

## RESEARCH ARTICLE

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

 Birgitte I. McDonald<sup>1,2,\*</sup> and Paul J. Ponganis<sup>2</sup>

## 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;  $50 \pm 9$  beats  $\text{min}^{-1}$ ) decreased compared with surface rates ( $113 \pm 5$  beats  $\text{min}^{-1}$ ), with all dives exhibiting an instantaneous  $f_H$  below resting ( $< 54$  beats  $\text{min}^{-1}$ ) 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  $\text{min}^{-1}$  in dives longer than 6 min in duration; (2) a slight increase in  $f_H$  to  $\sim 10$ – $40$  beats  $\text{min}^{-1}$  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  $\text{O}_2$  stores and the rate at which the  $\text{O}_2$  stores are depleted. Cardiovascular regulation is critical during diving because changes in heart rate ( $f_H$ ) and cardiac output affect not only blood  $\text{O}_2$  uptake from the lung but also the rate and magnitude of blood  $\text{O}_2$  delivery to tissues. Therefore, decreased  $f_H$

has long been considered central to a decreased rate of  $\text{O}_2$  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  $\text{min}^{-1}$  (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 than  $80$  beats  $\text{min}^{-1}$  (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  $\text{O}_2$  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 ( $S_{\text{O}_2}$ )

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**List of symbols and abbreviations**

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

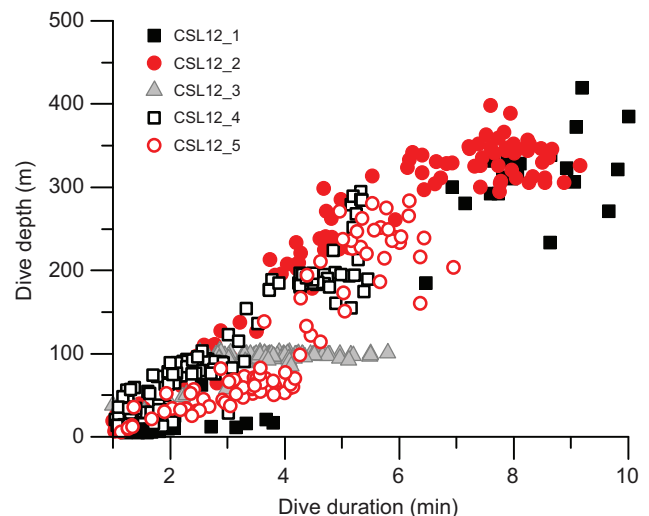
previously documented in these animals (McDonald and Ponganis, 2012; McDonald and Ponganis, 2013); and (4) the initial rate of decline in  $f_H$  would be slower in deeper dives, promoting gas exchange at shallower depths to take advantage of postulated larger respiratory  $O_2$  stores during deeper 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  $f_H$ ), 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  $f_H$  and sinus arrhythmia**

Resting  $f_H$  (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  $54\pm 6$  beats  $min^{-1}$  ( $N=5$ ; Table 2). There was a significant negative relationship between sea lion mass and resting  $f_H$  ( $y=-0.58x+100.27$ ,  $r^2=0.81$ ,  $F_{1,3}=12.37$ ,  $P=0.039$ ). While lying



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

on the beach, sea lions exhibited sinus arrhythmia with mean minimum instantaneous  $f_H$  (minimum beat-to-beat  $f_H$ ) of  $42\pm 9$  beats  $min^{-1}$  and mean maximum instantaneous  $f_H$  (maximum beat-to-beat  $f_H$ ) of  $87\pm 12$  beats  $min^{-1}$  (Fig. 2). Although respirations could not be observed, if inhalation is associated with the increased  $f_H$  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  $6.1\pm 0.8$  breaths  $min^{-1}$ ) (Table 2).

**Diving  $f_H$** 

In all dives, sea lions decreased dive  $f_H$  from pre-dive rates (Figs 3, 4), with all dives exhibiting an instantaneous  $f_H$  (beat-to-beat  $f_H$ ) 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  $f_H$  lower than resting  $f_H$  (Table 3).

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

**Table 1. Individual and pooled  $f_H$  data from California sea lions**

Seal lion ID	Dive duration (min)	Dive depth (m)	Dive $f_H$ (beats $min^{-1}$ )	Minimum $f_H$ (beats $min^{-1}$ )	Bottom $f_H$ (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)

$f_H$ , 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 $\text{min}^{-1}$ )	Minimum $f_H$ (beats $\text{min}^{-1}$ )	Maximum $f_H$ (beats $\text{min}^{-1}$ )	Respiration rate (breaths $\text{min}^{-1}$ )
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  $\text{min}^{-1}$ ). 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  $\text{min}^{-1}$ ), compared with average dive bout surface interval  $f_H$  (113±5 beats  $\text{min}^{-1}$ ) (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  $\text{min}^{-1}$  (Fig. 5B), and

average  $f_H$  during the bottom of the dive was around 20 beats  $\text{min}^{-1}$  (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.

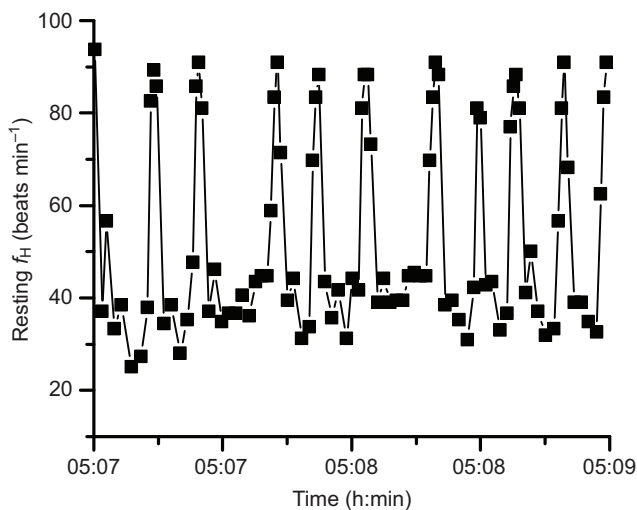
The total number of heart beats during a dive was positively related to dive duration (Table 4, Fig. 7A). Total beats in a dive appeared to begin to level out near 300–350 heart beats at a dive duration of 8–10 min, but only two sea lions exhibited such long-duration dives. The number of heart beats 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  $\text{min}^{-1}$ , suggesting that pulmonary blood flow and gas exchange are greatly reduced even before complete lung collapse.

## DISCUSSION

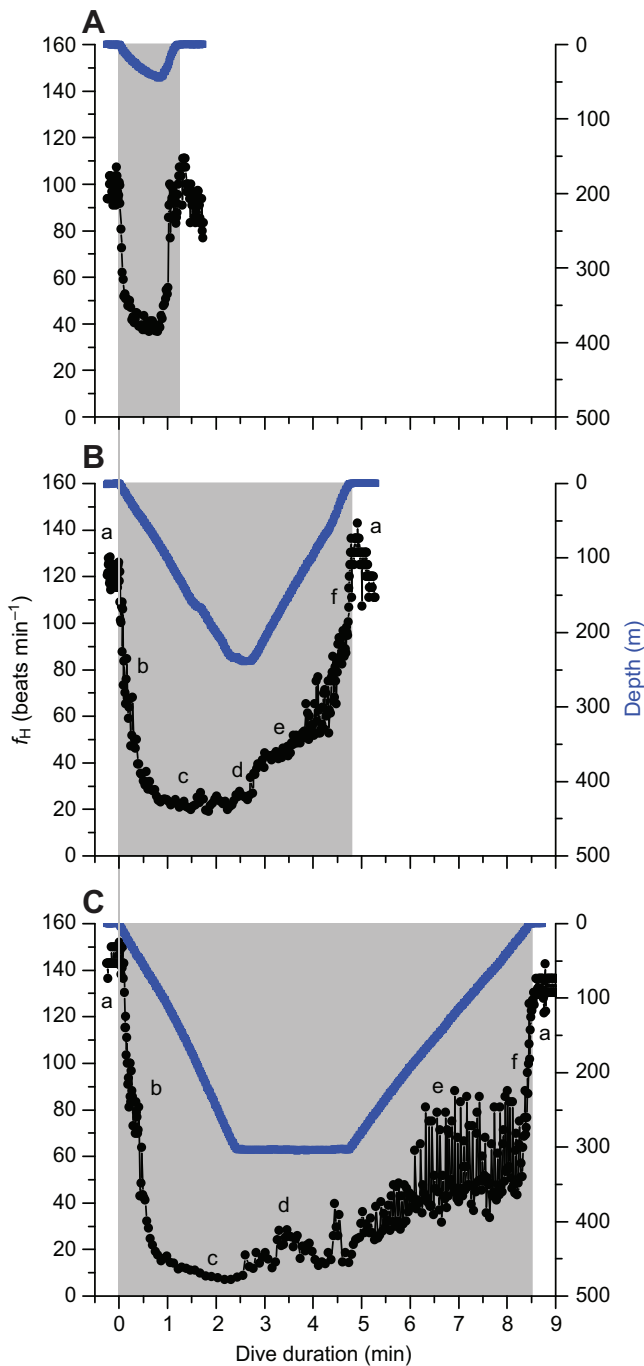
### Resting $f_H$

The resting  $f_H$  measured in this study (54±6 beats  $\text{min}^{-1}$ ) was lower than predicted for an animal of similar size (~80 beats  $\text{min}^{-1}$  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  $\text{min}^{-1}$ , average mass 30 kg) (Ponganis et al., 1997) and wild Antarctic fur seals (78±5 beats  $\text{min}^{-1}$ , 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  $\text{min}^{-1}$ , 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  $\text{min}^{-1}$ , 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  $\text{min}^{-1}$ ), indicating that in dives less than 3 min (estimated cADL),  $f_H$  only decreased to levels observed during

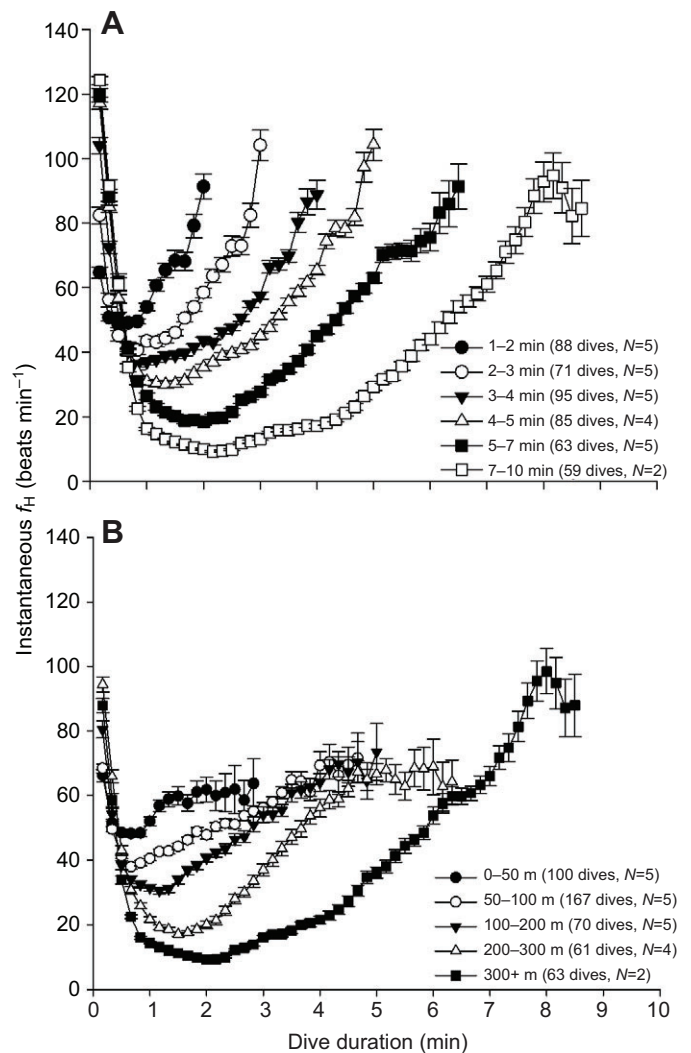


**Fig. 2. Instantaneous resting heart rate ( $f_H$ ) of a California sea lion lying on the beach.** Note the periodic, brief increase in  $f_H$ . This suggests sea lions display the typical respiratory sinus arrhythmia present in other diving mammals, and that each increase indicates an inhalation.



**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  $\text{min}^{-1}$  in the short dive (A), 19 beats  $\text{min}^{-1}$  in the mid-duration dive (B) and 7 beats  $\text{min}^{-1}$  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.

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



**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.

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 <sup>-1</sup> )	105±21 <sup>a</sup>	135±17 <sup>b</sup>	138±9 <sup>b</sup>
Dive $f_H$ (beats min <sup>-1</sup> )	55±8 <sup>a</sup>	51±6 <sup>b</sup>	40±6 <sup>c</sup>
Minimum $f_H$ (beats min <sup>-1</sup> )	37±9 <sup>a</sup>	25±18 <sup>b</sup>	11±5 <sup>c</sup>
Post-dive $f_H$ (beats min <sup>-1</sup> )	115±15 <sup>a</sup>	131±10 <sup>b</sup>	132±7 <sup>b</sup>
Heart beats to resting $f_H$	13±11 <sup>a</sup>	35±13 <sup>b</sup>	41±14 <sup>b</sup>
%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<sup>-1</sup> (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 Stellar sea lions was similar, Stellar sea lions regularly exhibited lower minimum  $f_H$ , with minimum  $f_H$  almost always less than 20 beats min<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup> and sometimes as low as 6 beats min<sup>-1</sup> (Fig. 5B), which is similar to extreme divers such as emperor penguins (3 beats min<sup>-1</sup>), elephant seals (3 beats min<sup>-1</sup>), grey seals (2 beats min<sup>-1</sup>) and Weddell seals (<10 beats min<sup>-1</sup>), 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.

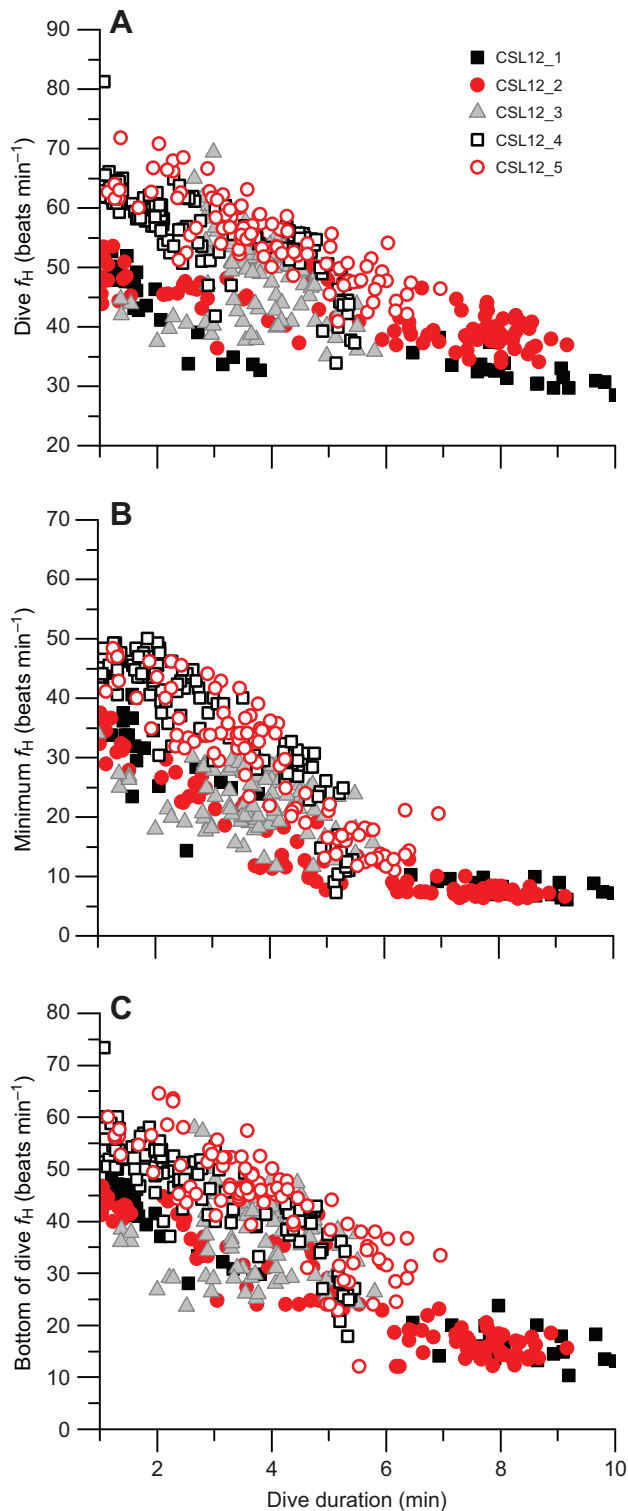
While the degree of bradycardia observed in long dives of California sea lions was similar to the extreme bradycardia observed in phocids, the  $f_H$  profiles were quite different. In general, phocid  $f_H$  decreases abruptly upon submergence. The intensity of the initial phocid bradycardia either 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<sub>2</sub> for ascent (Andersson et al., 2002). Additionally, with  $f_H$  less than 20 beats min<sup>-1</sup> 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<sub>2</sub> 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<sub>O<sub>2</sub></sub> rapidly decreased during descent, while arterial S<sub>O<sub>2</sub></sub> remained high, resulting in an arterio-venous (a–v) O<sub>2</sub> 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<sub>2</sub> difference suggests extreme hypoperfusion of tissue allowing for complete blood O<sub>2</sub> extraction, which is consistent with the low  $f_H$  measured during the end of descent and bottom portion of long-duration dives (Fig. 8).

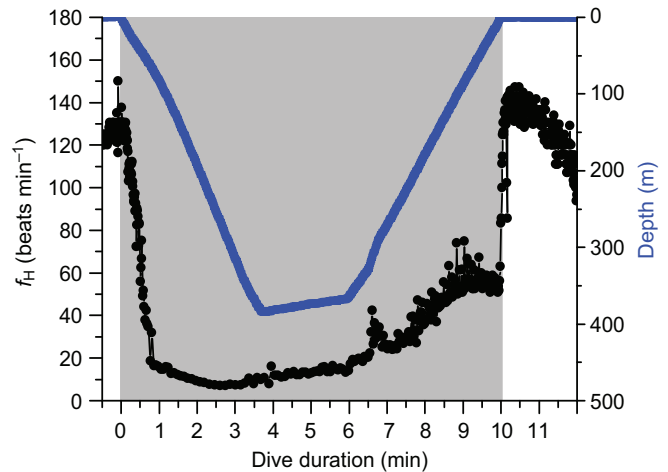
Another notable difference between short- (<3 min) and mid- to long-duration (>3 min) dives was the significantly higher pre-dive  $f_H$  in the longer dives (Table 3). This is consistent with the pre-dive arterialisation of venous blood often exhibited before long, deep dives (McDonald and Ponganis, 2013). The higher  $f_H$  before long dives will allow sea lions to ensure blood and muscle are fully loaded with O<sub>2</sub> 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<sub>2</sub> 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).

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,



**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  $\text{min}^{-1}$  and was less than 20 beats  $\text{min}^{-1}$  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  $\text{min}^{-1}$  towards the end of the surface interval.

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  $\text{O}_2$ . 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  $\text{O}_2$  stores (~16% of total body  $\text{O}_2$  stores) throughout the dive (Weise and Costa, 2007). This is supported by venous blood  $\text{O}_2$  profiles, where, occasionally, there was no decrease in venous blood  $\text{O}_2$  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  $\text{O}_2$  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  $\text{O}_2$  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**

Model	Model variables		Results						
			Fixed effect					Random effect	
	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						
	<b>Duration</b>	<b>Sea lion ID (intercept + slope)</b>	<b>2739.0</b>	-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						
	<b>Duration</b>	<b>Sea lion ID (intercept + slope)</b>	<b>2736.0</b>	-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						
	<b>Duration</b>	<b>Sea lion ID (intercept + slope)</b>	<b>3978.9</b>	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						
	<b>Duration</b>	<b>Sea lion ID (intercept + slope)</b>	<b>3505.8</b>	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<sub>2</sub> 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<sup>-1</sup>. 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<sub>2</sub> and N<sub>2</sub> 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<sub>2</sub> store, the extreme decline in  $f_H$  during later descent of deep dives should help decrease lung O<sub>2</sub> utilisation, and, importantly, should also minimise the risks of excess N<sub>2</sub> 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<sub>2</sub> delivery to O<sub>2</sub>-depleted tissues, thus lowering the  $P_{O_2}$  in the blood and increasing the blood/lung O<sub>2</sub> gradient, resulting in shorter surface intervals by maximisation of O<sub>2</sub> uptake at the surface (Thompson and Fedak, 1993). This hypothesis was supported in diving elephant seals by a more rapid decline in venous  $P_{O_2}$  at the end of the dive (Meir et al., 2009). In contrast, we found an increase in venous  $P_{O_2}$  during ascent from deep dives (Fig. 8), which is consistent with arterial  $P_{O_2}$  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,

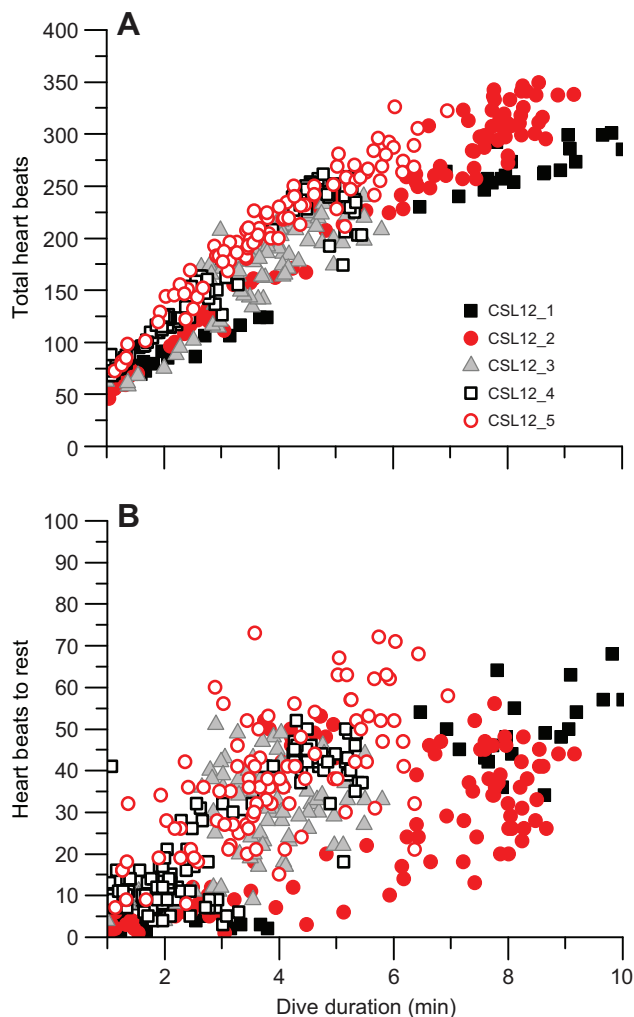
increased pulmonary blood flow, secondary to the ascent tachycardia, could also facilitate O<sub>2</sub> uptake of the blood and maintain or increase arterial O<sub>2</sub> content.

#### 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<sup>-1</sup>, and stayed below 20 beats min<sup>-1</sup> for more than a minute. At a  $f_H$  of 20 beats min<sup>-1</sup>, 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<sup>-1</sup>), 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  $54 \text{ beats min}^{-1}$ .

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 ( $\pm 0.2 \text{ kg}$ , MSI-7200 Dyna-link; Measurement Systems International, Seattle, WA, USA), and placed in a kennel to recover from anaesthesia (recovery time  $53 \pm 21 \text{ min}$ ). After one to four trips to sea, females were located using the radio transmitter, recaptured and instruments removed while the female was manually restrained ( $19 \pm 6 \text{ min}$ ).

### Instrumentation

While under anaesthesia, two subcutaneous, sterile ECG electrodes were inserted aseptically,  $\sim 5 \text{ cm}$  left of the dorsal midline; one electrode was anterior to the axilla and one electrode was placed posterior to the flipper,  $\sim 30 \text{ cm}$  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  $\times$  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;  $5 \times 3 \times 1.5 \text{ cm}$ ) and VHF radio transmitter (mm160B; Advanced Telemetry Systems, Isanti, MN, USA;  $6 \times 1.8 \text{ cm}$ ) 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

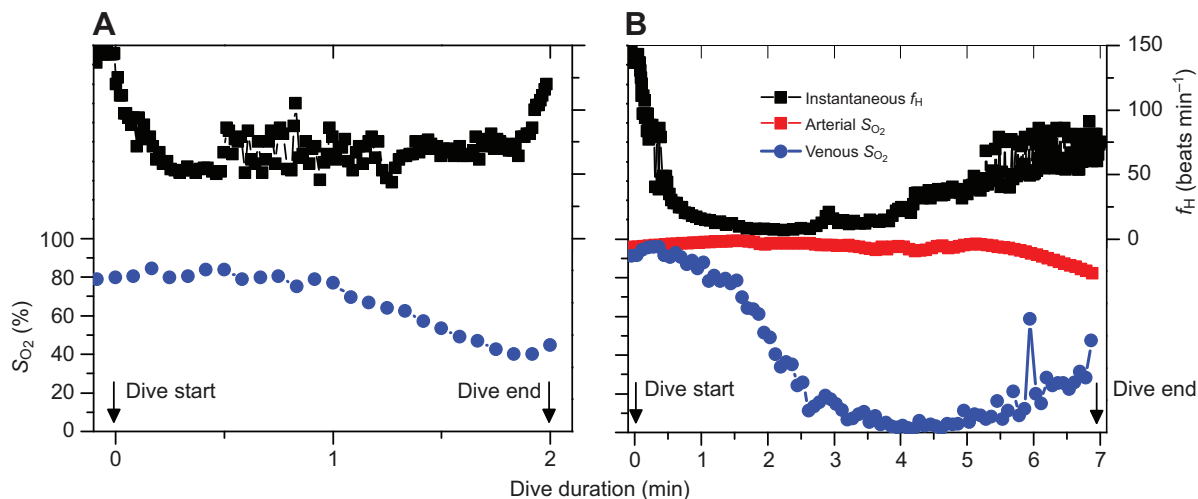
TDR data were processed and analysed in MatLab (The MathWorks, Natick, MA, USA) using a custom-written dive analysis program (IKNOS; Y. Tremblay). After calculating a zero offset correction at the surface, dives were identified on the basis of a minimum duration (20 s) and depth (5 m). For each dive, 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, Northampton, 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  $f_H = 54 \text{ beats min}^{-1}$ ). 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 assessed by examination of the residuals. All means are expressed  $\pm$ 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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