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RESEARCH ARTICLE

Deep-diving sea lions exhibit extreme bradycardia in long-duration dives

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ABSTRACT

Heart rate and peripheral blood flow distribution are the primary determinants of the rate and pattern of oxygen store utilisation and ultimately breath-hold duration in marine endotherms. Despite this, little is known about how otariids (sea lions and fur seals) regulate heart rate (f_H) while diving. We investigated dive f_H in five adult female California sea lions (*Zalophus californianus*) during foraging trips by instrumenting them with digital electrocardiogram (ECG) loggers and time depth recorders. In all dives, dive f_H (number of beats/duration; 50±9 beats min^{-1}) decreased compared with surface rates (113±5 beats min^{-1}), with all dives exhibiting an instantaneous f_H below resting (<54 beats min^{-1}) at some point during the dive. Both dive f_H and minimum instantaneous f_H significantly decreased with increasing dive duration. Typical instantaneous f_H profiles of deep dives (>100 m) consisted of: (1) an initial rapid decline in f_H resulting in the lowest instantaneous f_H of the dive at the end of descent, often below 10 beats min^{-1} in dives longer than 6 min in duration; (2) a slight increase in f_H to ~10–40 beats min^{-1} during the bottom portion of the dive; and (3) a gradual increase in f_H during ascent with a rapid increase prior to surfacing. Thus, f_H regulation in deep-diving sea lions is not simply a progressive bradycardia. Extreme bradycardia and the presumed associated reductions in pulmonary and peripheral blood flow during late descent of deep dives should (a) contribute to preservation of the lung oxygen store, (b) increase dependence of muscle on the myoglobin-bound oxygen store, (c) conserve the blood oxygen store and (d) help limit the absorption of nitrogen at depth. This f_H profile during deep dives of sea lions may be characteristic of deep-diving marine endotherms that dive on inspiration as similar f_H profiles have been recently documented in the emperor penguin, another deep diver that dives on inspiration.

KEY WORDS: Dive response, Otariid, Electrocardiogram, ECG, Heart rate, f_H, Resting, Pinniped

INTRODUCTION

The diving physiology and capabilities of breath-hold divers are crucial to their ability to exploit prey resources, thereby influencing their role in the ecosystem. The dive capacity of breath-hold divers is dependent on both the available O_2 stores and the rate at which the O_2 stores are depleted. Cardiovascular regulation is critical during diving because changes in heart rate (f_H) and cardiac output affect not only blood O_2 uptake from the lung but also the rate and magnitude of blood O_2 delivery to tissues. Therefore, decreased f_H has long been considered central to a decreased rate of O_2 consumption in breath-hold divers, and, ultimately, to their dive capacity. During forced submersion, a severe bradycardia (decrease in f_H to below resting values) results in the isolation of muscle and peripheral organs from blood flow, thereby conserving blood oxygen for the heart and brain (Scholander, 1940; Scholander et al., 1942; Zapol et al., 1979; Blix et al., 1983). However, more recent studies on trained and freely diving animals indicate that this 'dive response' is variable and often more moderate, with declines in f_H dependent on dive duration and activity (Andrews et al., 1997; Hindle et al., 2010; Davis and Williams, 2012; Noren et al., 2012).

Although the cardiovascular responses of phocid seals have been extensively studied (Scholander, 1940; Kooyman and Campbell, 1972; Thompson and Fedak, 1993; Andrews et al., 1997; Jobsis et al., 2001), there have been relatively few investigations of cardiac responses in otariids (fur seals and sea lions). A severe bradycardia did occur during forced submersion and simulated dives (Irving et al., 1963; Kooyman and Sinnett, 1982), but during trained submersions, the decline in f_H was much less (Elsner et al., 1964). And during relatively short, shallow trained dives of both California and Steller sea lions, f_H declined moderately, but could reach values of less than 10–20 beats min^{-1} (Ponganis et al., 1997; Hindle et al., 2010). However, in wild, shallow-diving fur seals, although f_H during diving declined below surface rates, it was rarely less that 80 beats min^{-1} (Boyd et al., 1999). In this study, we measured f_H in freely diving wild California sea lions [*Zalophus californianus* (Lesson 1828)], which, for the first time, documents the level of bradycardia in a deep-diving otariid.

The California sea lion is an excellent model species because its dive behaviour and physiology have been extensively studied (Feldkamp et al., 1989; Weise and Costa, 2007; McDonald and Ponganis, 2012; Villegas-Amtmann et al., 2012; McDonald and Ponganis, 2013). Although often considered a shallow diver, current research shows that they routinely perform long deep dives, often exceeding their calculated aerobic dive limit of 3–5 min (cADL – estimation of aerobic dive limit obtained by dividing usable oxygen stores by estimated diving O_2 consumption) (Weise and Costa, 2007; Villegas-Amtmann et al., 2011; McDonald and Ponganis, 2013). This dive behaviour provides a broad range of dive durations and depths in which to examine the cardiac response in a naturally diving animal.

We investigated the dive f_H response in naturally diving adult California sea lions on maternal foraging trips using a self-contained electrocardiogram (ECG) recorder and a time depth recorder (TDR). We hypothesised that: (1) sea lions would exhibit a true bradycardia upon submergence in mid- and long-duration dives; (2) long deep dives would have a slower dive f_H (number of beats during a dive/dive duration) than short shallow dives, with dive f_H even reaching levels observed in forced submersions; (3) the most severe bradycardias in deep dives would occur during late descent, at the time of minimum venous haemoglobin (Hb) saturation (S_{O_2})

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List of symbols and abbreviations

ADL	aerobic dive limit
a–v	arterio-venous
cADL	calculated aerobic dive limit
ECG	electrocardiogram
$f_{\rm H}$	heart rate
Hb	haemoglobin
$P_{\rm O_2}$	partial pressure of oxygen
$S_{\rm O_2}$	%Hb saturation
TDR	time–depth recorder

previously documented in these animals (McDonald and Ponganis, 2012; McDonald and Ponganis, 2013); and (4) the initial rate of decline in $f_{\rm H}$ would be slower in deeper dives, promoting gas exchange at shallower depths to take advantage of postulated larger respiratory O_2 stores during deeper dives (McDonald and Ponganis, 2012).

RESULTS

General results

ECG and diving data were successfully obtained from five sea lions. All the females exhibited diving behaviour typical of foraging trips on trips ranging from 1.7 to 12.0 days (Feldkamp et al., 1989; Kuhn, 2006; Villegas-Amtmann et al., 2011). The quality of the ECG traces varied between sea lions and across the deployment because of masking of the signal from muscle artefact and possible movement of the electrodes. For four of the five sea lions, ~100 dives (93–116 dives) were processed and analysed (10–17 h of diving bout $f_{\rm H}$), representing the range of dive durations the sea lion performed. Because of poor quality ECG data for most of the deployment, only 43 dives were analysed from one of the sea lions (6 h). A total of 461 dives with simultaneous ECG and depth data were analysed from dives greater than 1 min in duration. Dive duration and depth data for each female are given in Table 1. Median dive duration was 3.7 min (range 1.0–10.0 min) and median depth was 98 m (range 5–420 m). Dive duration and depth were significantly correlated (Fig. 1, Pearson's R=0.91, P<0.001); therefore, we just used dive duration in all the models.

Resting $f_{\rm H}$ and sinus arrhythmia

Resting $f_{\rm H}$ (total beats during 1 h when the sea lion was lying motionless on the beach/60 min) ranged from 46 to 62 beats min^{-1} with a mean of 54±6 beats min^{-1} (N=5; Table 2). There was a significant negative relationship between sea lion mass and resting $f_{\rm H}$ (y=−0.58x+100.27, r^2=0.81, $F_{1,3}$=12.37, P=0.039). While lying on the beach, sea lions exhibited sinus arrhythmia with mean minimum instantaneous $f_{\rm H}$ (minimum beat-to-beat $f_{\rm H}$) of 42±9 beats min^{-1} and mean maximum instantaneous $f_{\rm H}$ (maximum beat-to-beat $f_{\rm H}$) of 87±12 beats min^{-1} (Fig. 2). Although respirations could not be observed, if inhalation is associated with the increased $f_{\rm H}$ as observed in other diving animals (Lin et al., 1972; Ponganis et al., 1997; Meir et al., 2008), average respiration rate (total sinus arrhythmia peaks during 1 h/60 min) while resting ranged from 5.3 to 7.3 breaths min^{-1} (mean 6.1±0.8 breaths min^{-1}) (Table 2).

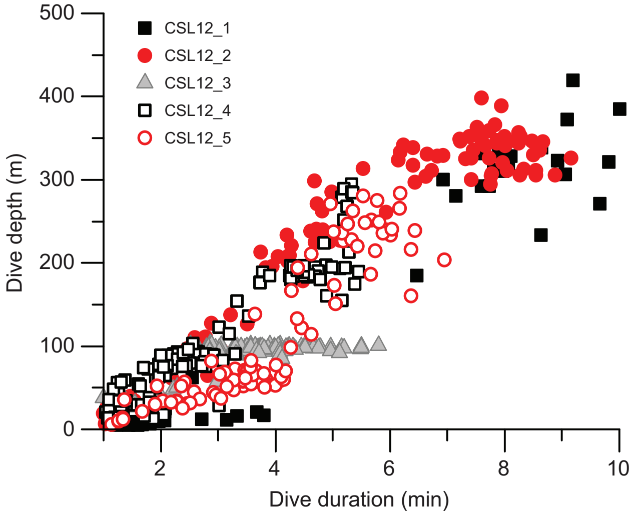

Fig. 1. Dive duration and dive depth are significantly correlated. Data are from five California sea lions. Pearson's r=0.91, P<0.001.

Diving $f_{\rm H}$

In all dives, sea lions decreased dive $f_{\rm H}$ from pre-dive rates (Figs 3, 4), with all dives exhibiting an instantaneous $f_{\rm H}$ (beat-to-beat $f_{\rm H}$) below resting (<54 beats min^{-1}) at some point during the dive (Table 1, Fig. 5B). However, it was only in the mid- and long-duration dives that sea lions consistently exhibited a true bradycardia with dive $f_{\rm H}$ lower than resting $f_{\rm H}$ (Table 3).

Dive $f_{\rm H}$ profiles, in general, exhibited one of two shapes depending on dive duration. In short, shallow dives, $f_{\rm H}$ profiles were usually a U-shape with an initial rapid decline, a relatively stable $f_{\rm H}$ near the bottom, and a rapid increase as the animal ascended (Fig. 3A). Pre- and post-dive $f_{\rm H}$ of these short dives (less than <3 min) were 105±21 and 115±15 beats min^{-1}, respectively,

Table 1. Individual and pooled $f_{\rm H}$ data from California sea lions

Seal lion ID	Dive duration (min)	Dive depth (m)	Dive $f_{\rm H}$ (beats min^{-1})	Minimum $f_{\rm H}$ (beats min^{-1})	Bottom $f_{\rm H}$ (beats min^{-1})
CSL12_1, N=43	4.9±3.3	151±156	39±8	21±13	29±13
95.0 kg	3.7 (1.2–10.0)	20.5 (5–420)	35 (28–53)	23 (6–43)	30 (10–48)
CSL12_2, N=106	5.3±2.6	233±122	43±6	15±10	27±12
81.0 kg	5.7 (1.0–9.2)	296 (6–398)	42 (34–55)	10 (6–38)	23 (12–48)
CSL12_3, N=103	3.7±0.9	92±17	49±7	23±5	39±7
73.8 kg	3.8 (1.0–5.8)	98 (27–103)	50 (35–69)	24 (12–34)	40 (24–58)
CSL12_4, N=116	2.9±1.4	107±77	56±7	37±11	46±10
72.8 kg	2.5 (1.1–5.5)	83 (13–294)	57 (34–81)	41 (7–50)	49 (18–73)
CSL12_5, N=93	3.9±1.4	111±86	55±7	29±11	44±10
70.4 kg	3.7 (1.2–7.0)	67 (6–284)	55 (41–72)	32 (11–48)	45 (12–65)
Grand mean ± s.d.	4.0±2.1	138±108	50±9	26±13	38±13
Grand median (range)	3.7 (1.0–10.0)	98 (5–420)	50 (28–81)	26 (6–50)	41 (10–73)

$f_{\rm h}$, heart rate; N, number of dives. The mean (±s.d.) and median (range) are given for each sea lion.

Table 2. Individual and pooled resting f_H data from California sea lions

Sea lion ID	Mass (kg)	Resting f_H (beats min^{-1})	Minimum f_H (beats min^{-1})	Maximum f_H (beats min^{-1})	Respiration rate (breaths min^{-1})
CSL12_1	95	46	35±8	85±9	5.3
CSL12_2	81	52	37±6	76±8	5.7
CSL12_3	73.8	53	37±8	88±8	5.7
CSL12_4	72.8	60	48±7	93±13	7.3
CSL12_5	70.4	62	49±6	90±12	6.7
Grand mean ± s.d.	78.6±10.0	54±6	42±9	87±12	6.1±0.8

Respiration rates were determined by counting the sinus arrhythmia peaks during 1 h of resting.

significantly less than pre- and post-dive f_H of mid- and long-duration dives (>3 min) (Table 3). These pre- and post-dive f_H were similar to the average dive bout surface interval f_H (113±5 beats min^{-1}). Dive f_H was only less than resting f_H in 46.3% of dives and tended to be more variable, although much of the variability could be explained by individual (Tables 3, 4, Fig. 5A).

Longer duration dives (>3 min) exhibited a more complex profile (Fig. 3B,C, Fig. 6). Prominent features typical of mid- and long-duration dives include: (a) a surface interval tachycardia (pre- and post-dive); (b) a steady rapid decrease in f_H during initial descent; (c) a gradual decline in f_H towards the end of descent with the lowest f_H of the dive at the end of descent: (d) a slight increase and sometimes variable f_H during the bottom portion of the dive: (e) a slow increase in f_H during ascent and; (f) a rapid increase in f_H just before surfacing. Often, towards the middle of ascent, large oscillations in f_H occurred (Fig. 3C), although this was not the case in the longest dives recorded (Fig. 6). Immediately preceding and following dives, sea lions exhibited elevated f_H (~130–140 beats min^{-1}), compared with average dive bout surface interval f_H (113±5 beats min^{-1}) (Table 3, Fig. 3B,C, Fig. 6).

The severity of the bradycardia was related to dive duration (Table 4, Figs 4, 5). The longest, deepest dives reached the lowest instantaneous f_H and remained low for extended periods of time, resulting in the lowest dive f_H (Figs 4, 5). Over 98% of dives greater than 5 min in duration exhibited a true bradycardia (Table 3, Fig. 5A). In dives greater than 6 min in duration, minimum instantaneous f_H was usually less than 10 beats min^{-1} (Fig. 5B), and average f_H during the bottom of the dive was around 20 beats min^{-1} (Fig. 5C). Although the negative relationship between dive duration and dive and minimum f_H was apparent in all five sea lions, individual sea lions exhibited significantly different f_H, accounting for 75–81% of the variation in the relationship between dive duration and f_H (Table 4), with only two sea lions performing dives greater than 7 min in duration.

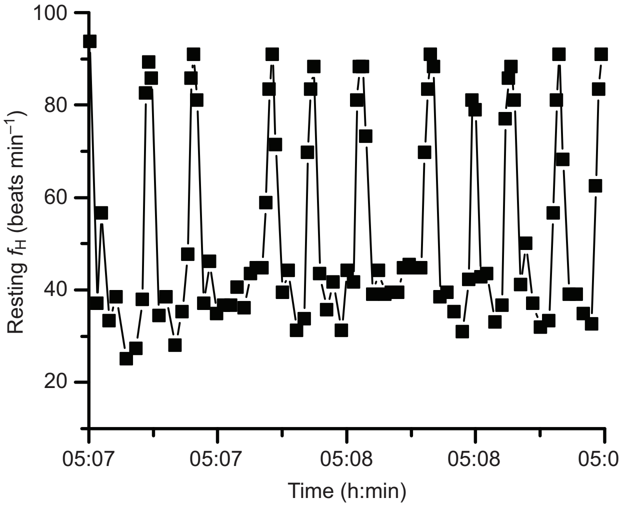

Fig. 2. Instantaneous resting heart rate (f_H) of a California sea lion lying on the beach. Note the periodic, brief increase in f_H. This suggests sea lions display the typical respiratory sinus arrhythmia present in other diving mammals, and that each increase indicates an inhalation.

The total number of heart beats during a dive was positively related to dive duration (Table 4, Fig. 7A). Total beats in a dive appeared to begin to level out near 300–350 heart beats at a dive duration of 8–10 min, but only two sea lions exhibited such long-duration dives. The number of heart beats until resting f_H was reached (a proxy for potential gas exchange early in the dive) was significantly greater in mid- and long-duration dives (>3 min) than in short-duration dives (Table 3, Fig. 7B). This is probably a result of both the elevated pre-dive f_H and the slight delay or slow onset of bradycardia often observed in the initial seconds post-submergence of longer duration dives. In dives greater than 200 m in depth, the mean number of heart beats before a depth of 200 m was obtained (a proxy for potential gas exchange before lung collapse) was 73±14 beats. At this depth, instantaneous f_H was 14±6 beats min^{-1}, suggesting that pulmonary blood flow and gas exchange are greatly reduced even before complete lung collapse.

DISCUSSION

Resting f_H

The resting f_H measured in this study (54±6 beats min^{-1}) was lower than predicted for an animal of similar size (~80 beats min^{-1} for an 80 kg mammal) (Stahl, 1967). In part, this may be due to the fact that the sea lions were probably sleeping (Snyder et al., 1964). The resting f_H in our study was also lower than previous measurements in captive juvenile California sea lions (87±17 beats min^{-1}, average mass 30 kg) (Ponganis et al., 1997) and wild Antarctic fur seals (78±5 beats min^{-1}, body mass 30–50 kg) (Boyd et al., 1999). However, we found a significant negative relationship between mass and resting f_H even with our small sample size of five sea lions (resting f_H=–0.58M_b+100.26, r^2=0.81, $F_{1,3}$=12.37, P=0.039). For a 30 kg sea lion, this equation predicts a resting f_H of 83 beats min^{-1}, which is similar to what was measured previously in juvenile sea lions (Ponganis et al., 1997), suggesting this equation may be useful in estimating resting f_H in sea lions.

The sea lions exhibited a distinct sinus arrhythmia fluctuating between a minimum of 42±9 and a maximum of 87±12 beats min^{-1}, comparable to the sinus arrhythmias described in other diving birds and mammals, including sea lions (Lin et al., 1972; Castellini et al., 1994; Andrews et al., 1997; Ponganis et al., 1997; Meir et al., 2008). The minimum instantaneous f_H during the sinus arrhythmia was similar to the mean minimum f_H in dives less than 3 min (37±7 beats min^{-1}), indicating that in dives less than 3 min (estimated cADL), f_H only decreased to levels observed during

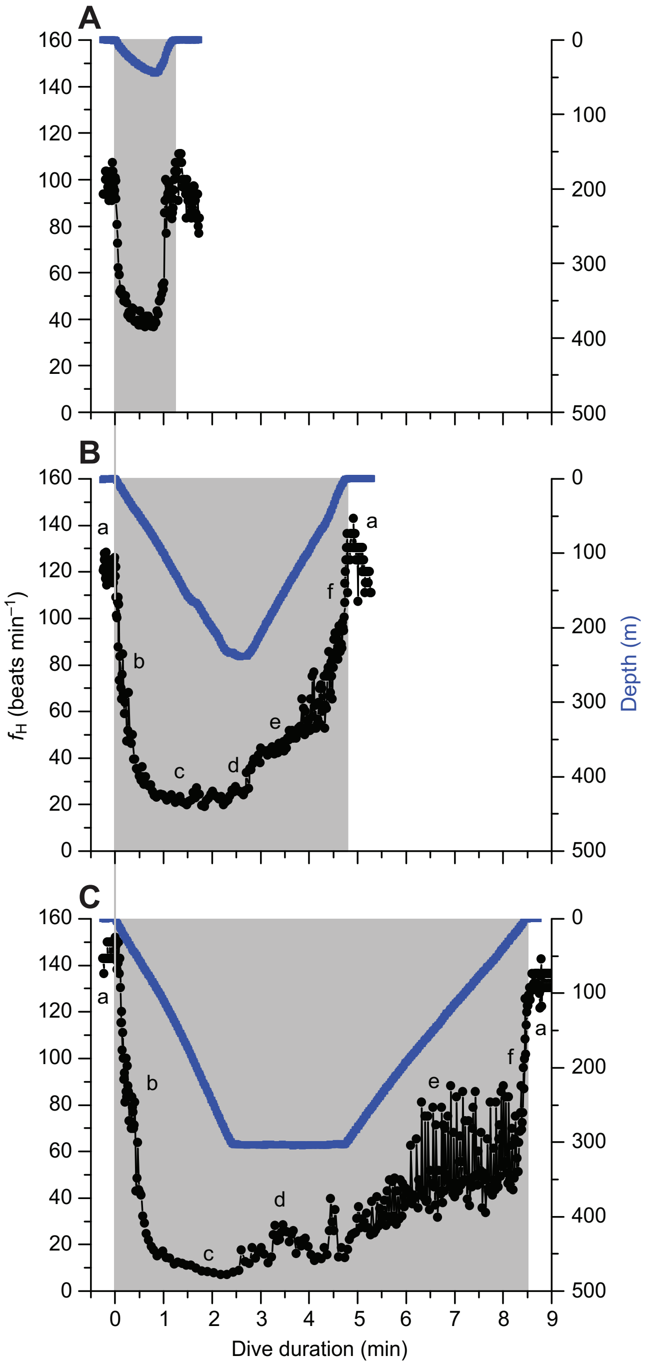

Fig. 3. Instantaneous f_H and dive depth profiles of a California sea lion (CSL12_2). Data are from (A) a short, shallow dive (1.3 min, 45 m), (B) a mid-duration dive (4.8 min, 239 m) and (C) a long-duration dive (8.5 min, 305 m). Minimum instantaneous f_H reached 37 beats min^{-1} in the short dive (A), 19 beats min^{-1} in the mid-duration dive (B) and 7 beats min^{-1} in the long-duration dive (C). Prominent features typical of mid- and long-duration dives include (a) a surface interval tachycardia (pre- and post-dive); (b) a steady rapid decrease in f_H during initial descent; (c) a gradual decline in f_H towards the end of descent with the lowest f_H of the dive at the end of descent; (d) a slight increase and sometimes variable f_H during the bottom portion of the dive; and (e) a slow increase in f_H during ascent, (f) often ending in a rapid increase just before surfacing.

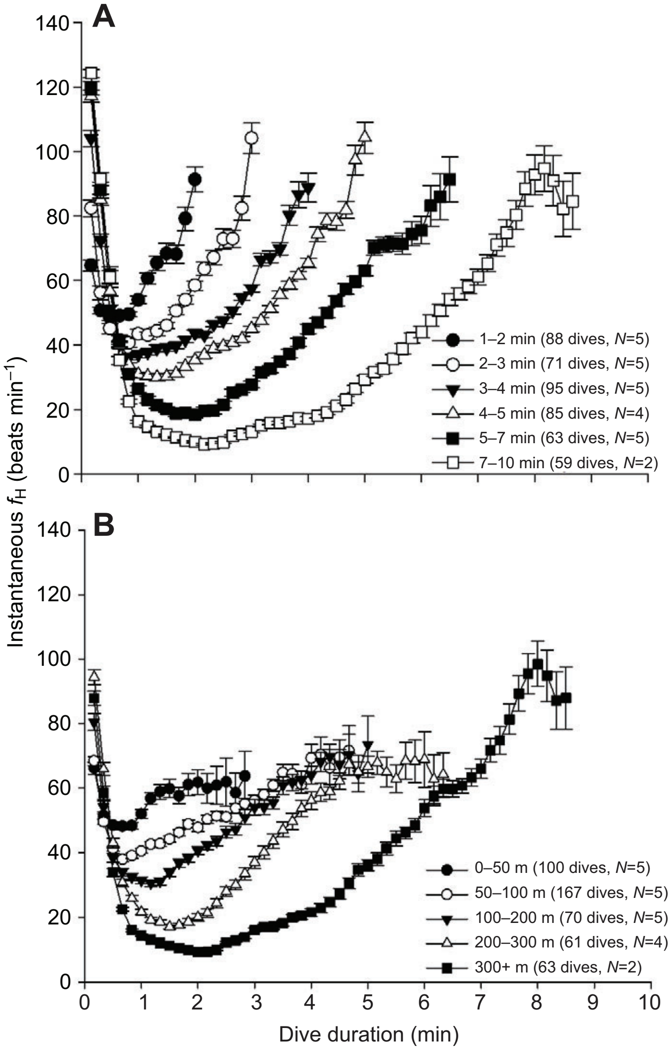

Fig. 4. Profiles of mean f_H at 10 s intervals of dives for (A) six duration categories and (B) five depth categories. Standard error bars are shown. Data were pooled from 461 dives performed by five sea lions. The number of dives in each category and the number of sea lions performing the dives in each category are provided in the keys.

exhalation at rest. This is consistent with observations in emperor penguins and elephant seals, where it was proposed that in dives shorter than the aerobic dive limit (ADL) the reduction in f_H is regulated by a mechanism of cardiorespiratory control similar to that governing the respiratory sinus arrhythmia, with a further reduction only occurring in dives longer than the ADL (Castellini et al., 1994; Meir et al., 2008).

Diving f_H

We obtained the first diving f_H data from wild sea lions on natural foraging trips, demonstrating how they regulate f_H over a range of dive durations. Sea lions always decreased dive f_H from surface f_H values; however, individual sea lions exhibited different dive f_H, accounting for a significant amount of the variation in the relationship between dive duration and f_H (intra-individual correlation: 75–81%) (Table 4). The individual differences in dive f_H exhibited in this study suggest that different dive capacities of individual sea lions may partially account for the range of dive strategies exhibited in a previous study (Villegas-Amtmann et al., 2011). Despite the individual differences in f_H, the pattern of the dive f_H response was similar in all the sea lions. As predicted, sea lions only consistently displayed a true bradycardia on mid- to long-

Table 3. Comparison of diving f_H between three dive duration categories

	Dives <3 min (N=159)	Dives 3–5 min (N=180)	Dives ≥5 min (N=122)
Dive duration (min)	1.9±0.6	4.0±0.5	6.9±1.3
Pre-dive f_H (beats min^{-1})	105±21^a	135±17^b	138±9^b
Dive f_H (beats min^{-1})	55±8^a	51±6^b	40±6^c
Minimum f_H (beats min^{-1})	37±9^a	25±18^b	11±5^c
Post-dive f_H (beats min^{-1})	115±15^a	131±10^b	132±7^b
Heart beats to resting f_H	13±11^a	35±13^b	41±14^b
%Dives with dive f_H below resting f_H	43.4	68.3	98.4

Significant differences between dive duration categories are indicated by different superscript letters. Results obtained from *post hoc* Tukey tests after running a mixed effect ANOVA.

duration dives (>4 min) (Fig. 5A). Additionally, as seen in freely diving phocids (Thompson and Fedak, 1993; Andrews et al., 1997), dive f_H and minimum f_H were negatively related to dive duration, with the longest duration dives having the lowest dive f_H and displaying the most intense bradycardia, often below 10 beats min^{-1} (Fig. 5A,B).

The mild bradycardia and the dive f_H profiles observed in the shorter duration dives (<3 min) were similar to those observed in trained juvenile California sea lions and adult Stellar sea lions (Ponganis et al., 1997; Hindle et al., 2010), but much more intense than f_H observed in freely diving Antarctic fur seals (Boyd et al., 1999). Surprisingly, although dive f_H of trained Steller sea lions was similar, Steller sea lions regularly exhibited lower minimum f_H, with minimum f_H almost always less than 20 beats min^{-1} in dives less than 2 min in duration. In the wild, California sea lions rarely exhibited a minimum f_H less than 20 beats min^{-1} in similar duration dives (Fig. 5B), suggesting greater blood oxygen transport during these natural short-duration dives.

Although California sea lions are not usually considered exceptional divers, they exhibited extreme bradycardia, comparable to that of the best diving phocids (Hill et al., 1987; Thompson and Fedak, 1993; Andrews et al., 1997), during their deep dives. In dives greater than 6 min in duration, minimum f_H was usually less than 10 beats min^{-1} and sometimes as low as 6 beats mins^{-1} (Fig. 5B), which is similar to extreme divers such as emperor penguins (3 beats min^{-1}), elephant seals (3 beats min^{-1}), grey seals (2 beats min^{-1}) and Weddell seals (<10 beats min^{-1}), and even as low as what was observed in forced submersion studies (Scholander, 1940; Hill et al., 1987; Thompson and Fedak, 1993; Andrews et al., 1997; Meir et al., 2008). Thus, similar to phocids, the extreme bradycardia exhibited during forced submersions is also a routine component of the sea lion's physiological repertoire, allowing them to perform long-duration dives.

While the degree of bradycardia observed in long dives of California sea lions was similar to the extreme bradycardia observed in phocids, the f_H profiles were quite different. In general, phocid f_H decreases abruptly upon submergence. The intensity of the initial phocid bradycardia either remains relatively stable or intensifies as the dive progresses, and does not start to increase until the seal begins its ascent (Thompson and Fedak, 1993; Andrews et al., 1997). In contrast, the f_H profiles of sea lions were more complex, showing a more gradual decrease during descent, with the minimum f_H of the dive usually towards the end of descent (Figs 3, 6). There was often a slight increase in f_H during the bottom portion of the dive, and as soon as the sea lions started to ascend, the f_H slowly started to increase, often becoming irregular during the middle of ascent, before increasing rapidly as the sea lion approached the surface. As proposed for emperor penguins with similar f_H profiles during deep dives (Wright et al., 2014), the extreme low f_H towards the end of descent and at the bottom of the dive (especially in dives less than 200 m) may limit pulmonary gas exchange and peripheral tissue perfusion. At deeper depths, but before the depth of complete lung collapse (McDonald and Ponganis, 2012), the bradycardia and lower cardiac output would limit pulmonary gas exchange, thereby limiting nitrogen absorption at depth, while also conserving O_2 for ascent (Andersson et al., 2002). Additionally, with f_H less than 20 beats min^{-1} during late descent, perfusion of muscle is unlikely. Because of this similarity to emperor penguin heart rates during deep dives, we hypothesise that this f_H profile may be characteristic of deep-diving higher vertebrates that dive on inspiration.

The notable differences in f_H profiles between the short- and long-duration dives of sea lions are consistent with our previous investigation of blood oxygen depletion in diving sea lions, where we also found distinct differences between short- and long-duration dives (Fig. 8) (McDonald and Ponganis, 2013). In short-duration dives, venous blood O_2 depletion was usually moderate, but highly variable (McDonald and Ponganis, 2013). This is consistent with the moderate and more variable f_H observed in dives of less than 3 min in duration compared with longer duration dives (Fig. 5). In long-duration dives, venous S_{O_2} rapidly decreased during descent, while arterial S_{O_2} remained high, resulting in an arterio-venous (a–v) O_2 content difference in the middle of the dive of more than 4 times the value in most animals at rest (McDonald and Ponganis, 2013). This large a–v O_2 difference suggests extreme hypoperfusion of tissue allowing for complete blood O_2 extraction, which is consistent with the low f_H measured during the end of descent and bottom portion of long-duration dives (Fig. 8).

Another notable difference between short- (<3 min) and mid- to long-duration (>3 min) dives was the significantly higher pre-dive f_H in the longer dives (Table 3). This is consistent with the pre-dive arterialisation of venous blood often exhibited before long, deep dives (McDonald and Ponganis, 2013). The higher f_H before long dives will allow sea lions to ensure blood and muscle are fully loaded with O_2 before the initiation of a dive, and may facilitate the possible use of a–v shunts to arterialise venous blood, thereby maximising the amount of O_2 they are able to take down on a dive (McDonald and Ponganis, 2013). Elevated pre-dive f_H and arterialisation of venous blood were also observed in freely diving emperor penguins (Meir et al., 2008; Meir and Ponganis, 2009; Wright et al., 2014). The higher pre-dive f_H, combined with recent evidence that sea lions inhaled greater air volumes before longer dives, suggests that, like penguins, sea lions plan their dives (McDonald and Ponganis, 2012).

The total number of heart beats during a dive increased with dive duration (Fig. 7A). Grey seals and emperor penguins exhibited a similar relationship between dive duration and total beats in shorter

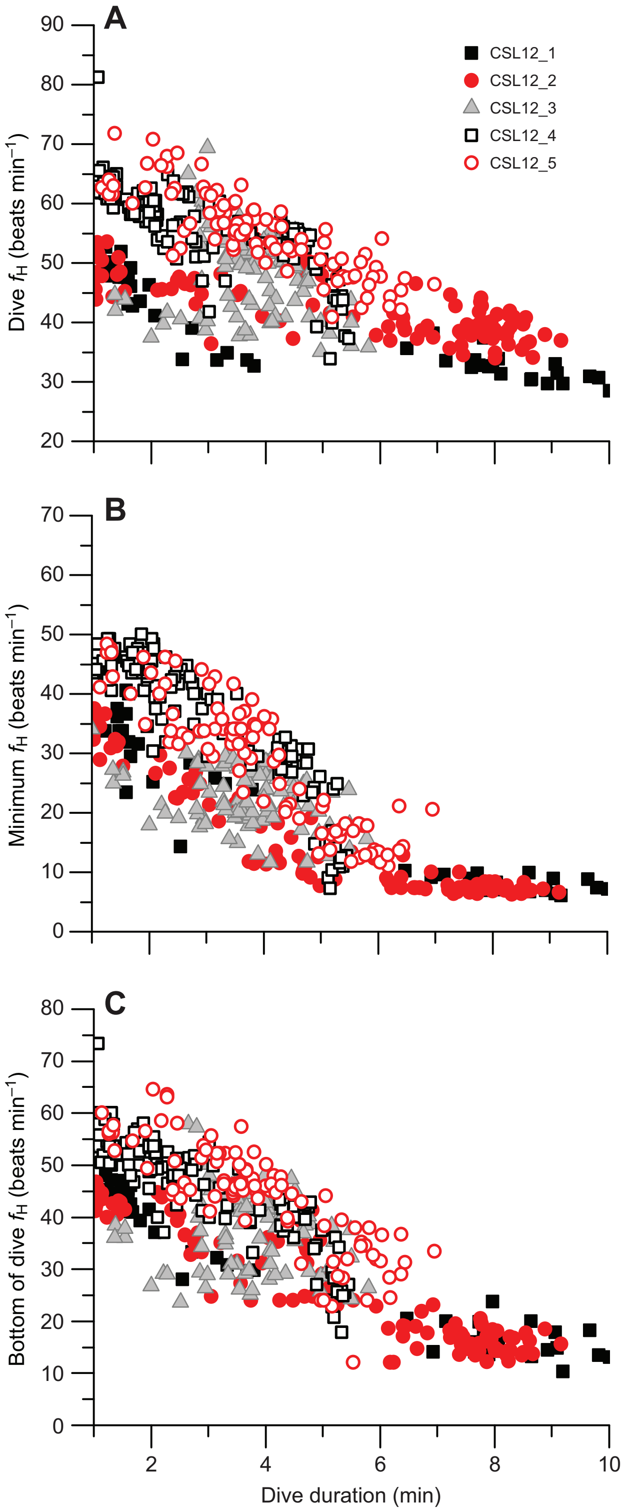

Fig. 5. f_H decreases with increasing dive duration. Dive duration versus (A) dive f_H (total number of beats/dive duration), (B) minimum instantaneous f_H and (C) bottom f_H (total beats at bottom of dive/bottom time) for California sea lions (461 dives from five sea lions).

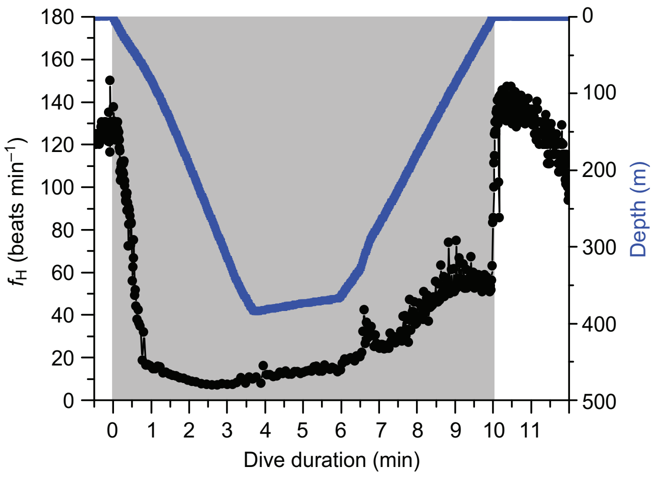

Fig. 6. Instantaneous f_H and dive depth profiles of the longest dive (10.0 min, 385 m) from a California sea lion (CSL12_1). During this dive, instantaneous f_H reached 7 beats min^{-1} and was less than 20 beats min^{-1} for over 5.5 min. Post-dive f_H was high in the first 0.5–1 min after surfacing, but then declined to ~100 beats min^{-1} towards the end of the surface interval.

duration dives; however, for dives greater than ~7 min in these two species, the total number of beats levelled off (Thompson and Fedak, 1993; Wright et al., 2014). It was suggested that they may be partitioning out a maximum number of heart beats during a dive. While there was no clear limit of total dive heart beats in this study, there was some evidence that the total number of beats might be starting to level off around 300 beats in dives greater than 8 min. This might be more apparent if we had a larger sample size and more longer duration dives.

Implications for pulmonary gas exchange

The moderate dive f_H in short, shallow dives compared with the much slower f_H of deep long-duration dives suggests more pulmonary blood flow and greater potential for reliance on lung O_2. Most of these dives were to depths of less than 100 m (well below the estimated depth of lung collapse near 200 m) (McDonald and Ponganis, 2012), so maintenance of a moderate f_H during these dives may allow sea lions to maximise use of the potentially significant lung O_2 stores (~16% of total body O_2 stores) throughout the dive (Weise and Costa, 2007). This is supported by venous blood O_2 profiles, where, occasionally, there was no decrease in venous blood O_2 between the beginning and end of the dive; this can only occur if pulmonary gas exchange continues throughout the dive (McDonald and Ponganis, 2013). Greater utilisation of the lung O_2 store in sea lions is consistent with higher dive f_H in other species that both dive on inspiration and typically perform shallow dives (dolphins, porpoises, some penguin species), and in deeper diving species when they perform shallow dives (emperor penguins) (Williams et al., 1999; Reed et al., 2000; Green et al., 2001; Froget et al., 2004; Wright et al., 2014).

In deeper dives of sea lions, although f_H was lower and bradycardia more extreme, the diving f_H profiles suggest that pulmonary gas exchange is also important. In long-duration dives, even though f_H started to decrease upon or shortly after submergence, the decrease was not as abrupt as in phocids. Additionally, in long deep dives, despite having overall low dive f_H, there were more heart beats before resting f_H was reached compared with short, shallow dives. In dives less than 3 min in duration, there were ~10–15 beats until instantaneous f_H reached resting values. In longer duration dives (>3 min), there were usually ~30–40 beats before instantaneous f_H reached resting values. We suggest the greater number of heart beats early in these deeper dives enables more gas exchange and blood O_2 uptake at shallow depths, thus allowing utilisation of the postulated larger respiratory

Table 4. Mixed effect model results examining the relationship between dive duration and heart rate and heart beats

			Results						
	Model variables		Fixed effect						Random effect
Model	Fixed effect	Random effect	AIC	Coeff.	Error	d.f.	*t*-value	*P*	IC (%)
Duration vs dive f_{H}	Duration		2999.4						
	Duration	Sea lion ID (intercept)	2807.8						
	Duration	**Sea lion ID (intercept + slope)**	**2739.0**	−2.73	0.54	455	−5.07	<0.001	75.1
		Sea lion ID (intercept)	3084.2						
Duration vs min. f_{H}	Duration		3054.6						
	Duration	Sea lion ID (intercept)	2887.2						
	Duration	**Sea lion ID (intercept + slope)**	**2736.0**	−4.59	1.04	455	−4.4	<0.001	81.1
		Sea lion ID (intercept)	3313.2						
Duration vs total heart beats	Duration		4302.0						
	Duration	Sea lion ID (intercept)	4082.5						
	Duration	**Sea lion ID (intercept + slope)**	**3978.9**	41.23	3.42	455	12.05	<0.001	57.4
		Sea lion ID (intercept)	5033.7						
Duration vs beats to resting (resting=54)	Duration		3639.3						
	Duration	Sea lion ID (intercept)	3517.8						
	Duration	**Sea lion ID (intercept + slope)**	**3505.8**	7.2	0.65	455	11.06	<0.001	38.4
		Sea lion ID (intercept)	3839.6						

The AICs for all models are reported. The fixed effects and intraclass correlation (IC) are only presented for the top model (indicated in bold). Dive duration is always the fixed effect and individual sea lion is always the random effect (with random intercept or random intercept and slope).

O_2 stores in deeper dives (McDonald and Ponganis, 2012). The less abrupt decline in f_{H} we observed in sea lions is similar to the more gradual declines documented in emperor penguins and porpoises (Reed et al., 2000; Meir et al., 2008; Wright et al., 2014), where it has also been proposed that the gradual decrease in f_{H} allows them to maximise pulmonary gas exchange at shallower depths. However, as sea lions swam deeper, f_{H} decreased further (Figs 3, 6), and by 200 m depth (the approximate depth of lung collapse) (McDonald and Ponganis, 2012), instantaneous f_{H} was 14 beats min^{-1}. Such an extreme decline in f_{H} in conjunction with increased pulmonary shunting due to lung compression at greater depths will result in minimisation of both O_2 and N_2 uptake by blood, even before the depth of full lung collapse (100% pulmonary shunt) is reached (Kooyman and Sinnett, 1982). Thus, while a higher f_{H} and greater number of heart beats early in the dive should allow for utilisation of the respiratory O_2 store, the extreme decline in f_{H} during later descent of deep dives should help decrease lung O_2 utilisation, and, importantly, should also minimise the risks of excess N_2 absorption.

As in other freely diving mammals and penguins studied (Millard et al., 1973; Hill et al., 1987; Thompson and Fedak, 1993; Andrews et al., 1997; Boyd et al., 1999; Williams et al., 1999; Green et al., 2003; Froget et al., 2004; Meir et al., 2008), California sea lions also exhibited a gradual tachycardia as they began ascent, with the rate of increase escalating during the last 15–30 s of the dive. It has been proposed that the ascent tachycardia may increase blood flow and O_2 delivery to O_2-depleted tissues, thus lowering the $P_{\mathrm{O_2}}$ in the blood and increasing the blood/lung O_2 gradient, resulting in shorter surface intervals by maximisation of O_2 uptake at the surface (Thompson and Fedak, 1993). This hypothesis was supported in diving elephant seals by a more rapid decline in venous $P_{\mathrm{O_2}}$ at the end of the dive (Meir et al., 2009). In contrast, we found an increase in venous $P_{\mathrm{O_2}}$ during ascent from deep dives (Fig. 8), which is consistent with arterial $P_{\mathrm{O_2}}$ profiles of deep dives, which indicated resumption of gas exchange in re-expanded lungs during ascent (McDonald and Ponganis, 2013). We propose that in sea lions, increased pulmonary blood flow, secondary to the ascent tachycardia, could also facilitate O_2 uptake of the blood and maintain or increase arterial O_2 content.

Implications for blood flow

f_{H} is often used as a proxy to estimate blood flow and perfusion during diving because of the relative ease of its measurement. This is based on the assumption that stroke volume does not change during diving in sea lions (Elsner et al., 1964; Ponganis et al., 1991), and, hence, changes in f_{H} directly reflect changes in cardiac output. As breath-hold divers maintain arterial pressure while diving (Scholander, 1940; Irving et al., 1942; Zapol et al., 1979), changes in cardiac output should be associated with changes in peripheral vascular resistance and changes in blood flow to tissues (Scholander, 1940; Zapol et al., 1979; Blix et al., 1983). In Weddell seals, a decrease in cardiac output of ~85% during forced submersions resulted in an 80–100% decrease in tissue perfusion in all tissues excluding the brain, adrenal glands and lung (Zapol et al., 1979).

Sea lions exhibited extremely low instantaneous f_{H} values that often remained low for significant portions of the dive (Figs 4, 6), suggesting severe decreases in tissue perfusion in dives greater than 5 min in duration. In almost all dives greater than 6 min in duration, instantaneous f_{H} reached 10 beats min^{-1}, and stayed below 20 beats min^{-1} for more than a minute. At a f_{H} of 20 beats min^{-1}, cardiac output will be ~36% of resting cardiac output and only about 18% of average surface cardiac output. At these levels of cardiac suppression, most of this flow should be directed towards the brain and heart (Dormer et al., 1977; Zapol et al., 1979).

In contrast to the extreme bradycardia exhibited in the long-duration dives, the decrease in f_{H} exhibited in short dives (<3 min) was much less intense (dive f_{H} ~50 beats min^{-1}), suggesting total peripheral perfusion during these dives was similar to perfusion levels at rest. The tissue distribution of such peripheral blood flow is unknown during these short-duration dives of sea lions. Similarly, it remains to be determined whether the f_{H} response during these dives, and even the f_{H} during the deeper dives of sea lions, is

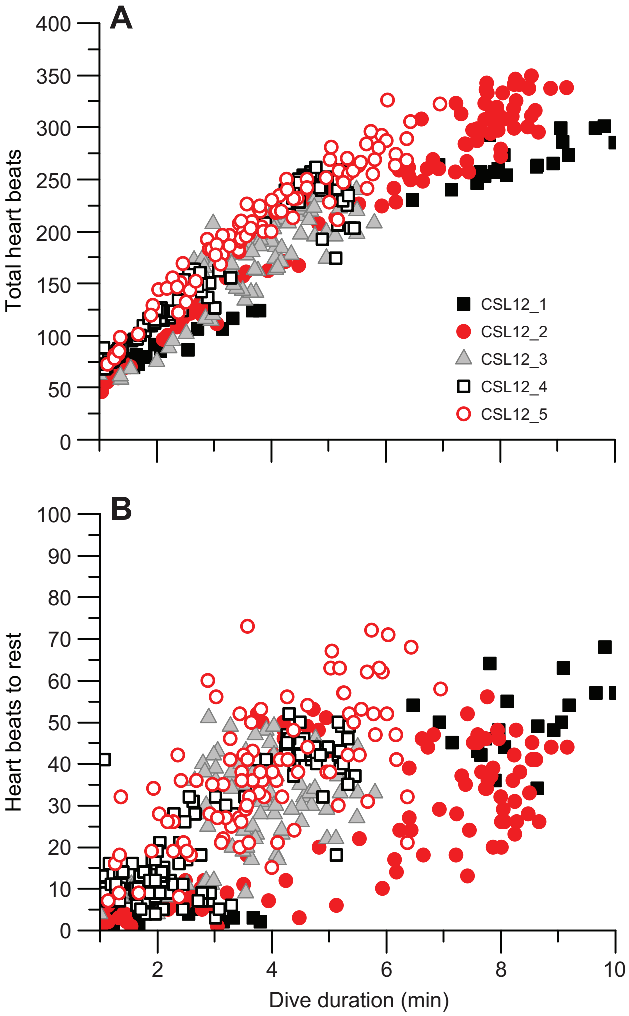

Fig. 7. Dive duration versus (A) total dive heart beats and (B) heart beats to reach resting f_H. Resting f_H is 54 beats min^{-1}.

exercise-modulated as has been proposed in other marine mammals (Davis and Kanatous, 1999; Davis and Williams, 2012; Noren et al., 2012). It is not known whether heart rate and muscle blood flow are linked to muscle work effort in diving sea lions. These topics should be further addressed with future investigations of (a) the relationship of f_H to stroke rate, (b) the relationship of the rate of blood O_2 depletion to both f_H and stroke rate, and (c) the relationship of myoglobin desaturation rate to f_H and stroke rate.

Conclusions

We successfully obtained diving f_H profiles from a deep-diving otariid during natural foraging trips. We found that (1) f_H decreases during all dives, but true and more intense bradycardia only occurred in longer duration dives and (2) in the longest duration dives, f_H and presumed cardiac output were as low as 20% of resting values. We conclude that, although initial high f_H promotes gas exchange early in deep dives, the extremely low f_H in late descent of deep dives (a) preserves lung O_2, (b) conserves blood O_2, (c) increases the dependence of muscle on myoglobin-bound O_2 and (d) limits N_2 absorption at depth. This f_H profile, especially during the late descent/early bottom phase of deep dives is similar to that of deep-diving emperor penguins, and may be characteristic of deep-diving endotherms that dive on inspiration.

MATERIALS AND METHODS

This study was conducted on San Nicolas Island, California, during November 2012. Five adult female California sea lions were captured using customised hoop nets and anaesthetised with isoflurane gas with O_2 using a portable field vaporiser set up (McDonald and Ponganis, 2013). Instruments were only deployed on lactating females in order to increase the likelihood of instrument recovery. Females were instrumented with an ECG/Pressure recorder, 3D accelerometer TDR and a radio transmitter. After instrumentation, females were weighed (±0.2 kg, MSI-7200 Dyna-link; Measurement Systems International, Seattle, WA, USA), and placed in a kennel to recover from anaesthesia (recovery time 53±21 min). After one to four trips to sea, females were located using the radio transmitter, recaptured and instruments removed while the female was manually restrained (19±6 min).

Instrumentation

While under anaesthesia, two subcutaneous, sterile ECG electrodes were inserted aseptically, ~5 cm left of the dorsal midline; one electrode was anterior to the axilla and one electrode was placed posterior to the flipper, ~30 cm apart. Electrodes were secured with a suture and covered with a glued neoprene patch. The electrodes were attached to a custom-built digital ECG/pressure recorder (3991 BioLog; UFI, Morro Bay, CA, USA; sampled ECG at 50 Hz and external pressure at 1 Hz) in a waterproof housing (Meer Instruments, Palomar Mountain, CA, USA; 3 cm diameter×15 cm length). The ECG recorder was attached with 5 min epoxy glue (Loctite; Henkel Corp., Westlake, OH, USA) to the fur of the midline back between the electrode insertion sites, and the electrode leads were covered with coban glued to the fur. Additionally, a 3D accelerometer tag (tdr10-X; Wildlife Computers, Redmond, WA, USA; sampled 3-axis acceleration at 16 Hz, depth at 1 Hz; 5×3×1.5 cm) and VHF radio transmitter (mm160B; Advanced Telemetry Systems, Isanti, MN, USA; 6×1.8 cm) were attached caudal to the ECG recorder using 5 min epoxy. All procedures were performed under a National Marine Fisheries Service Marine Mammal Permit (no. 14676) and with University of California, San Diego Animals Subjects Committee approval (S11303).

Data processing and analysis

TDR data were processed and analysed in MatLab (The MathWorks, Natick, MA, USA) using a custom-written dive analysis program (IKNOS; Y. Tremblay). After calculating a zero offset correction at the surface, dives were identified on the basis of a minimum duration (20 s) and depth (5 m). For each dive, maximum depth, dive duration, post-dive interval and bottom time (time spent at depths greater than 80% of maximum depth) were determined. The ECG data were processed in Origin (version 8.6, OriginLab, Northhampton, MA, USA) using a custom-written peak detection program (K. Ponganis) to mark R-wave peaks and calculate the R–R intervals (instantaneous f_H). Data were visually inspected to confirm correct identification of R-wave peaks.

Resting f_H was determined for each sea lion by analysing 2 h of ECG data from when the sea lion was lying motionless on the beach (determined from accelerometer and TDR data). The hour with the lowest f_H was used to determine resting f_H (total beats during the hour/60 min). Resting respiration rate was determined by counting the number of sinus arrhythmia peaks during the hour used to determine resting f_H and dividing by 60 min.

The TDR and ECG data were synchronised using the pressure and depth data from the ECG logger and TDR, respectively. Instantaneous f_H was used in diving f_H profiles. For dives greater than 1 min in duration, a custom-written MatLab code was used to determine the total number of heart beats during a dive (total heart beats), dive f_H (total heart beats/dive duration), minimum instantaneous f_H during the dive, pre- and post-dive f_H (number of heart beats in 5 s preceding or following the dive/0.083 min), bottom f_H (heart beats during bottom of dive/bottom time duration) and the number of heart beats until instantaneous f_H was less than resting f_H (average resting f_H=54 beats min^{-1}). Heart rate at 10 s intervals was also determined throughout the dive. In addition to dive f_H, average surface f_H during diving bouts was determined by averaging the instantaneous f_H from all surface intervals during analysed dive bouts.

The relationships between dive duration and dive f_H, minimum f_H, total heart beats during a dive, and heart beats to resting were investigated using

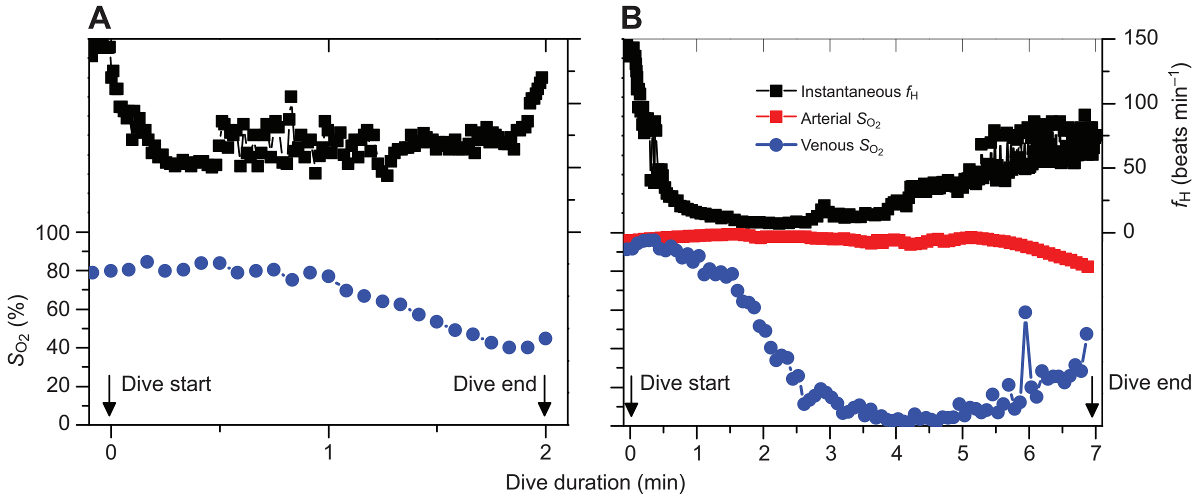

Fig. 8. Instantaneous f_H and hemoglobin saturation (S_{O_2}) profiles. Data are from (A) two, 2 min dives to 33 m and (B) three, 7 min dives to ~310–350 m depth. Heart rate and venous S_{O_2} profiles are from two (A) or three (B) different sea lions performing dives with similar depth profiles. S_{O_2} data are from a previous publication (McDonald and Ponganis, 2013). The S_{O_2} profiles displayed were converted from P_{O_2} profiles with an O$_2$–haemoglobin dissociation curve determined at pH 7.4 throughout the entire dive to maintain consistency and to provide a conservative estimate of continuous S_{O_2}.

linear mixed effects models (Cran R 2.12.2, package nlme). Dive duration was the fixed effect in all models, and to account for the lack of independence caused by having many dives from the same individual, individual (sea lion ID) was included as a random effect. Covariance and random effect structures of the full models were evaluated using Akaike's information criterion (AIC) and examination of residual plots (Zuur et al., 2009). AICs from all the tested models are presented with the best model in bold.

Additionally, dives were classified as short-duration (less than 3 min, minimum cADL) (Weise and Costa, 2007), mid-duration (3–5 min, range of cADLs) or long-duration (>5 min) dives. Differences in pre-dive f_H, dive f_H, minimum f_H, post-dive f_H, and heart beats to resting between the categories were investigated using mixed effects ANOVA, followed by *post hoc* Tukey tests. In all models, dive duration category was the fixed effect and individual (sea lion ID) was included as a random effect. Model fit was accessed by examination of the residuals. All means are expressed ±s.d. and results of the Tukey tests were considered significant at $P<0.05$. Statistical analysis was performed in R.

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Competing interests
The authors declare no competing financial interests.

Author contributions
B.I.M. and P.J.P. conceived and performed the study, B.I.M. conducted data analysis, and B.I.M. and P.J.P. wrote the manuscript.

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References
Andersson, J. P. A., Linér, M. H., Rünow, E. and Schagatay, E. K. A. (2002). Diving response and arterial oxygen saturation during apnea and exercise in breath-hold divers. *J. Appl. Physiol.* **93**, 882-886.

Andrews, R. D., Jones, D. R., Williams, J. D., Thorson, P. H., Oliver, G. W., Costa, D. P. and Le Boeuf, B. J. (1997). Heart rates of northern elephant seals diving at sea and resting on the beach. *J. Exp. Biol.* **200**, 2083-2095.

Blix, A. S., Elsner, R. and Kjekshus, J. K. (1983). Cardiac output and its distribution through capillaries and A-V shunts in diving seals. *Acta Physiol. Scand.* **118**, 109-116.

Boyd, I. L., Bevan, R. M., Woakes, A. J. and Butler, P. J. (1999). Heart rate and behavior of fur seals: implications for measurement of field energetics. *Am. J. Physiol.* **276**, H844-H857.

Castellini, M. A., Milsom, W. K., Berger, R. J., Costa, D. P., Jones, D. R., Castellini, J. M., Rea, L. D., Bharma, S. and Harris, M. (1994). Patterns of respiration and heart rate during wakefulness and sleep in elephant seal pups. *Am. J. Physiol.* **266**, R863-R869.

Davis, R. W. and Kanatous, S. B. (1999). Convective oxygen transport and tissue oxygen consumption in Weddell seals during aerobic dives. *J. Exp. Biol.* **202**, 1091-1113.

Davis, R. W. and Williams, T. M. (2012). The marine mammal dive response is exercise modulated to maximize aerobic dive duration. *J. Comp. Physiol. A* **198**, 583-591.

Dormer, K. J., Denn, M. J. and Stone, H. L. (1977). Cerebral blood flow in the sea lion (*Zalophus californianus*) during voluntary dives. *Comp. Biochem. Physiol.* **58A**, 11-18.

Elsner, R. W., Franklin, D. L. and Vancitters, R. L. (1964). Cardiac output during diving in an unrestrained sea lion. *Nature* **202**, 809-810.

Feldkamp, S. D., Delong, R. L. and Antonelis, G. A. (1989). Diving patterns of California sea lions, *Zalophus californianus*. *Can. J. Zool.* **67**, 872-883.

Froget, G., Butler, P. J., Woakes, A. J., Fahlman, A., Kuntz, G., Le Maho, Y. and Handrich, Y. (2004). Heart rate and energetics of free-ranging king penguins (*Aptenodytes patagonicus*). *J. Exp. Biol.* **207**, 3917-3926.

Green, J. A., Butler, P. J., Woakes, A. J., Boyd, I. L. and Holder, R. L. (2001). Heart rate and rate of oxygen consumption of exercising macaroni penguins. *J. Exp. Biol.* **204**, 673-684.

Green, J. A., Butler, P. J., Woakes, A. J. and Boyd, I. L. (2003). Energetics of diving in macaroni penguins. *J. Exp. Biol.* **206**, 43-57.

Hill, R. D., Schneider, R. C., Liggins, G. C., Schuette, A. H., Elliott, R. L., Guppy, M., Hochachka, P. W., Qvist, J., Falke, K. J. and Zapol, W. M. (1987). Heart rate and body temperature during free diving of Weddell seals. *Am. J. Physiol.* **253**, R344-R351.

Hindle, A. G., Young, B. L., Rosen, D. A. S., Haulena, M. and Trites, A. W. (2010). Dive response differs between shallow- and deep-diving Steller sea lions (*Eumetopias jubatus*). *J. Exp. Mar. Biol. Ecol.* **394**, 141-148.

Irving, L., Scholander, P. F. and Grinnell, S. W. (1942). The regulation of arterial blood pressure in the seal during diving. *Am. J. Physiol.* **135**, 0557-0566.

Irving, L., Peyton, L. J., Bahn, C. H. and Peterson, R. S. (1963). Action of the heart and breathing during the development of fur seals (*Callorhinus ursinus*). *Physiol. Zool.* **36**, 1-20.

Jobsis, P. D., Ponganis, P. J. and Kooyman, G. L. (2001). Effects of training on forced submersion responses in harbor seals. *J. Exp. Biol.* **204**, 3877-3885.

Kooyman, G. L. and Campbell, W. B. (1972). Heart rates in freely diving Weddell Seals, *Leptonychotes weddelli*. *Comp. Biochem. Physiol.* **43A**, 31-36.

Kooyman, G. L. and Sinnett, E. E. (1982). Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. *Physiol. Zool.* **55**, 105-111.

Kuhn, C. E. (2006). Measuring at sea feeding to understand the foraging behavior of pinnipeds. PhD dissertation, University of California, Santa Cruz, CA, USA.

Lin, Y.-C., Matsuura, D. T. and Whittow, G. C. (1972). Respiratory variation of heart rate in the California sea lion. *Am. J. Physiol.* **222**, 260-264.

McDonald, B. I. and Ponganis, P. J. (2012). Lung collapse in the diving sea lion: hold the nitrogen and save the oxygen. *Biol. Lett.* **8**, 1047-1049.

McDonald, B. I. and Ponganis, P. J. (2013). Insights from venous oxygen profiles: oxygen utilization and management in diving California sea lions. *J. Exp. Biol.* **216**, 3332-3341.

Meir, J. U. and Ponganis, P. J. (2009). High-affinity hemoglobin and blood oxygen saturation in diving emperor penguins. *J. Exp. Biol.* **212**, 3330-3338.

Meir, J. U., Stockard, T. K., Williams, C. L., Ponganis, K. V. and Ponganis, P. J. (2008). Heart rate regulation and extreme bradycardia in diving emperor penguins. *J. Exp. Biol.* **211**, 1169-1179.

Meir, J. U., Champagne, C. D., Costa, D. P., Williams, C. L. and Ponganis, P. J. (2009). Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. *Am. J. Physiol.* **297**, R927-R939.

Millard, R. W., Johansen, K. and Milsom, W. K. (1973). Radiotelemetry of cardiovascular responses to exercise and diving in penguins. *Comp. Biochem. Physiol.* **46A**, 227-240.

Noren, S. R., Williams, T. M., Kendall, T. and Cuccurullo, V. (2012). Bradycardia redefined: a variable cardiovascular dive response in dolphins. *J. Exp. Biol.* **215**, 2735-2741.

Ponganis, P. J., Kooyman, G. L. and Zornow, M. H. (1991). Cardiac output in swimming California sea lions, *Zalophus californianus*. *Physiol. Zool.* **64**, 1296-1306.

Ponganis, P. J., Kooyman, G. L., Winter, L. M. and Starke, L. N. (1997). Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions, *Zalophus californianus*. *J. Comp. Physiol. B* **167**, 9-16.

Reed, J. Z., Chambers, C., Hunter, C. J., Lockyer, C., Kastelein, R., Fedak, M. A. and Boutilier, R. G. (2000). Gas exchange and heart rate in the harbour porpoise, *Phocoena phocoena*. *J. Comp. Physiol. B* **170**, 1-10.

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

Scholander, P. F., Irving, L. and Grinnell, S. W. (1942). Aerobic and anaerobic changes in seal muscles during diving. *J. Biol. Chem.* **142**, 431-440.

Snyder, F., Hobson, J. A., Morrison, D. F. and Goldfrank, F. (1964). Changes in respiration, heart tate, and systolic blood pressure in human sleep. *J. Appl. Physiol.* **19**, 417-422.

Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *J. Appl. Physiol.* **22**, 453-460.

Thompson, D. and Fedak, M. A. (1993). Cardiac responses of grey seals during diving at sea. *J. Exp. Biol.* **174**, 139-154.

Villegas-Amtmann, S., Simmons, S. E., Kuhn, C. E., Huckstadt, L. A. and Costa, D. P. (2011). Latitudinal range influences the seasonal variation in the foraging behavior of marine top predators. *PLoS ONE* **6**, e23166.

Villegas-Amtmann, S., Atkinson, S., Paras-Garcia, A. and Costa, D. P. (2012). Seasonal variation in blood and muscle oxygen stores attributed to diving behavior, environmental temperature and pregnancy in a marine predator, the California sea lion. *Comp. Biochem. Physiol.* **162A**, 413-420.

Weise, M. J. and Costa, D. P. (2007). Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. *J. Exp. Biol.* **210**, 278-289.

Williams, T. M., Haun, J. E. and Friedl, W. A. (1999). The diving physiology of bottlenose dolphins (*Tursiops truncatus*). I. Balancing the demands of exercise for energy conservation at depth. *J. Exp. Biol.* **202**, 2739-2748.

Wright, A. K., Ponganis, K. V., McDonald, B. I. and Ponganis, P. J. (2014). Heart rate of emperor penguins at sea: implications for oxygen store management. *Mar. Ecol. Prog. Ser.* **496**, 85-98.

Zapol, W. M., Liggins, G. C., Schneider, R. C., Qvist, J., Snider, M. T., Creasy, R. K. and Hochachka, P. W. (1979). Regional blood flow during simulated diving in the conscious Weddell seal. *J. Appl. Physiol.* **47**, 968-973.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York, NY: Springer Science.