diving response, as originally proposed by Scholander and Irving in their early work with forced submersion studies (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942).

An increase in f_H during ascent (prior to surfacing) was a common feature in most dives. Such a response has been observed in many diving species, including other penguins, and has been termed an 'ascent' or 'anticipatory tachycardia' (Andrews et al., 1997; Froget et al., 2004; Green et al., 2003; Millard et al., 1973; Thompson and Fedak, 1993). It has been hypothesized that this response may serve to replenish the O_2 supply to depleted tissues, thereby lowering the P_{O2} in blood, and providing a larger gradient to maximize O_2 uptake at the surface, and perhaps to minimize recovery time (Butler and Jones, 1997; Millard et al., 1973; Thompson and Fedak, 1993). If the number of beats in this period of increased f_H during ascent are counted and multiplied by the stroke volume of the emperor penguin (Kooyman et al., 1992), an estimate of the amount of blood circulating in that time period can be obtained. Such a calculation provides the percentage of blood available that could contribute to the supply of depleted tissues at the end of the dive. For example, considering the longest dive in this study (Fig. 2C) and the known blood volume of an emperor penguin (Kooyman and Ponganis, 1998), 92% of the blood volume would be circulated during this ascent period. For the 10 min and 11 min dives in Fig. 6B,C, these values are 47% and 43%, respectively. For shorter dives of 1.5 min and 5.8 min (Fig. 2A,B), 96% and 69% of the blood volume is circulated during this ascent period. These values imply that over a wide range of dive durations, a significant portion of the blood volume is circulated during the period of increased heart rate during ascent. The distribution of this increased blood flow to tissue (brain, heart, central organs and/or muscle) is unknown but would be dependent on sympathetic responses in individual birds during the ascent.

Heart rate profiles: surface intervals

Heart rate during surface intervals was measured over 1 min immediately prior to and following a dive because total surface interval data (i.e. the entire time period between dives) were not considered relevant for the present study. Time spent on the surface post-dive at the isolated dive hole is not necessarily an indicator of diving recovery, as these emperor penguins are subject to other influences and distractions such as interactions with other birds in the corral or activities and personnel at the Penguin Ranch campsite.

Pre- and post-dive periods of tachycardia presumably serve to load O_2 stores pre-dive, and to eliminate carbon dioxide and replenish O_2 stores post-dive. Tachycardia and high respiration rates facilitate rapid gas exchange during the surface interval and allow for a quick recovery from dives (Fedak et al., 1988; Kooyman et al., 1971; Le Boeuf et al., 2000). The maximum instantaneous surface interval f_H in this study is the highest ever recorded for emperor penguins (256 beats min^{-1}), and is in the range of f_H

recorded during maximum O_2 consumption ($\dot{V}_{O2max}$) of emperor penguins swimming in a flume (Kooyman and Ponganis, 1994). The similarity of the peak values obtained in these two studies, and the consistent range of the f_H values immediately prior to and following dives among individual birds suggest that emperor penguins are operating at a maximum of oxygen uptake during these periods of the surface interval.

While f_H is at its highest immediately pre- and post-dive, there is little variation in f_H and a distinct RSA pattern is not evident. As the f_H decreased after the initial extreme tachycardia during the post-dive surface period, the RSA pattern did emerge in many cases. Similar RSA patterns have been demonstrated in sleep apnea studies of elephant seals. Although the RSA is present in seals throughout the post-apneic period, it is much more pronounced as the tachycardia decreases during recovery (Castellini et al., 1994a). A decrease in the sinus arrhythmia index (the difference between inspiratory and expiratory f_H) at high f_H has also been observed in other mammals (Mazza et al., 1980). The time until the RSA pattern emerged after dives in this study ranged from 11–311 s with a mean of 107±10 s (N=43). By this time, when respiration can once again be inferred by the RSA, it is not significantly elevated above the resting range. Previous data on emperor penguins document vigorous hyperventilation after dives greater than 1 min in duration, with rates of up to 25 breaths min^{-1} (mean ~15 breaths min^{-1}) during the first minute after surfacing (Kooyman et al., 1971). The time required for post-dive respiratory rate to return to resting levels (~3 min), as inferred by post-dive RSA patterns, is consistent with that observed in the previous study (Kooyman et al., 1971).

Stroke frequency and heart rate

Consistent with a previous study (van Dam et al., 2002), emperor penguins diving at the isolated dive hole stroked continuously throughout the dive, with a stroke-glide pattern but no prolonged periods of gliding. The highest stroke frequencies (1.5–2.2 Hz) occurred during initial descent, as the penguin overcame its positive buoyancy at shallow depth (Sato et al., 2002; van Dam et al., 2002). High stroke frequencies also occurred occasionally later in the dive, during descents from shallow depths.

The classical exercise response includes increases in f_H and muscle blood flow in response to increasing work effort, in both terrestrial and marine animals (Fedak et al., 1988; Williams et al., 1991). Consequently, if working skeletal muscle were perfused during diving, f_H during the dive would be expected to increase with stroke frequency, reflecting the increase in work effort. As discussed in the results, it is clear that there is no significant correlation between mean stroke frequency and dive f_H (Fig. 7A). This is also apparent when considering individual dive profiles. Review of simultaneous depth, stroke frequency, and instantaneous f_H profiles reveals that high stroke frequencies are always associated with descents but not usually associated with corresponding changes in f_H (Fig. 6). As discussed previously, f_H profiles during the dive are characterized by an immediate decrease in f_H upon submersion, followed by a readjustment period where f_H briefly increases before gradually decreasing. Despite this immediate and quite drastic reduction in f_H, stroke frequency is at its maximum during this initial descent period. According to classical exercise physiology, if f_H were significantly influenced by stroke frequency, high f_H would be expected during the descent when stroke frequency is at its maximum, however, the reverse holds true. Heart rates are conversely at their most significant rate of decrease in this period, reaching the level of f_H at rest within the first 20 s in 71% of dives, whereas maximum stroke frequency is sustained. Excluding descents, dives are characterized by constant stroke frequencies for the entire duration, yet f_H is variable and toward the end of longer dives, is often 2–4 times lower than during the first half of the dive (Fig. 6). An increase in f_H during ascent was also present in most dives, while stroke frequency remains constant in this period.

Circulatory adjustments are expected in order to maintain blood pressure because of reduced cardiac output during diving (reductions in f_H, and unchanged or slightly decreased stroke volume) (Butler and Jones, 1997; Irving, 1938). A substantial redistribution of blood flow during forced submersion and diving studies has been documented in many birds and mammals, often with more pronounced changes as the dive approaches or exceeds the ADL (Butler and Jones, 1997; Davis et al., 1983; Irving, 1938; Kooyman, 1989; Millard et al., 1973; Murdaugh et al., 1966). The 'true bradycardia' and the lack of coupling of f_H and stroke frequency in dives of emperor penguins at the isolated dive hole are consistent with decreased or absent muscle blood flow during dives. This assumption is supported by prior studies. In emperor penguins, the temperature of the pectoral muscle, one of the two primary underwater locomotory muscles of penguins, consistently increased during diving and decreased during the surface interval (Ponganis et al., 2003). The magnitude and pattern of the temperature fluctuations were consistent with what would be predicted if there were little to no muscle blood flow during dives, and if the metabolic rate of the muscle were calculated on the basis of complete depletion of myoglobin-bound O_2 stores within the ADL (Ponganis et al., 2003). Recent data also demonstrate that blood lactate levels in emperor penguins do not increase significantly during the dive, even in dives longer than the ADL (P. J. Ponganis, unpublished data). Blood lactate does not increase until the post-dive surface interval period (Ponganis et al., 1997). The true bradycardia, the lack of a f_H–stroke frequency relationship, pectoral muscle temperature increases and lactate washout into the blood during the post-dive period all support the concept of peripheral vasoconstriction, isolation of muscle from the blood O_2 store and reliance of muscle metabolism on a myoglobin-bound O_2 store.

Review of f_H profiles also reveals that during the initial descent of a dive, f_H immediately decreases, then transiently increases, and gradually decreases again (Figs 2, 6). This pattern has also been seen in diving king and macaroni penguins (Froget et al., 2004; Green et al., 2003). It might be argued that this initial readjustment in f_H could imply a redistribution of blood back to muscle after intense stroking effort associated with the descent. This suggestion was offered to explain the comparable f_H readjustment pattern in deep dives of king penguins and a delayed increase of pectoral muscle temperature observed in these birds (Froget et al., 2004). The hypothesis posed for king penguins was based on the fact that the readjustment was seen only in deep (long) dives in that study (not in shallow, short dives), and was thereby suggested to reflect an increase in work effort possibly due to increased buoyancy from a higher inspired air volume before deep, long dives (Froget et al., 2004; Sato et al., 2002). Contrary to these king penguin results, however, emperor penguin dives at the isolated dive hole demonstrated this initial f_H readjustment in all dives. Emperor and king penguins exhibit differences in diving behavior, diving capacity, and ecological constraints, and thus physiological disparities may simply be due to the fact that the two species rely on different diving strategies. In order to make a more appropriate comparison between these two species, however, it would be necessary to obtain f_H profiles of emperor penguins during deep dives while foraging at sea. Regardless of the distribution of blood flow during the transient increase in f_H during descent, increased cardiac output should facilitate respiratory gas exchange and the transfer of O_2 from the respiratory store to the blood O_2 store during this time period.

The value of digital ECG

Although digital ECG records result in more laborious data processing than their R-wave detector (f_H data logger) counterparts, they reveal the full extent of the cardiac response, allowing validation of extremely high f_H values associated with surface intervals and extremely low f_H during the latter parts of long dives. Application of digital ECG could resolve studies employing f_H data loggers, which have resulted in disparate f_H data for the same species (Green et al., 2005; Green et al., 2003). The detailed beat-to-beat analysis afforded by digital ECG also reveals physiological patterns such as the RSA.

Differences in diving f_H between this and the previous study on f_H in emperor penguins (Kooyman et al., 1992) can most likely be attributed to the difference in technology, as the previous study used an R-wave recorder which averaged f_H over 15 s intervals. Most of the dives in that study were much shorter than those in this one, which also contributes to the higher dive f_H found by Kooyman et al. (Kooyman et al., 1992). However, even in a 10 min dive in the previous study, f_H was reported to decline to a minimum of only about 30 beats min^{-1}, as compared to instantaneous minimum f_H values of 5–10 beats min^{-1} for dives of similar duration in this study. Although some of the dive f_H values for particular durations were consistent between the two studies, Kooyman et al. (Kooyman et al., 1992) did not find a correlation between f_H and dive duration, as compared to the present study (Fig. 4). These differences in findings may be secondary to differences in sample size and the range of dive durations, to a loss of variation in f_H due to averaging by the R-wave detector, or to possible miscounting of T-waves or other waveforms in the ECG profile by the R-wave detector in the earlier study. Digital ECG avoids the latter two potential problems by providing the entire record of the ECG signal. It also eliminates imposing an upper limit for f_H, as was necessary with the R-wave recorder to avoid the incorrect counting of T waves as R waves (Kooyman et al., 1992). In order to avoid such double counting in the previous study, it was necessary to impose an upper limit of 120 beats min^{-1}, which was significantly below surface interval f_H revealed by both the post-dive analog ECG in that study and the digital ECG in this study.

In summary, we have validated the hypotheses posed in this study, and have determined that: (1) in contrast to any other free diving bird, a true bradycardia and extremely low f_H occur routinely during dives of emperor penguins at the isolated dive hole, (2) peak surface interval f_Hs (the highest surface interval f_Hs ever recorded for this species) are likely consistent with maximum oxygen uptake, (3) respiratory rate can be inferred from the RSA in this species, and (4) f_H and stroke frequency are not coupled in diving emperor penguins.

LIST OF SYMBOLS AND ABBREVIATIONS

ADL	aerobic dive limit
DLT	diving lactate threshold
ECG	electrocardiogram
f_H	heart rate
P_{O_2}	partial pressure of oxygen
RSA	respiratory sinus arrhythmia
SAI	sinus arrhythmia index
TDR	time depth recorder
$\dot{V}_{O_2max}$	maximum rate of oxygen consumption

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