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RESEARCH ARTICLE

Stroke effort and relative lung volume influence heart rate in diving sea lions

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ABSTRACT

The dive response, bradycardia (decreased heart rate) and peripheral vasoconstriction, is the key mechanism allowing breath-hold divers to perform long-duration dives while actively swimming and hunting prey. This response is variable and modulated by factors such as dive duration, depth, exercise and cognitive control. This study assessed the potential role of exercise and relative lung volume in the regulation of heart rate (f_H) during dives of adult female California sea lions instrumented with electrocardiogram (ECG), depth and tri-axial acceleration data loggers. A positive relationship between activity (minimum specific acceleration) and f_H throughout dives suggested increased muscle perfusion associated with exercise. However, apart from late ascent, f_H during dives was still less than or equal to resting f_H (on land). In addition, the activity–f_H relationship was weaker in long, deep dives consistent with prioritization of blood oxygen conservation over blood oxygen delivery to muscle in those dives. Pulmonary stretch receptor reflexes may also contribute to f_H regulation as f_H profiles generally paralleled changes in relative lung volume, especially in shallower dives and during early descent and late ascent of deeper dives. Overall, these findings support the concept that both exercise and pulmonary stretch receptor reflexes may influence the dive response in sea lions.

KEY WORDS: Depth, Dive response, Exercise, Minimum specific acceleration, Parasympathetic, Stroke rate, Sympathetic

INTRODUCTION

Marine mammals face an extraordinary foraging challenge compared with their terrestrial counterparts in that they must hold their breath while actively searching for and capturing prey. It is essential to understand the physiological mechanisms that underlie their ability to perform such behaviors if we are to evaluate their role in the ecosystem and their ability to exploit prey resources in a changing environment. The impressive breath-hold capabilities of marine mammals are made possible by both an enhanced oxygen storage capacity and a suite of physiological adjustments during dives that regulate the rate of blood oxygen consumption (the 'dive response'). The dive response is characterized by selective peripheral vasoconstriction that is matched by a proportional decrease in cardiac output, thereby maintaining blood pressure throughout the dive. This variable response regulates the magnitude and distribution of peripheral blood flow, and, in extreme cases, limits blood flow to non-essential organs and working muscle, conserving oxygen for critical tissues such as the brain and heart (Scholander et al., 1942; Scholander, 1940).

The dive response is a dynamic process, which is influenced by a variety of factors such as dive duration, depth, exercise intensity, temperature and even volition (Davis and Williams, 2012; Elmegaard et al., 2016; Kaczmarek et al., 2018; McDonald et al., 2017; McDonald and Ponganis, 2014; Noren et al., 2012; Thompson and Fedak, 1993; Williams et al., 2015). Based on recent publications that emphasized the potential role of both exercise and depth in regulation of the dive response of marine mammals (Davis and Williams, 2012; Williams et al., 2015), Ponganis et al. (2017) reviewed the literature to examine how the parasympathetic and sympathetic nervous systems interact during controlled dive studies, and concluded that in laboratory-based studies (a) the parasympathetic nervous system dominated over the sympathetic nervous system in the regulation of heart rate (f_H) during dives (Elliott et al., 2002; Signore and Jones, 1995, 1996), (b) changes and fluctuations in f_H were primarily due to changes in parasympathetic activity (Blix and Folkow, 1983; Butler and Jones, 1997), and (c) exercise, changes in lung volume and volitional control were three primary factors that likely influence the parasympathetic response during a dive (Angell-James et al., 1981; Elmegaard et al., 2016; Ridgway et al., 1975; Signore and Jones, 1996).

California sea lions (*Zalophus californianus*) perform a wide variety of dives, with durations ranging from 1–2 min on dives <100 m maximum depth to 10 min for dives of >400 m maximum depth (Kuhn and Costa, 2014; McDonald and Ponganis, 2013). As in other pinnipeds (Andrews et al., 1997; Boyd et al., 1999; Hill et al., 1987; Hindle et al., 2010; Kooyman and Campbell, 1972; Thompson and Fedak, 1993), f_H profiles vary during different types of dives of sea lions. In general, as dives become deeper and longer, the f_H profiles of sea lions are characterized by higher initial f_H and more extreme mid-dive bradycardias (decreased f_H) (McDonald and Ponganis, 2014). This range of dive behaviors and f_H profiles make the California sea lion an ideal model to investigate regulation of the cardiovascular response during dives at sea.

In this paper, we assessed the potential roles of exercise and relative lung volume in the regulation of f_H during dives of California sea lions. We used f_H data from the sea lions in our previous study (McDonald and Ponganis, 2014), and analyzed simultaneously collected tri-axial acceleration data to evaluate two indices of workload: flipper stroke rate and minimum specific acceleration (MSA) (Simon et al., 2012; Tift et al., 2017). We also estimated relative lung volume throughout the dive, using depth profiles and Boyle's law, to evaluate the potential role of pulmonary stretch receptors and lung volume reflexes on f_H regulation during dives

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(Andersson and Schagatay, 1997; Angell-James et al., 1981; Angell-James and Daly, 1978; Drummond and Jones, 1979). These relative lung volumes, as calculated, represent the change in air volume due to pressure, and serve as an index of potential compression and expansion of the lung. Calculation or modeling of actual lung volumes would require parameters that are not available, i.e. airway, alveolar and chest wall compliance in a living sea lion, dead space and alveolar volume (Bostrom et al., 2008; Fahlman et al., 2017; Moore et al., 2011). Nonetheless, this approach provides an initial assessment of the potential effects of changes in lung volume on heart rate regulation via pulmonary stretch receptors.

We hypothesized that there would be a relationship between activity and f_H during diving, but the relationship would weaken in long, deep dives because conservation of blood oxygen would take priority over blood flow to muscles. Indeed, such differences in the dive response relative to activity and depth had been reported in another otariid, the Steller sea lion (*Eumetopias jubatus*) (Hindle et al., 2010). This dissociation of f_H from flipper stroke effort might be secondary to the effects of depth on the f_H response (Williams et al., 2015) and/or to volitional control (managing f_H based on expected dive duration) (Elmegaard et al., 2016; Ridgway et al., 1975). We also hypothesized that f_H profiles would parallel relative lung volume profiles during dives because the greatest changes in f_H occurred during descent and ascent, the times at which lung volumes change the most during a dive. Lastly, we hypothesized that the previously observed, large beat-to-beat fluctuations in f_H would be associated with activity level and/or changes in relative lung volume due to adjustments in vagal activity.

MATERIALS AND METHODS

Animal capture and instrumentation

This study was conducted on five lactating adult female California sea lions, *Zalophus californianus* (Lesson 1828), on San Nicolas Island CA, USA (33°16′27″ N, 119°34′30″ W) in November 2012. Experimental details, including capture/release, anesthesia, instrumentation and f_H analysis, were as described in a prior publication and will only briefly be described here (McDonald and Ponganis, 2014). Sea lions were captured and anesthetized with isoflurane gas with O_2 using a portable field vaporizer set up (McDonald and Ponganis, 2013). Females were instrumented with an electrocardiogram (ECG)/pressure recorder (3991 BioLog, UFI, Morro Bay, CA, USA) that sampled ECG at 50 Hz and external pressure at 1 Hz in a custom-made waterproof housing (Meer Instruments, Palomar Mountain, CA, USA; 3 cm diameter×15 cm length), a 3D accelerometer time depth recorder (TDR: tdr10-X, Wildlife Computers, Redmond, WA, USA; sampling tri-axial acceleration at 16 Hz, depth at 1 Hz; 5×3×1.5 cm) and a radio transmitter (mm160B, Advanced Telemetry Systems, Isanti, MN, USA; 6×1.8 cm). After instrumentation, females were weighed and placed in a kennel to recover from anesthesia. Females were recaptured and instruments recovered after one to four trips to sea. All procedures were approved by the University of California, San Diego Animal Subjects Committee (no. S11303) and National Marine Fisheries Services (no. 14676). The time on the TDR and ECG logger was synchronized to the same internet-synced computer clock before deployment. Synchronization of the two recorders was also confirmed by analysis of the simultaneous depth profiles from the two devices.

Data processing and analysis

Depth data were processed using a custom-written dive analysis program in Matlab (The MathWorks, Natick, MA, USA; IKNOS, Y. Tremblay) as described in McDonald and Ponganis (2014). Briefly, after calculating a zero-offset correction at the surface, dives were identified based on a minimum duration (20 s) and depth (5 m). For each dive, maximum depth, dive duration and post-dive surface interval were determined. Relative lung volume throughout the dive was calculated as: 1/(1+depth/10) with depth in meters.

ECG data have previously been published and analysis methods are described in McDonald and Ponganis (2014). Briefly, the ECG data were processed in Origin (version 8.6, OriginLab, Northampton, MA, USA) using a custom-written peak detection program to mark R-wave peaks and measure the R–R intervals and calculate instantaneous f_H. Data were visually inspected to confirm the correct identification of R-wave peaks.

Two activity indices were calculated using the tri-axial acceleration data: (1) flipper stroke rate and (2) MSA. Fore-flipper stroke rate was calculated using a custom-written algorithm in Matlab using the *x*- or *z*-axis (Tift et al., 2017). The low-frequency static acceleration was eliminated using a 0.2 Hz high-pass Butterworth filter. The resulting dynamic acceleration was analyzed using power spectral density analysis to identify the dominant frequency of a flipper stroke for each axis (~0.8–1.2 strokes s^{-1}). Flipper strokes were then identified using a Matlab peak detection algorithm similar to those in other studies (Jeanniard-du-Dot et al., 2016; Sato et al., 2011). A single flipper stroke was identified when there was a prominent acceleration peak ($\geq$0.45 m s^{-2}) in either the forward surge or the heave surge direction. Instantaneous stroke rate was calculated based on the time difference between two strokes. MSA was calculated as the absolute value of the norm of acceleration and is a measure of how much the total acceleration deviates from the acceleration due to gravity. This is an underestimate on the specific acceleration generated by the animal (Simon et al., 2012). The relationship between MSA and flipper stroke rate was investigated using a linear mixed-effect model, with sea lion ID as a random effect (R 3.5.3, package nlme; Pinheiro et al., 2017). While stroke rate and MSA were positively related (the best model included stroke rate), stroke rate only explained 24.2% of the variation in MSA (Table 1). MSA is likely a better indicator of workload because it incorporates the force of the stroke (M. Cole and B.I.M., unpublished data). Additionally, it is a simple metric to calculate that is less prone to error. For this reason, MSA was primarily used to investigate the relationship between activity and f_H.

For dives greater than 1 min in duration, a custom-written Matlab code was used to determine dive f_H (total number of heart beats in a dive divided by dive duration), post-dive f_H (30 s immediately after surfacing; if the surface interval was <30 s, post-dive f_H was calculated based on the entire surface interval), mean MSA (cumulative dive MSA divided by the number of MSA measurements during the dive) and dive flipper stroke rate (total flipper strokes in a dive divided by dive duration). Mean instantaneous f_H, mean MSA, mean depth and mean relative lung volume were also determined for 10 s intervals throughout the dive. Although this study focused on dive f_H, we processed 30–35 min of surface swimming f_H and MSA for 4 sea lions for comparison with dive f_H.

The relationships of dive duration, dive depth and activity (mean MSA) to dive f_H and post-dive f_H were investigated using linear mixed-effects models (Cran R 3.5.3, package nlme). Dive duration, dive depth and mean MSA were fixed effects, 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. Similar analyses were performed with the 10 s interval data. The influence of activity (mean interval MSA) and depth (mean for

Table 1. Mixed-effects model results examining the relationship between behavior and heart rate (f_{H}) in California sea lions

Model variables				Fixed effects					Random	Variance explained	
Response variable	Fixed effects	Random effect	AIC	Intercept	Error	Coefficient	Error	t	icc	Marginal R^2	Conditional R^2
Activity index (n=418 dives from 4 sea lions)											
Mean dive MSA	Dive stroke rate	ID (intercept)	−541	0.21	0.039	0.90	0.08		0.30	0.24	0.36
Surface interval f_{H} (n=821 surface intervals from 4 sea lions)											
Surface f_{H}	Surface MSA	ID (intercept and slope)	6756	47.75	5.34	34.59	7.95	4.35	0.24	0.10	0.39
Dive f_{H} (n=452 dives from 5 sea lions)											
Dive f_{H}	Max. depth	ID	2486	49.46	2.426	−0.07	0.01	−8.45	0.50	0.34	0.66
	Mean dive MSA	(intercept)				11.84	1.39	8.51			
	Interaction					0.04	0.02	2.25			
Dive f_{H}	Duration	ID	2362	51.91	2.198	−0.03	0.01	−6.35	0.46	0.40	0.65
	Mean dive MSA	(intercept)				14.34	1.53	9.40			
	Interaction					−0.02	0.01	−2.19			
Post-dive f_{H}	Duration	ID	3394	106.07	5.033	−0.02	0.02	−1.04	0.45	0.35	0.61
	Mean dive MSA	(intercept)				6.01	5.32	1.13			
	Interaction					0.16	0.03	4.73			
Dive interval f_{H} (n=9852 intervals from 5 sea lions)											
Mean interval f_{H}	Depth	ID	69,368	56.54	1.175	−0.18	0.00	−38.24	0.08	0.66	0.77
	Mean MSA	(intercept and slope)				28.06	3.96	7.08			
	Interaction					−0.11	0.00	−27.17			
Mean interval f_{H}	Relative lung vol.	ID	68,331	29.38	2.271	56.59	10.44	5.42	0.11	0.27	0.39
	Mean MSA	(intercept and slope)				1.92	0.41	4.67			
	Interaction					74.03	2.43	30.42			

Marginal R^2 indicates the amount of variance accounted for by fixed variables. Conditional R^2 indicates the amount of variance accounted for by the entire model. t is the test statistic and icc is the intra-correlation coefficient that indicates the amount of variance attributed to an individual sea lion (ID).

interval) on mean interval instantaneous f_{H} was examined using linear mixed-effects models with sea lion ID as a random effect. Intervals with a mean dive depth <10 m were excluded to prevent surface f_{H} influencing the relationships at the beginning and end of each dive. These models also included a correlation structure to account for the autocorrelation in the time series data. 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). Some of the fixed effects were correlated (i.e. dive duration with dive depth) and therefore only one was included in comparable models which were then compared to determine which model accounted for more variation in the data. The best model was selected by removing a single variable and comparing the full and reduced model using a log-likelihood chi-squared test. If the reduced model was better, another variable was removed until removing variables did not improve the model. Only the best models are presented in Table 1.

To evaluate the occurrence and distribution of marked fluctuations in f_{H} during dives, f_{H} profiles were analyzed with a custom-written Matlab program designed to detect an anomalous heartbeat. To detect the most significant fluctuations, an anomalous heartbeat was defined as a heartbeat with a 10 beat min^{-1} change in f_{H} from the prior heartbeat, followed by a subsequent change of ≥30% from the anomalous f_{H} in the opposite direction that the anomaly occurred. Goodness-of-fit chi-squared tests were performed to determine whether these anomalies occurred more or less than due to chance at different depths (0–100, 100–200, 200–300 and >300 m depth bins), dive phase (descent, bottom and ascent) and flipper stroke rates (0–0.19, 0.2–0.39, 0.4–0.59, 0.6–0.79, 0.8–1 Hz bins).

RESULTS

General results

Analyses were performed on 452 dives from five sea lions with simultaneous depth, ECG and tri-axial acceleration data. Dives had a median duration of 3.4 min (range: 1–10 min) and a median depth of 98 m (range: 5–420 m depth) (McDonald and Ponganis, 2014). Complete reviews of animal characteristics and f_{H} profiles are given in McDonald and Ponganis (2014). Sea lions exhibited stroke–glide patterns with instantaneous flipper stroke rates as high as 1.5 Hz and with prolonged glides during descents of deep dives (Fig. 1). Mean dive flipper stroke rate was 0.37±0.11 Hz (range: 0.01–0.70 Hz). Dive duration and depth were significantly correlated (Pearson's R=0.91, P<0.001); therefore, models with duration and depth were run separately, and compared. Duration explained more of the variation in f_{H}, so dive duration results are presented below.

While the match was not perfect, the shape of the instantaneous f_{H} profile most closely resembled the relative lung volume profile in both shallow and deep dives (Fig. 1B,D). There were more fluctuations in the f_{H} profile than in the relative lung volume profile during both shallow and deep dives, and there was also a continuous gradual rise in f_{H} during the long ascent from deep dives that did not parallel the relative lung volume profile. Increases in f_{H} were often associated with increased activity (flipper stroke rate or MSA), but also occurred without increased activity. For example, the onset of flipper stroking at the end of the prolonged glide during descent of deep dives was usually associated with a small increase in f_{H} (see Fig. 1D,E). f_{H} also increased slightly at the initiation of ascent during the bottom phase of the dive; however, this increase in f_{H} continued even when flipper stroke rate and MSA were in decline towards the end of the dive (Fig. 1). f_{H} profiles paralleled MSA

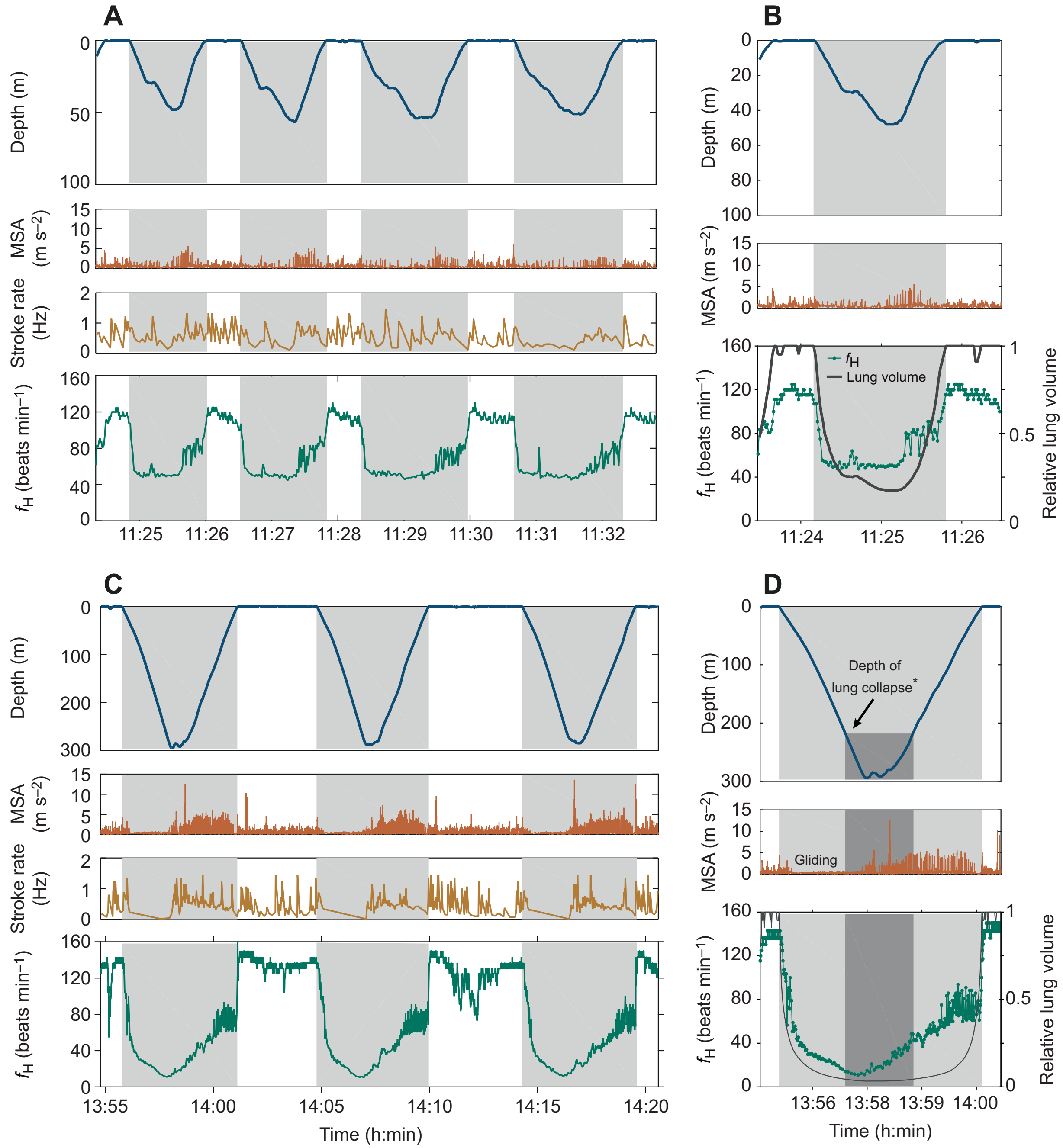

Fig. 1. Depth, activity and heart rate profiles from a California sea lion. (A) Example of four ∼2 min dives (gray area) to ∼70 m, demonstrating the typical activity [minimum specific acceleration (MSA) and flipper stroke rate] and heart rate (f_H) response in short shallow dives. (B) Close-up of the first dive in A. (C) Example of three 5 min dives to ∼300 m. (D) Close-up of the first dive in C. The dark gray box indicates lung collapse (from McDonald and Ponganis, 2012). In all dives, f_H decreases, but to lower levels in the longer deep dives. The f_H profiles have a similar shape to the relative lung volume profile; however, in the deep dives, f_H increases earlier than the relative lung volume and may be associated with increased activity. f_H at rest on the beach averages 54 beats min^{-1} in sea lions (McDonald and Ponganis, 2014).

profiles during shallow dives of 1–2 min duration (Fig. 2). However, as dives became deeper and longer, the f_H profile diverged further from the MSA profile.

Influence of dive behavior on dive f_H

Both dive duration and activity (MSA) influenced dive f_H in wild sea lions (Table 1). The more active the dive, the higher the f_H (Fig. 3A, Table 1; coefficient and error=14.34±1.53, t=9.4). Dive f_H was negatively related to dive duration (and depth) (Table 1; duration coefficient and error=−0.03±0.01, t=−6.35). Although at all durations, f_H increased with increasing activity, the relationship varied with dive depth (Table 1, Fig. 2; interaction t=2.72). Together, dive duration and activity accounted for 40% of the variation in dive f_H. Neither dive duration nor activity influenced post-dive f_H, but the interaction term was significant (Fig. 3B, Table 1).

In addition to examining the relationship between f_H and activity at the level of the dive, we examined the influence of MSA and depth on f_H at 10 s intervals within the dives. Both depth and activity

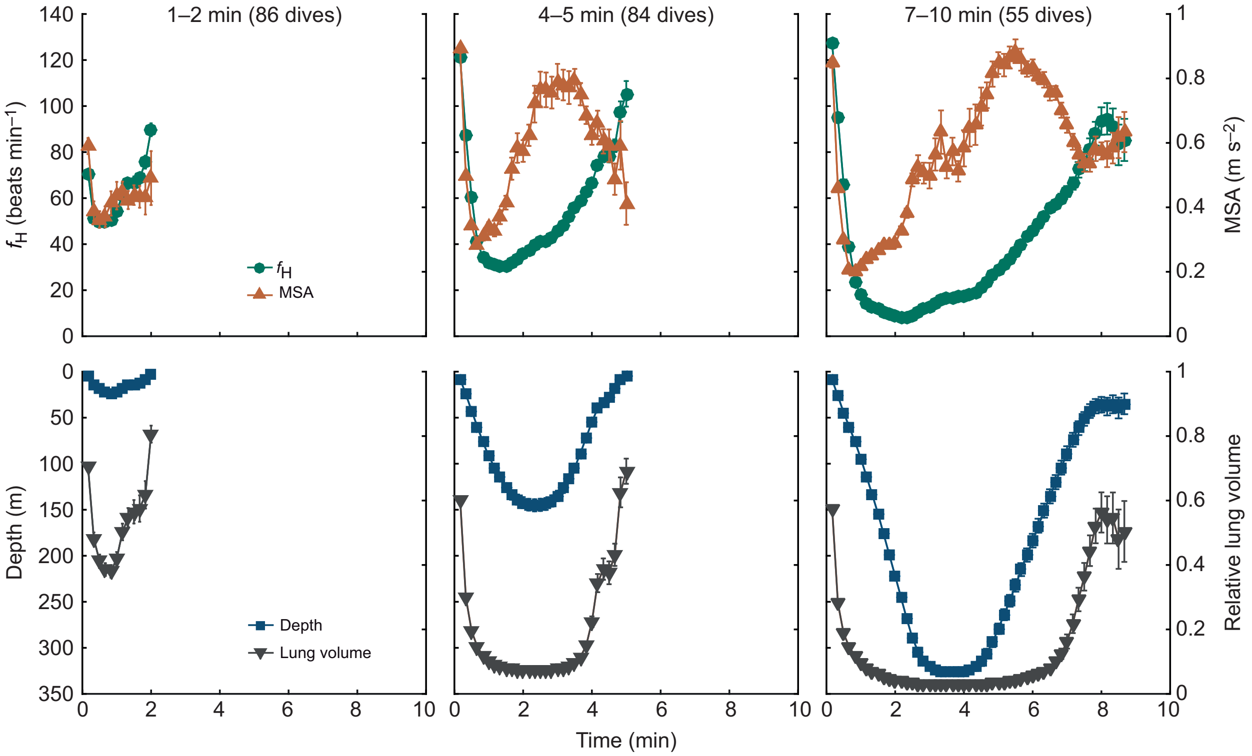

Fig. 2. Mean f_H, MSA, relative lung volume and depth profiles in short (1–2 min), intermediate (4–5 min) and long duration (>7 min), deep dives. Although f_H and MSA appear linked during short-duration dives, f_H diverges from activity as dives become deeper and longer. Data are binned in 10 s intervals. The f_H profile parallels the relative lung volume profile in shallow dives. In the intermediate and long duration dives, the f_H profile is similar to the relative lung volume profile, although they start to diverge later in the dive, likely due to intense stroking effort at the beginning of ascent. Relative lung volume starts and ends at ∼60–70% because the mean depths of the first and last intervals were between 5 and 10 m. Data represent 225 dives from 5 sea lions.

influenced f_H (Table 1, Figs 2 and 4). At a given depth, f_H increased as activity (mean MSA) increased (Table 1; coefficient and error=28.06±3.96, t=7.08). As reported previously in this species, deeper depths were associated with lower f_H (Table 1; coefficient and error=−0.18±0.005, t=−38.24); however, the relationship between activity and f_H varied with interval depth (Table 1; coefficient and error=−0.11±0.004, t=−27.17). The slope of the activity and f_H relationship was lower at depths >300 m (Fig. 4). Together, dive depth and activity accounted for 66% of the variation in interval f_H.

Surface swimming f_H

During surface swimming, sea lions exhibited a bimodal distribution of instantaneous f_H with one peak between 75 and 90 beats min^{-1}, likely associated with respirations, and a second larger peak between 40 and 50 beats min^{-1} (Fig. 5B). There was a positive relationship between surface interval MSA and surface interval f_H, similar to what was observed in the fine-scale dive data; however, the relationship only explained 10% of the variation in surface f_H (Table 1, Fig. 5C).

Anomalies

We documented 3134 f_H anomalies. Eighty-four percent of anomalies occurred at depths less than 100 m, which was greater than predicted based on the amount of time sea lions spent at those depths (63%), resulting in fewer anomalies at depths greater than 200 m than predicted (Fig. 6A; goodness-of-fit χ^2=611.0, d.f.=3, P<0.0001). Forty-eight percent of anomalies occurred during the bottom phase of the dive, which was greater than predicted (32%) (Fig. 6B; goodness-of-fit χ^2=426.2, d.f.=2, P<0.0001). Most anomalies occurred at flipper stroke rate frequencies between 0.20 and 0.39 Hz (44%), which was more than predicted based on the amount of time they exhibited flipper stroke rates within this range (33%). There were significantly fewer anomalies at flipper stroke rates greater than 0.4 Hz (24%) than predicted (37%) (Fig. 6C; goodness-of-fit χ^2=295.3, d.f.=4, P<0.0001).

DISCUSSION

Dive duration, depth and activity influence f_H

One of the knowledge gaps in the diving physiology of marine mammals is the importance of muscle perfusion during natural dives (Williams et al., 2018). Early studies suggested that blood flow was significantly reduced, resulting in animals relying on muscle oxygen stores and anaerobic metabolism (Scholander et al., 1942; Scholander, 1940). More recently, it has been proposed that the dive response, and resulting blood flow, are modulated by exercise, depth and expectations for the upcoming dive (i.e. projected maximum depth or duration) (Davis and Williams, 2012; Elmegaard et al., 2016; McDonald et al., 2017; Williams et al., 2015). Although this is difficult to measure directly, the role of muscle perfusion during diving is often evaluated by examining the relationship between f_H and activity (Davis and Williams, 2012; Williams et al., 2015). We found support for our hypothesis that there is a positive relationship between activity and f_H during diving, suggesting possible changes in muscle perfusion associated with exercise. f_H was lowest in the long, deep dives and higher in more active dives (irrespective of depth); however, the impact of activity varied with dive duration, as indicated by the significant interaction term, which suggests that conservation of blood oxygen takes priority over blood flow to muscles (Table 1, Fig. 3). Together, dive duration and activity accounted for 40% of the variation in f_H.

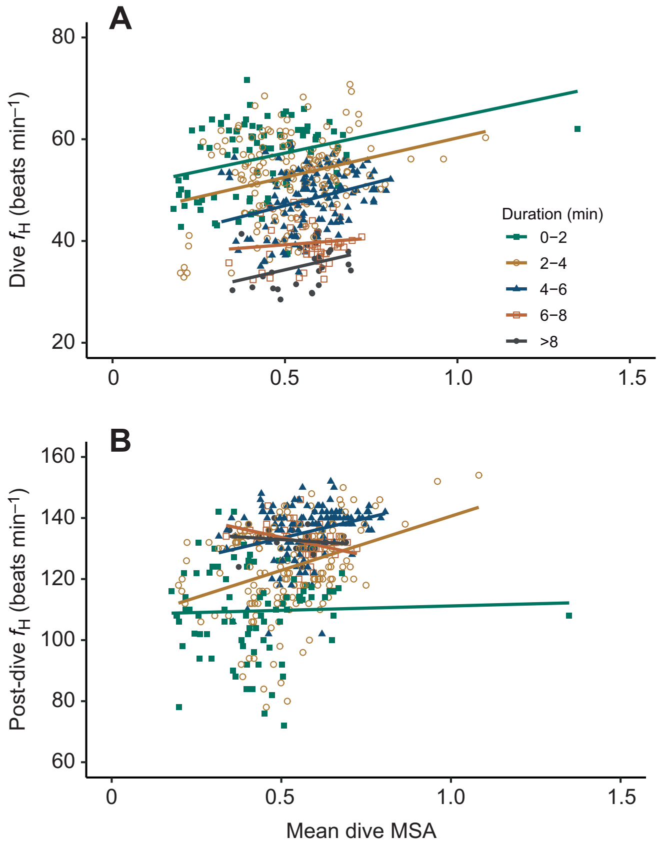

Fig. 3. Dive duration and activity (MSA) influence dive f_H, while neither influence post-dive f_H. (A) Dive f_H and (B) post-dive f_H. Dive f_H decreases with increasing dive duration. f_H increased with increased activity when dive duration was controlled for; however, the relationship between activity and f_H varied with duration (significant interaction term). To facilitate visualization of this, dives are grouped into dive duration categories. Data represent 452 dives from 5 sea lions.

To further evaluate the relationship between f_H and activity, we qualitatively and quantitatively examined the influence of depth (and relative lung volume) and MSA on f_H in 10 s intervals throughout the dives (Figs 2 and 4). During 1–2 min shallow dives, the f_H profile paralleled the MSA profile (Fig. 2), suggesting that exercise intensity may affect f_H during shallow dives. However, it should be noted that hemoglobin saturation in the anterior vena cava (which drains the active locomotory pectoral muscles) often increased during many of these shallow dives (Tift et al., 2018). Consequently, although f_H may increase with MSA during shallow dives, the blood flow pattern and delivery of blood oxygen to exercising muscle during shallow dives is still unclear.

During longer deep dives, the f_H response became more independent of MSA (Fig. 2). When statistically controlling for depth, there was a positive relationship between activity and f_H, but the relationship was influenced by depth (Table 1, Fig. 4). At depths greater than 300 m, the increase in f_H associated with increased activity was significantly less. For example, at depths between 200 and 300 m, an increase in MSA of 1 m s^{-2} resulted in an increase in f_H of ~22 beats min^{-1}; however, the same increase in MSA at depths greater than 300 m only resulted in an increase of ~6 beats min^{-1} (Fig. 4). This further suggests that during long, deep dives, blood oxygen conservation is prioritized over muscle perfusion. These results are similar to f_H measurements of diving trained Steller sea lions, in which f_H correlated with overall dynamic body acceleration (ODBA, an activity index) during shallow dives, but not during deeper dives (Hindle et al., 2010). The occurrence of lower f_H at greater depths for a given stroke rate has also been documented in cetaceans (Williams et al., 2015).

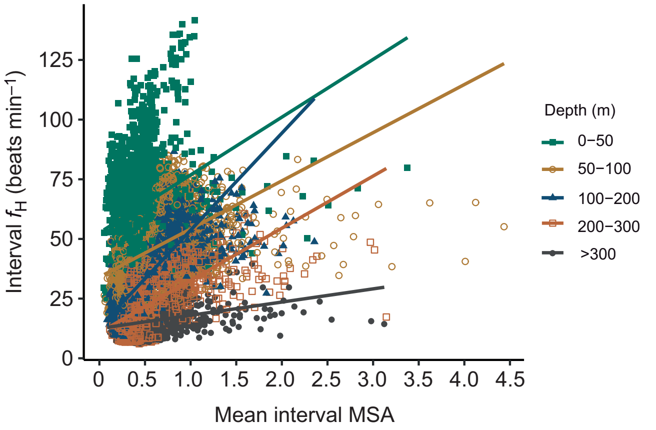

Fig. 4. Activity (MSA) and dive depth influence dive interval (10 s) f_H. f_H is lower at deeper depths; intervals with increased activity have higher f_H, but the relationship between activity and f_H is weaker at depths deeper than 300 m. Intervals with mean depth <10 m were excluded to avoid including surface f_H. The elevated f_H values at low MSA and shallow depths are from the 10–20 s prior to surfacing. Least-square lines have been added to visualize trends. Data represent 9851 intervals from 5 sea lions.

In addition to the above quantitative analyses, qualitative evaluation of the f_H and activity profiles (i.e. Figs 1 and 2) revealed that exercise and f_H were not always linked. For example, at the beginning of descent when f_H and flipper stroke effort were both high, f_H was declining from elevated pre-dive levels. In addition, during early descent, hemoglobin saturation is often greater than 90% in the anterior vena cava, the vein that drains the pectoral and shoulder muscles that power the fore-flipper (Tift et al., 2018). Such high venous hemoglobin saturations are not consistent with muscle blood flow and increased extraction of blood oxygen by exercising muscle. Thus, it is unlikely that the high f_H at the start of a dive is due to the onset of exercise, but rather it is a result of the surface tachycardia proceeding the dive.

Although f_H profiles diverged from MSA profiles in deeper dives, f_H gradually increased during ascent, a time at which there is active flipper stroking. Some muscle blood flow and delivery of blood oxygen to muscle probably occurs during this gradual rise in f_H during ascent. In every case in which muscle blood flow has been examined in seals, even small elevations in f_H are accompanied by small increases in muscle blood flow (Grinnell et al., 1942; Jobsis et al., 2001; Ponganis et al., 2008, 2006). The magnitude of blood oxygen delivery relative to muscle oxygen consumption is unknown but is probably small because the small increase in cardiac output due to a small elevation in f_H during a dive will be accompanied by a small but also widespread decrease in sympathetic vasoconstriction with blood redistribution to multiple tissues, not just muscle (Elsner et al., 1966). Notably, the previously observed changes in anterior and posterior vena caval hemoglobin saturation during ascent support the concept of such increased widespread peripheral blood flow (McDonald and Ponganis, 2013; Tift et al., 2017, 2018).

f_H and relative lung volume during dives

The general contour of the f_H profile resembled the shape of the relative lung volume profile during shallow and deep dives (Fig. 1). This similarity was most evident during descent (especially during

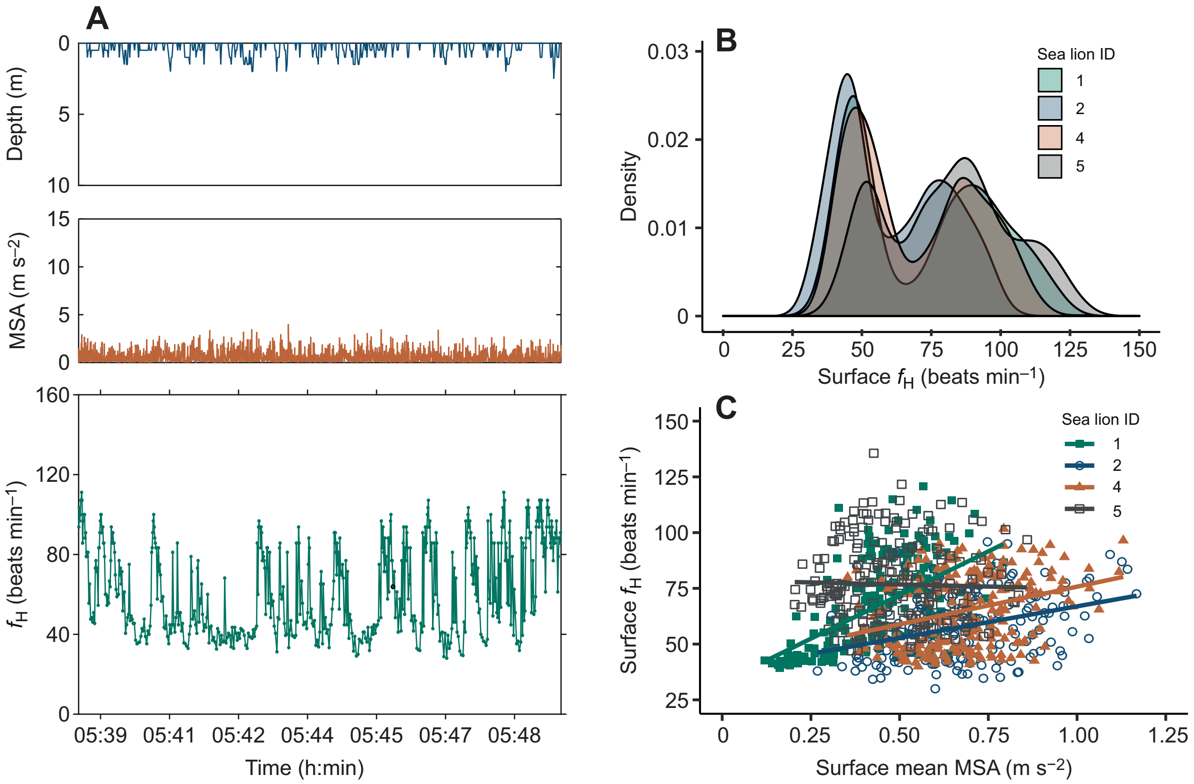

Fig. 5. Depth, MSA and f_H profiles from a surface swimming California sea lion. (A) Example of a 10 min segment of surface swimming. (B) All sea lions had a bimodal distribution with a peak in f_H between 40 and 50 beats min^{-1} and another between 75 and 90 beats min^{-1}. (C) Relationship between mean MSA and surface f_H for 10 s intervals (831 intervals from 4 sea lions). There was a positive relationship between MSA and f_H at the surface, although the best model indicated that the relationship differed between the sea lions and only explained 10% of the variation.

the prolonged glides of deep dives) and during the rapid increase in f_H during final ascent (usually in the final 10 m of the ascent). These findings suggest that the activity of pulmonary stretch receptors contributes to changes in f_H during dives. The relative lung volumes, calculated from depth profiles via Boyle's law, are the change in a gas volume secondary to a change in pressure. Actual lung volumes during a dive are a function lung volume (both dead space and alveolar volume), compliance of airways, alveoli and the chest wall, and the depth profile of a dive (Fahlman et al., 2017). Such data from a living animal are not available for sea lions. Consequently, we have used our relative lung volume calculations as a simple, first index of lung volume to emphasize the potential role of pulmonary stretch receptors in f_H regulation during dives. Furthermore, because pulmonary stretch receptors are associated with airway smooth muscle (Widdicombe, 2001), activation of such receptors may be dependent on volume changes in a particular segment of the tracheo-bronchial tree.

The pulmonary stretch reflex is probably only one of many factors that contribute to the regulation of f_H during dives. Multiple reflexes in the dive response and volitional control cannot be ruled out, and, indeed, may be the primary factors involved in regulation of the dive response (Kaczmarek et al., 2018; Panneton, 2013; Ridgway et al., 1975). After all, sea lions decrease f_H to 20–30 beats min^{-1} during face submersion on land (Kaczmarek et al., 2018), and can decrease their f_H to 10 beats min^{-1} on command in air (Ridgway et al., 1975).

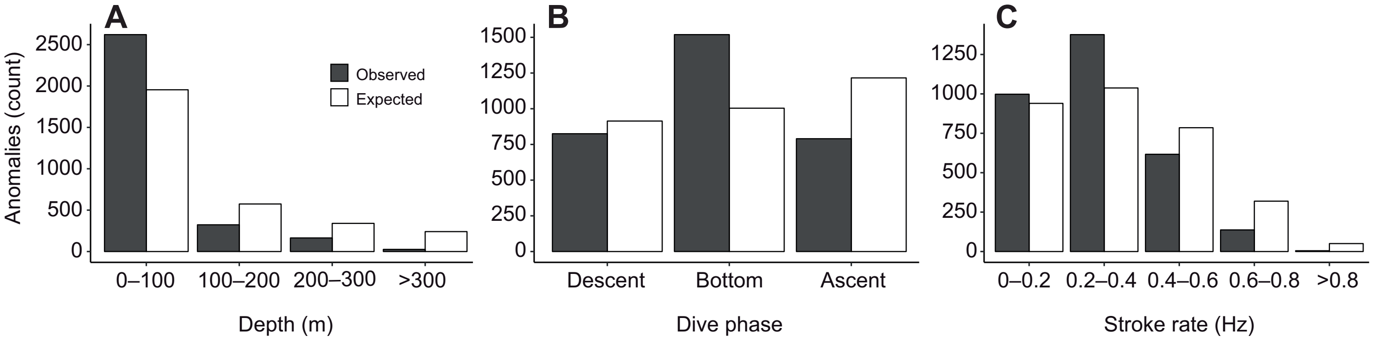

Fig. 6. The distribution of large beat-to-beat fluctuations in f_H of diving sea lions. Fluctuations were defined by a change of ≥10 beats min^{-1} in f_H followed by a ≥30% change in f_H in the opposite direction. Anomalies occurred more at depths <100 m (χ^2=611.0, d.f.=3, P<0.0001), during the bottom phase of the dive (χ^2=2, d.f.=426.22, P<0.0001), and flipper stroke rates between 0.2 and 0.4 Hz (χ^2=295.3, d.f.=4, P<0.0001). Data represent 3134 anomalies from 5 sea lions.

During ascent from deep dives, the deviation of the f_{H} profile from relative lung volume profile may be associated with flipper stroke effort. However, as demonstrated in Figs 1 and 2, the increase in f_{H} and the fluctuations in MSA during ascent were not parallel. During the latter half of ascent, f_{H} increased while MSA decreased (Fig. 1).

The instantaneous f_{H} profiles have several other notable features in relation to relative lung volume (Fig. 1). First, large beat-to-beat fluctuations in f_{H} can occur without simultaneous large changes in relative lung volume (and often in the absence of large changes in locomotory effort). Second, f_{H} increases at the start of ascent, irrespective of stroke activity, depth of dive or prior changes in f_{H}. This often subtle increase appears instantaneously with the start of ascent and occurs before any significant change in relative lung volume. These observations suggest a component of cognitive control over f_{H}. Lastly, there did not appear to be any characteristic changes in the f_{H} profile that were associated with boundaries of the estimated zone of 'lung collapse' (depths below which absence of significant gas exchange is assumed Fig. 1D).

Autonomic regulation of f_{H} during surface swimming versus diving

f_{H} profiles and the relationship of f_{H} to activity in the sea lion differed between surface swimming and diving (Fig. 1 versus Fig. 5). During surface swimming, instantaneous f_{H} typically oscillated between 50 and 100 beats min^{-1}, likely associated with alternating periods of breathing and shallow swimming (Fig. 5). The lower surface swimming f_{H} (~50–60 beats min^{-1}) is similar to what is observed during the bottom phase of 1–2 min dives and the on-land resting f_{H} (54 beats min^{-1}) (McDonald and Ponganis, 2014). These surface f_{H} are also similar to the surface and submerged f_{H} of young sea lions swimming in a flume (Williams et al., 1991). Similar to results of dive analyses, there was a positive relationship between surface interval MSA and surface interval f_{H}; however, the relationship only explained 10% of the variation in surface f_{H} (Table 1, Fig. 5B). Other factors that we could not measure, such as respiration rate, likely mask a stronger relationship. The regulation of f_{H} in sea lions during surface swimming probably reflects a typical mammalian exercise response with an increase in f_{H} secondary to parasympathetic (vagus nerve) withdrawal at low exercise intensity, and, additionally, to sympathetic activation (cardiac accelerator fibers) at higher workloads (Michael et al., 2017; Ponganis et al., 2017). In contrast, during the surface intervals following dives reaching depths between 50 and 300 m, f_{H} was much higher, typically 130–150 beats min^{-1}, even though the sea lions exhibited similar surface MSA after both shallow and deep dives. The higher f_{H} during surface intervals suggests maximal vagal withdrawal and high sympathetic cardiac accelerator fiber activity, especially immediately prior to and after deep dives. Such regulation of f_{H} during the surface interval is consistent with autonomic blockade studies (Elliott et al., 2002; Ponganis et al., 2017). Both sea lions and emperor penguins (*Aptenodytes forsteri*) have higher f_{H} before deep dives, suggesting maximal vagal withdrawal and greater sympathetic activation prior to deeper dives (McDonald and Ponganis, 2014; Wright et al., 2014). Such high f_{H} during the surface interval allows for respiratory gas exchange and tissue re-perfusion, resulting in off-loading of carbon dioxide and restoration of oxygen stores.

In contrast to surface swimming and inter-dive surface intervals, f_{H} during dives declined, often to levels well below resting f_{H} (Fig. 1). The dive response, secondary to activation of the sympathetic and parasympathetic nervous systems, results in a slowing of the heart (bradycardia) via the vagus nerve and peripheral vasoconstriction that regulates blood flow distribution and maintains blood pressure (Blix and Folkow, 1983; Butler and Jones, 1997). It appears that the vagus nerve dominates over any activity of the sympathetic cardiac accelerator fibers to produce the bradycardia, and that any increase in f_{H} due to exercise during a dive is secondary to vagal withdrawal in the presence of increased sympathetic tone (Ponganis et al., 2017). We also expect that any effects due to relative lung volume changes would be mediated by vagal withdrawal (increased f_{H}) during lung expansion and vagal activation (decreased f_{H}) during lung compression (Angell-James et al., 1981).

After a review of the literature and our previously published f_{H} data in sea lions, we postulated that large beat-to-beat fluctuations in f_{H} were common in sea lions, and that these benign fluctuations were primarily due to adjustments in parasympathetic tone, secondary to changes in flipper stroke effort, relative lung volume and/or volitional control (Ponganis et al., 2017). At low exercise intensities, changes in f_{H} are primarily controlled by adjustments in parasympathetic tone (Carter et al., 2003; Michael et al., 2017). In sea lions, most f_{H} anomalies were at low flipper stroke frequencies (0.2–0.4 Hz), and were less than predicted at flipper stroke frequencies above 0.4 Hz. In addition, most f_{H} anomalies occurred at depths less than 100 m when relative lung volume changes the most. Consequently, although there may be some adjustments in vagal tone at these low exercise intensities in sea lions, we suspect that changes in relative lung volume contributed most to such fluctuations in f_{H} due to adjustments in parasympathetic activity via pulmonary stretch receptor reflexes. Although it has been suggested that cardiac arrhythmias in seals may result from autonomic conflict between a maximally stimulated parasympathetic (dive) response and a maximally stimulated sympathetic (exercise) response (Williams et al., 2015), we did not find evidence that maximum flipper stroke effort (and a presumed maximum exercise sympathetic response) was associated with these f_{H} anomalies. Such f_{H} anomalies also occur in several odontocete species even when the animals are at rest or stationary underwater (Bickett et al., 2019). It is unlikely that autonomic conflict between an exercise response and dive response occurred in those situations.

Conclusions

We found support for our first hypothesis that exercise influences diving f_{H}, but the relationship was weak in long, deep dives, consistent with prioritization of blood oxygen conservation over oxygen delivery to working muscles. This dissociation of f_{H} from activity in deeper dives might be secondary to the effects of depth on the f_{H} response and/or to volitional control. We also found support for our second hypothesis, that pulmonary stretch receptor reflexes contribute to f_{H} regulation as f_{H} profiles generally paralleled changes in relative lung volume during early descent and late ascent. Lastly, our data supported the hypothesis that f_{H} anomalies are associated with changes in depth/relative lung volume, but provided no evidence for the hypothesis that anomalies are associated with intense activity. These findings support the concept that exercise, pulmonary stretch receptor reflexes and anticipation influence the dive response.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: B.I.M., P.J.P.; Methodology: B.I.M., P.J.P.; Formal analysis: B.I.M., M.S.T., L.A.H., M.J.; Investigation: B.I.M., M.S.T., P.J.P.; Resources: B.I.M., P.J.P.; Data curation: B.I.M.; Writing - original draft: B.I.M., P.J.P.; Writing - review & editing: B.I.M., M.S.T., L.A.H., P.J.P.; Visualization: B.I.M.; Supervision: B.I.M., P.J.P.; Project administration: B.I.M., P.J.P.; Funding acquisition: B.I.M., P.J.P.

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Data availability

Data are available from the figshare digital repository: https://doi.org/10.6084/m9.figshare.11539197.v1

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