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RESEARCH ARTICLE

Respiratory sinus arrhythmia and submersion bradycardia in bottlenose dolphins (*Tursiops truncatus*)

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ABSTRACT

Among the many factors that influence the cardiovascular adjustments of marine mammals is the act of respiration at the surface, which facilitates rapid gas exchange and tissue re-perfusion between dives. We measured heart rate (f_H) in six adult male bottlenose dolphins (*Tursiops truncatus*) spontaneously breathing at the surface to quantify the relationship between respiration and f_H, and compared this with f_H during submerged breath-holds. We found that dolphins exhibit a pronounced respiratory sinus arrhythmia (RSA) during surface breathing, resulting in a rapid increase in f_H after a breath followed by a gradual decrease over the following 15–20 s to a steady f_H that is maintained until the following breath. RSA resulted in a maximum instantaneous f_H (if_H) of 87.4±13.6 beats min^{-1} and a minimum if_H of 56.8±14.8 beats min^{-1}, and the degree of RSA was positively correlated with the inter-breath interval (IBI). The minimum if_H during 2 min submerged breath-holds where dolphins exhibited submersion bradycardia (36.4±9.0 beats min^{-1}) was lower than the minimum if_H observed during an average IBI; however, during IBIs longer than 30 s, the minimum if_H (38.7±10.6 beats min^{-1}) was not significantly different from that during 2 min breath-holds. These results demonstrate that the f_H patterns observed during submerged breath-holds are similar to those resulting from RSA during an extended IBI. Here, we highlight the importance of RSA in influencing f_H variability and emphasize the need to understand its relationship to submersion bradycardia.

KEY WORDS: Cardiorespiratory coupling, Heart rate, Marine mammals

INTRODUCTION

Bradycardia, or the reduction in heart rate (f_H) compared with resting f_H, is considered a central component of the dive response which enables marine mammals to perform extended breath-holds underwater. By reducing cardiac output, bradycardia, in concert with peripheral vasoconstriction, limits the overall rate of O_2 depletion by the peripheral tissues and helps to conserve O_2 in the blood for hypoxia-intolerant tissues like the brain and heart (Scholander, 1940, 1963; Irving, 1939). In contrast, increased f_H and blood flow facilitate rapid gas exchange and reduce the time needed for recovery at the surface (Fedak et al., 1988; Le Boeuf et al., 2000). These differing cardiovascular and respiratory requirements underpin the need for fine-scale modulation of f_H in marine mammals to enable the management of differing physiological demands while diving and at the surface. As technological advancements increase the ease of f_H measurements in freely moving animals, an increasing number of studies have sought to examine f_H modulation in marine mammals across a range of behavioral and environmental conditions (Block, 2005; Ponganis, 2007; Wilson et al., 2015). It is becoming clear that the suite of physiological adaptations that support an increased capacity for diving in marine mammals (i.e. dive response) is complex and regulated by many factors both during submersion and at the surface during dive preparation and recovery (Bickett et al., 2019; Elmegaard et al., 2016; Fahlman et al., 2020b, 2019; Kaczmarek et al., 2018; Noren et al., 2012).

Given the importance of f_H in regulating hemodynamic changes during diving, many researchers have investigated factors that influence f_H in marine mammals including water temperature, area of facial submersion, dive depth, activity, voluntary control, age and nutritional state. In harbor seals (*Phoca vitulina*) and California sea lions (*Zalophus californianus*), bradycardia has been shown to occur without submersion in water, but colder water and an increased area of facial submersion can increase the degree of f_H reduction (Kaczmarek et al., 2018). Change in the relative lung volume of diving California sea lions was found to follow a similar shape to that of change in f_H, particularly during descent and late ascent, suggesting a potential role of pulmonary stretch receptors in determining diving f_H (McDonald et al., 2020; Ponganis et al., 2017). In bottlenose dolphins, lung compression and expansion associated with pressure changes during a dive did influence changes in diving f_H (Williams et al., 2015b). Studies in bottlenose dolphins, Weddell seals (*Leptonychotes weddellii*) and narwhals (*Monodon monoceros*) have hypothesized that f_H is elevated during activity associated with diving and may result in sympathetic and parasympathetic conflict, causing cardiac arrhythmias (Davis and Williams, 2012; Williams et al., 2015b). In contrast, others have concluded that when sympathetic and parasympathetic stimulation are highest, f_H variability is minimal and that arrhythmias due to autonomic adjustment are benign (Fahlman et al., 2020a; Ponganis et al., 2017). Conditioned cognitive adjustment of f_H has also been shown to play a role in f_H modulation as harbor porpoises (*Phocoena phocoena*), bottlenose dolphins and California sea lions trained to perform dives of different durations differentially adjusted f_H proportionally with dive duration (Elmegaard et al., 2016; Fahlman et al., 2020a, 2019; McDonald et al., 2018; Ridgway et al., 1975). Studies in bottlenose dolphins indicate that a dolphin's ability to exhibit bradycardia increases with age, such that mean diving f_H decreases with increasing age class (Noren et al., 2004). Similarly, in northern elephant seal pups (*Mirounga angustirostris*) and harbor seal pups performing apneas on land, mean f_H decreased with increasing age (Andrews et al., 1997; Castellini et al., 1994b; Hicks et al., 2004). In spite of these advancements in understanding the

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factors that influence diving f_H, little attention has been paid to the factors that influence surface f_H, despite its importance for dive recovery and preparation, and the importance of designating resting f_H to estimate the magnitude of the dive response. One aspect of surface f_H control that warrants further investigation is cardiorespiratory coupling through respiratory sinus arrhythmia (RSA) which drives respiration-associated changes in f_H (Andrews et al., 1997; Castellini et al. 1994a,b; Cauture et al., 2019; Fahlman et al., 2020b, 2019; Lin et al., 1972).

In terrestrial mammals, RSA is known to result in an increase in instantaneous f_H (if_H) during inspiration and a decrease during expiration (Hirsch and Bishop, 1981). Despite differences in breathing strategies between terrestrial and marine mammals, RSA has also been observed and recorded in several marine mammal species, including the gray whale (*Eschrichtius robustus*), killer whale (*Orcinus orca*), short-finned pilot whale (*Globicephala macrorhynchus*), beluga (*Delphinapterus leucas*), common dolphin (*Delphinus delphis*), bottlenose dolphin, harbor porpoise, northern elephant seal, hooded seal (*Cystophora cristata*), California sea lion and fur seal (*Callorhinus ursinus*) (Andrews et al., 1997; Castellini et al., 1994a,b; Cauture et al., 2019; Elsner et al., 1966; Fahlman et al., 2020a, 2019; Hamlin et al., 1972; Irving et al., 1963; Kanwisher and Ridgway, 1983; Kastelein and Meijler, 1989; Lin et al., 1972; Lyamin et al., 2016; McDonald and Ponganis, 2014; Påsche and Krog, 1980; Ponganis and Kooyman, 1999; Ridgway, 1986, 1972). Results from studies in northern elephant seals, hooded seals, California sea lions and bottlenose dolphins have shown that during the inter-breath interval (IBI), RSA results in dramatic changes in if_H, with high if_H values exhibited at the beginning of an IBI followed by a continuous decrease towards a low, stable if_H at the end of an IBI (Andrews et al., 1997; Castellini et al., 1994a; Cauture et al., 2019; Fahlman et al., 2020b, 2019; Lin et al., 1972; Påsche and Krog, 1980). Interestingly, in the California sea lion, the minimum f_H observed between breaths on land was comparable to the minimum f_H observed during dives (Lin et al., 1972). Additionally, previous studies in bottlenose dolphins suggested that, after the RSA was accounted for, the base f_H of dolphins breathing at the surface was similar to reported dolphin f_H values during diving bradycardia (Cauture et al., 2019; Fahlman et al., 2020a). Hooded seals performing submerged apneas demonstrated similar f_H to the minimum if_H measured during periods of breathing on land (Påsche and Krog, 1980). In emperor penguins (*Aptenodytes forsteri*) and California sea lions, the minimum if_H associated with RSA was comparable to the minimum if_H seen in dives shorter than the aerobic dive limit (ADL), suggesting that the same mechanism of cardiorespiratory control governs these patterns (McDonald and Ponganis, 2014; Meir et al., 2008). In cetaceans, however, because of the challenge of measuring cardiorespiratory parameters in fully aquatic animals, studies investigating cardiorespiratory coupling and its relationship to surface and diving f_H are limited (Bickett et al., 2019; Cauture et al., 2019; Fahlman et al., 2020a,b, 2019).

We examined differences in patterns of f_H modulation between surface breathing and submerged breath-holds to investigate how RSA influences surface f_H during rest and the relationship between RSA at the surface and changes in f_H observed during short submersions in bottlenose dolphins. We hypothesized that (1) IBI would influence the degree of RSA we observed and that RSA should increase with longer IBIs, as has been shown in humans (Hirsch and Bishop, 1981), (2) that the minimum if_H of the RSA would decrease with increasing IBI as the effect of lung inflation is diminished (Angell-James et al., 1981), and (3) that, as has been suggested in seals and manatees, the minimum if_H measured during long IBIs at the surface would be comparable to the minimum f_H during submerged breath-holds (Castellini et al., 1994a; Castellini and Zenteno-Savin, 1997; Gallivan et al., 1986). Additionally, we examined differences in RSA under fasted and non-fasted, resting conditions as we hypothesized that the increased metabolic cost of digestion under non-fasted conditions would result in increased resting surface f_H and reduced RSA compared with fasting conditions, but that rates measured under fasted conditions should reflect resting metabolic demands. Finally, we discuss the potential significance of similarities between f_H observed during long IBIs at the surface and during submerged breath-holds for our contemporary understanding of the dive response.

MATERIALS AND METHODS

All experiments were done with voluntary participation from the dolphins and an individual could end a trial at any point. The study protocols were accepted at Dolphin Quest Oahu, and also by the Animal Care and Welfare Committee at the Oceanogràfic (OCE-17-16, amendments OCE-29-18 and OCE-3-19i) and the Institutional Animal Care and Use Committee of Duke University (A045-17-02).

Animals

Six adult male, bottlenose dolphins, *Tursiops truncatus* (Montagu 1821), housed at Dolphin Quest Oahu (Honolulu, HI, USA) with an average age of 23.2±7.0 years (range 11–31 years) and body mass 189.3±36.3 kg (range 147.0–251.7 kg) participated in the study. Prior to the start of the study, all dolphins were desensitized to the research equipment used. Research trials consisted of stationary 10 min resting trials at the surface under fasted or non-fasted conditions, as well as 2 min breath-hold trials under non-fasted conditions. Fasted trials were only conducted in the morning with approximately 15 h having passed since the last meal on the previous day. All trials were preceded by 2 min of low-activity behavior during which the animal was either resting next to the trainer or swimming slowly.

To prepare for a research trial, the dolphin turned ventral side up to allow placement of the electrocardiogram (ECG) electrodes (Cauture et al., 2019; Fahlman et al., 2020b). Following placement of the ECG electrodes, the animal rolled back, dorsal side up. The trial continued if the ECG signal was visible, otherwise the electrodes were taken off, reattached, and the procedure repeated. Next, the pneumotachometer, a device used to measure changes in airflow, was placed over the blowhole and the trial began. For the 2 min breath-hold trials, the animal was asked to roll over onto its side with its blowhole fully submerged immediately following a breath, as previously detailed (Fahlman et al., 2019). The dolphin remained in this position until the end of the pre-determined breath-hold duration, at which time the animal was asked to roll back dorsal-side up to end the breath-hold and reinitiate spontaneous breathing. All experiments were conducted during a 2 week period in May 2019.

Respiration

A Fleisch type pneumotachometer (Mellow Design, Valencia, Spain) was used to measure breath-by-breath exhaled and inhaled respiratory flow as previously detailed (Fahlman et al., 2015). The flow signal was used to determine the beginning and end of a respiration, and the breathing frequency (f_R) and IBI were determined from the duration between breaths. Further analysis of the respiratory parameters was not performed for this study.

Heart rate

f_H was determined using a three-lead ECG data recording system. The ECG leads were connected to gold-plated electrodes (Disposable

GoldSelect Cup Electrodes, DE-003710, Rochester Med, LifeSync Neuro, Coral Springs, FL, USA) mounted inside custom-made silicone suction cups (Smooth-Sil 940, Smooth-On, Inc., Macungie, PA, USA) connected to a custom-built bio-amplifier (UUB/1-ECGb, UFI, Morro Bay, CA, USA) and with a BNC connector to the data acquisition system (Powerlabs 8/35, ADInstruments, Colorado Springs, CO, USA). The electrodes were placed on the ventral side of the animal with one electrode placed inside the top edge of the left and right pectoral fin, respectively, and the third electrode placed on the left side, 15 cm caudal to the upper left electrode. Each suction cup was filled with conductive paste (Ten20 Conductive Paste, Weaver and Company, Aurora, CO, USA) before being placed on the skin.

Data acquisition and processing

The respiratory flow and ECG were recorded at 400 Hz by a data acquisition system (Powerlabs 8/35, ADInstruments), and displayed in real-time by a laptop computer running LabChart (v.8.1, ADInstruments). The if_H was extracted from the ECG signal using the ECG Analysis Module in LabChart. All data were analyzed using MATLAB (version 2018b, ©2018 The MathWorks, Inc.). For each IBI, the RSA was estimated using two different methods. The percent RSA [RSA (%)] was calculated as the difference between the peak and the trough of the if_H signal in beats min^{-1}, normalized by the mean if_H ($i\bar{f}_H$) for that section: RSA (%)=$\Delta if_H/i\bar{f}_H$ (Mortola et al., 2016). RSA was also calculated using the peak-to-trough method [RSA (s)] (Lewis et al., 2012). For the calculation of averaged if_H responses for surface trials, f_H was resampled at 1 s intervals and averaged at every second for the duration of the 90th percentile IBI using the function *resample*.

Statistics

All statistical analyses were performed using R (v.3.6.2) (http://www.R-project.org/). Differences in mean if_H, maximum if_H, minimum if_H, RSA (%) and RSA (s) between fasted and non-fasted surface trials during short (IBI<10 s), intermediate (10 s≤IBI≤30 s) and long IBIs (IBI>30 s) were determined using a mixed effects ANOVA, followed by a *post hoc* Tukey test. Differences between long surface IBIs (IBI>30 s) and submerged breath-hold trials were determined using a mixed effects ANOVA. If there was no significant difference in a f_H parameter during fasted and non-fasted IBIs, those data were combined to compare with the breath-holds. Linear mixed effect models, using the package *nlme*, were used to determine the relationships between IBI and f_H and RSA measurements with animal ID as a random effect. All variables were $\log_{10}$-transformed prior to model fit. Differences in intercepts (B_0) and slopes (B_1) between fasted and non-fasted surface trials were evaluated as terms within the linear mixed effect model and if there was no significant difference in either parameter, the fasted and non-fasted data were combined and are described by a single model. Linear mixed effect models were also used to evaluate the relationship between the duration of the previous IBI and the maximum if_H of the IBI as well as the relationship between the duration of the previous IBI and the difference between the maximum if_H of the IBI and the minimum if_H of the previous IBI. The package *r2glmm* was used to determine r^2 values for each model. All statistical tests were done assuming that $P<0.05$ indicated a significant difference. Values are presented as means±s.d. unless stated otherwise.

RESULTS

A total of 22 trials were conducted with six male bottlenose dolphins (Table S1). These were composed of 8 fasted (n=241 breaths) and 7 non-fasted (n=246 breaths) surface trials, during which the blow-hole was above the water's surface and the dolphin could breathe spontaneously. Another 7 non-fasted breath-hold (n=7 breaths) trials were conducted with the dolphin rolled ventrally and the blow-hole submerged.

Fasted versus non-fasted surface breathing f_H and RSA

Mean f_H for fasted trials was 67.2±11.8 beats min^{-1} and mean f_H for non-fasted trials was 78.0±13.1 beats min^{-1} (Welch two-sample *t*-test, t=−9.5, P<0.0001). RSA was observed following respiration as an increase in the if_H immediately following the breath followed by a decrease in if_H until the next breath. There was large variation in the IBI within and between dolphins (Table S1). The mean IBI during fasted trials was 19.7±14.3 s as compared with 14.6±8.0 s during non-fasted trials. For all surface trials, fasted and non-fasted, maximum if_H was 87.4±13.6 beats min^{-1} and minimum if_H was 56.8±14.8 beats min^{-1} (range: 124.4–23.9 beats min^{-1}). Summary statistics for f_H, RSA and body mass (M_b) for all animals are displayed in Tables S1 and S2. The mean f_H during fasted surface trials (67.2±11.8 beats min^{-1}) closely agreed with the allometric prediction of 64.2 beats min^{-1} for the average body mass of the animals that participated in the fasted trials, whereas the non-fasted mean f_H (78.0±13.1 beats min^{-1}) was elevated as compared with the allometric prediction (Table S1) (Stahl, 1966).

We examined differences in both f_H and estimated indices of RSA during fasted and non-fasted surface trials binned by IBI (Table 1). Mean if_H decreased with increasing IBI across the three IBI categories (Table 2, Fig. 1) and mean if_H was lower during fasted trials as compared with non-fasted trials for short and intermediate IBIs, but not for long IBIs (Fig. 1A,B, Table 1). Minimum if_H also decreased with increasing IBI (Table 2) and, like mean if_H, was lower for short and intermediate IBIs during fasted trials than during non-fasted trials, but this difference was absent for long IBIs (Fig. 1C,D, Table 1). Notably, however, the regression describing the relationships between IBI and mean if_H as well as IBI and minimum if_H did not differ between fasted and non-fasted trials (mean if_H: B_0, P=0.97; B_1, P=0.22, minimum if_H: B_0, P=0.45; B_1, P=0.06) (Fig. 1B,D, Table 2).

Table 1. Inter-breath interval (IBI), heart rate (f_H) and respiratory sinus arrhythmia (RSA) statistics for fasted and non-fasted surface trials binned by IBI

IBI (s)	Fasted state	IBI (s)	Mean if_H (beats min^{-1})	Maximum if_H (beats min^{-1})	Minimum if_H (beats min^{-1})	RSA (%)	RSA (s)
IBI<10 s	Fasted (n=53)	7.9±1.7^{a}	74.5±9.7^{c}	83.2±10.6^{a,b}	63.8±11.4^{c,d}	26.6±13.7^{a}	0.25±0.19^{a,b}
	Non-fasted (n=80)	7.3±2.1^{a}	81.5±12.3^{d}	91.1±13.6^{b}	67.1±13.9^{d}	29.8±15.6^{a}	0.26±0.19^{a}
10 s≤IBI≤30 s	Fasted (n=152)	17.2±4.7^{b}	67.6±10.3^{b}	81.6±11.6^{a}	52.7±12.1^{b}	44.0±18.6^{b}	0.46±0.26^{c}
	Non-fasted (n=152)	16.4±5.0^{b}	77.3±13.0^{c}	93.3±13.9^{b}	58.9±13.3^{c}	45.5±15.8^{b}	0.42±0.20^{b}
IBI>30 s	Fasted (n=36)	47.9±15.7^{d}	55.2±11.6^{a}	84.3±13.8^{a,b}	35.7±9.8^{a}	91.1±23.8^{d}	1.1±0.34^{d}
	Non-fasted (n=14)	36.0±4.5^{c}	65.0±9.3^{a,b}	89.7±10.4^{a,b}	46.5±8.5^{a,b}	67.8±23.2^{c}	0.66±0.26^{c}

IBI category, trial type and an interaction term were the fixed effects and animal ID was the random effect in a mixed effects ANOVA followed by a *post hoc* Tukey test. Different lowercase letters indicate significant differences (*post hoc* tests).

Table 2. Linear mixed effect model parameters for relationships between $\log_{10}$-transformed IBI and $\log_{10}$-transformed f_H during fasted and non-fasted surface trials

	B_0±s.e.	P	B_1±s.e.	P	r^2
Mean if_H (beats min^{-1})	2.01±0.03	<0.0001	−0.12±0.01	<0.0001	0.25
Minimum if_H (beats min^{-1})	2.01±0.04	<0.0001	−0.23±0.01	<0.0001	0.38
Maximum if_H (beats min^{-1})	1.95±0.03	<0.0001	0.003±0.01	0.77	0.01
RSA (%)	0.95±0.06	<0.0001	0.56±0.02	<0.0001	0.53
RSA (s)	−1.22±0.08	<0.0001	0.67±0.03	<0.0001	0.49

Regressions are in the form $\log_{10}(Y)=B_0+B_1\cdot\log_{10}(X)$, where IBI is the independent variable. Animal ID was included as a random variable.

The regression analysis showed that maximum if_H did not vary significantly with IBI (Table 2) and pair-wise comparison across IBI categories revealed a significant difference in maximum if_H between fasted and non-fasted trials only for intermediate IBIs (Table 1). There was no significant difference in the slopes or in the exponents of the regression of IBI and maximum if_H for fasted as compared with non-fasted trials (B_0, P=0.18; B_1, P=0.68) and therefore they are described by a single equation (Table 2). There was a significant negative relationship between the duration of the previous IBI and the maximum if_H of the IBI, and a significant positive relationship between the duration of the previous IBI and the difference between the maximum if_H of the IBI and the minimum if_H of the previous IBI, a potential proxy for the effect of lung inflation (Table S3, Fig. S1). Both metrics of RSA, RSA (%) and RSA (s), increased with increasing IBI and there was no difference in the coefficients of the regression between fasted and non-fasted trials [RSA (%): B_0, P=0.77; B_1, P=0.92; RSA (s): B_0, P=0.75; B_1, P=0.83] (Fig. 2, Table 2). There was a significant interaction between IBI and fasted state for both metrics of RSA. Both metrics of RSA only differed for fasted as compared with non-fasted IBIs that were longer than 30 s (Table 1).

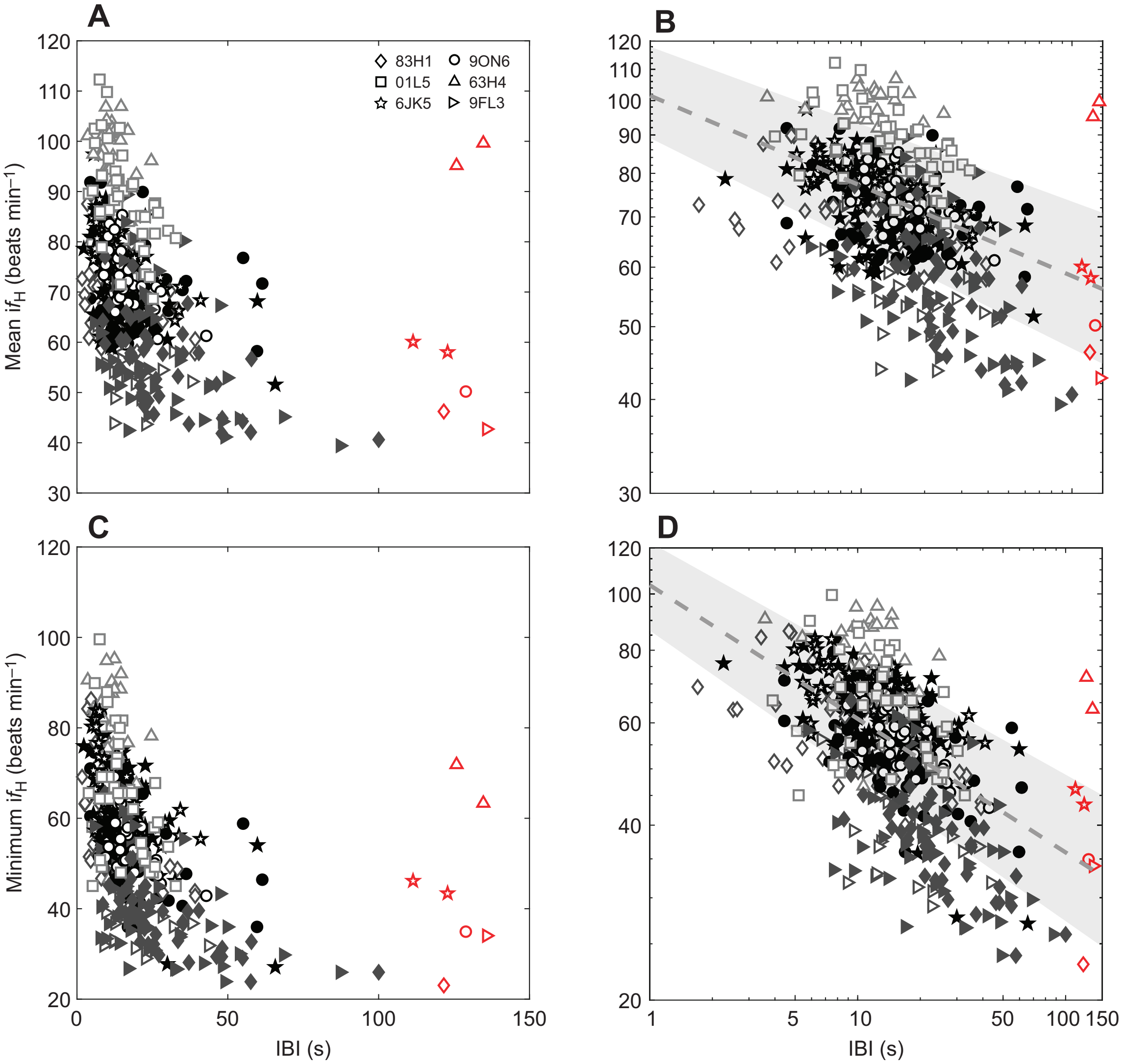

Fig. 1. Mean and minimum instantaneous heart rate (if_H) during surface breathing and submerged breath-holds. Mean if_H (A,B) and minimum if_H (C,D) against inter-breath interval (IBI) for fasted surface (filled grayscale symbols, n=241), non-fasted surface (open grayscale symbols, n=246) and breath-hold trials (open red symbols, n=7). A and C are plotted on a linear scale and B and D are plotted on a log–log scale with regressions and 95% confidence intervals for the surface data as detailed in Table 2.

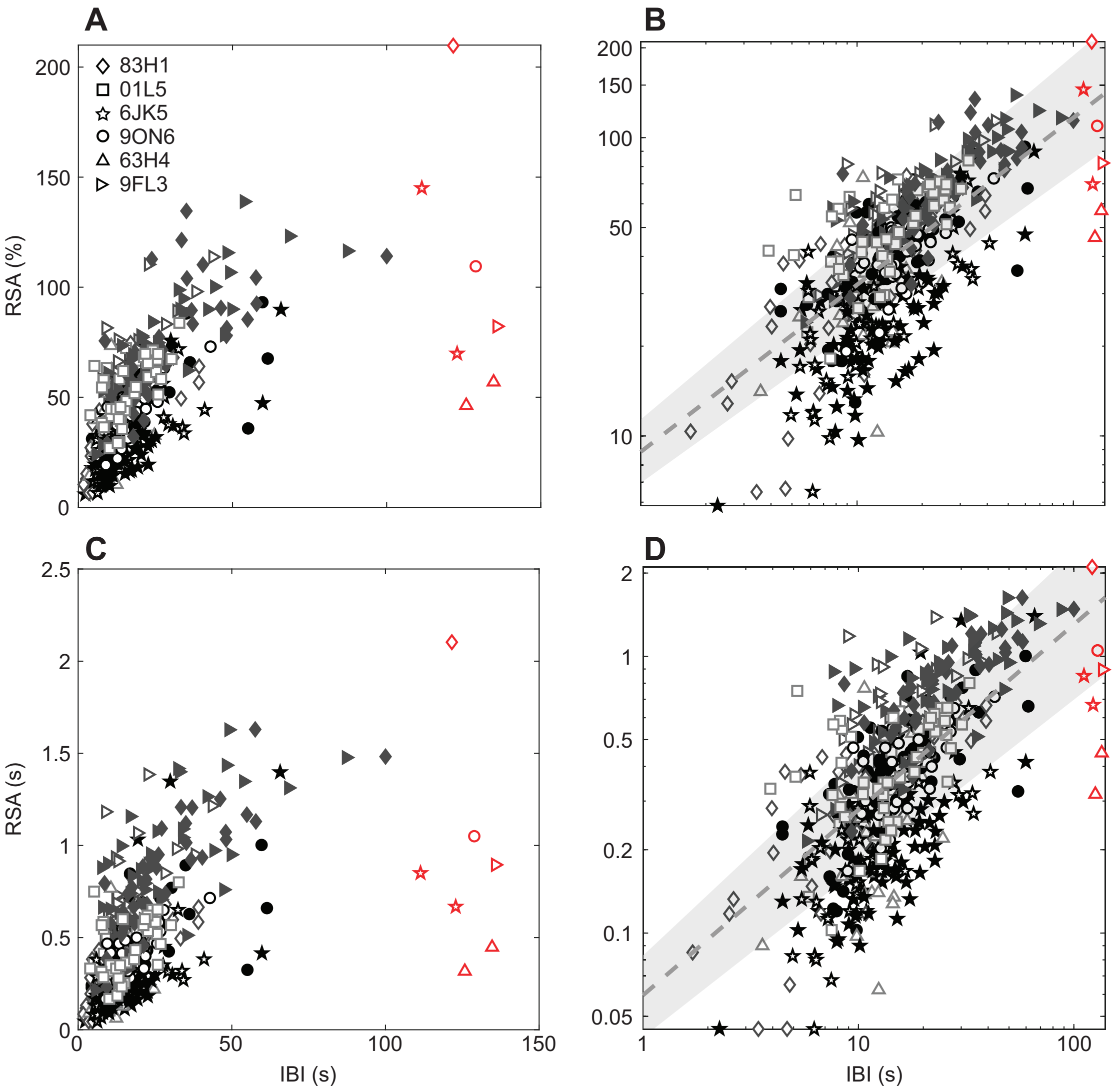

Fig. 2. Respiratory sinus arrhythmia (RSA) during surface breathing and submerged breath-holds. Data showing two methods to estimate the RSA as RSA (%) (A,B) and RSA (s) (C,D) against IBI for fasted surface (filled grayscale symbols, *n*=241) non-fasted surface (open grayscale symbols, *n*=246) and submerged breath-hold trials (open red symbols, *n*=7). A and C are plotted on a linear scale and B and D are plotted on a log–log scale with regressions and 95% confidence intervals for the surface data as detailed in Table 2.

In addition to RSA, two other distinct arrhythmic patterns were observed in the f_H measured during IBIs at the surface. The first was an oscillatory pattern within the gradual decrease in if_H of the RSA (Fig. 3). This oscillatory pattern, in addition to the initial peak in if_H, resulted in secondary and tertiary local maxima and minima within the if_H signal. These peaks occurred with a regular period of approximately 10 s (0.1 Hz, see Fig. 3A). The second arrhythmia was a series of paired heart beats that resulted in the oscillation of the if_H signal

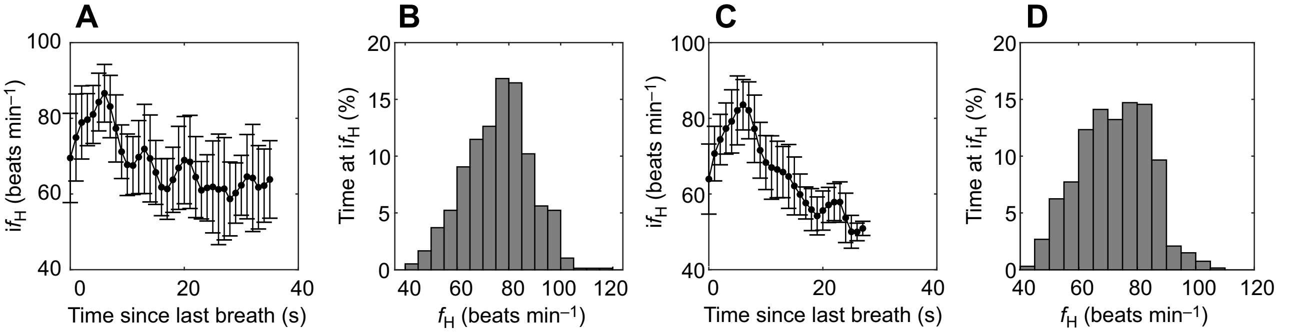

Fig. 3. RSA during a fasted and non-fasted surface trial. Mean±s.d. if_H responses (A,C) and distribution of if_H (B,D) of 9ON6 for a representative fasted surface trial (A,B) and a representative non-fasted surface trial (C,D) corresponding to the 90th percentile duration IBI for each respective trial.

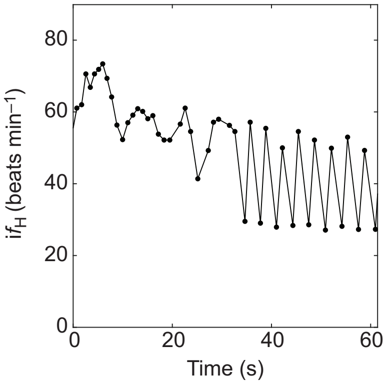

Fig. 4. A paired beat arrhythmia. if_H during an IBI shows an example of a paired beat arrhythmia.

between approximately 50 beats min^{-1} and 30 beats min^{-1} (Fig. 4). This pattern resulted from a short R–R interval between paired beats followed by a longer R–R interval until the next set of paired beats and was only observed during one surface IBI in a single dolphin (63H4).

Surface breathing versus submerged breath-hold f_H and RSA

The mean breath-hold duration was 125.9±8.4 s (range: 121.6–135.8 s) and the mean f_H during breath-holds was 64.6±23.3 beats min^{-1}. For all breath-hold trials, maximum if_H was 104.6±23.5 beats min^{-1} and minimum if_H was 45.3±17.1 beats min^{-1} (range: 133.3–23.1 beats min^{-1}). In all surface trials (combined fasted and non-fasted) mean IBI and mean f_H were 17.1±11.8 s and 72.7±13.6 beats min^{-1}, respectively. One dolphin (63H4) did not demonstrate submersion bradycardia during the submerged breath-holds.

There was no significant difference between the maximum if_H or minimum if_H of the RSA during long IBIs at the surface compared with breath-hold trials when dolphins demonstrated some degree of submersion bradycardia (excludes two trials with 63H4, Table 3). There was a significant difference in mean if_H, with lower mean if_H during the breath-holds as compared with the surface IBIs, but this was largely driven by the extended period of low if_H following the first 15–20 s of the breath-hold. In addition, there was a significant difference in both metrics of RSA, with higher RSA during breath-hold as compared with surface IBIs.

There were three general f_H patterns observed during breath-hold trials (Fig. 5). The first was a pattern similar in shape to that observed between breaths during surface trials. This pattern began with a brief increase in if_H directly following the breath and as the animal submerged followed by a gradual, oscillating decrease in if_H (see Fig. 5A,B). Interestingly, the minimum if_H during the breath-hold trial for an animal displaying this pattern was approximately 15 beats min^{-1} lower as compared with the minimum if_H during the same animal's non-fasted surface trials. The second pattern seen during the breath-hold trials was a rapid increase in if_H after the breath followed by a rapid decrease in if_H to a stable, reduced f_H (see Fig. 5C,D). The third distinct f_H pattern observed was a rapid oscillation with an amplitude of approximately 30 beats min^{-1} around an elevated f_H (see Fig. 5E,F). Notably, this pattern was only observed in one animal (63H4) and was consistent during both breath-hold trials with this individual.

DISCUSSION

We measured f_H and IBI in bottlenose dolphins and found that they exhibit large RSA during surface breathing and that, for long IBIs, the minimum if_H of the RSA was not significantly different from the minimum if_H observed during a 2 min, static submersion. In addition to RSA, we observed several other arrhythmias while the dolphins were breathing at the surface and during submerged breath-holds. We examined cardiorespiratory parameters during fasted and non-fasted surface trials and found that average f_H during fasted trials agreed with the previously published allometric relationship for resting f_H, whereas the average f_H during non-fasted trials was comparatively higher, and suggest that these differences are secondary to differences in average IBI (Stahl, 1966). The regression analysis revealed no differences between any if_H or RSA parameters during fasted and non-fasted trials when accounting for variation in IBI, but when data were binned by IBI, there were significant differences in the same parameters.

Importantly, this study does not account for other factors that may affect RSA and surface f_H including tidal volume (V_T) or exercise. We would expect to observe a stronger relationship between RSA and IBI duration if we had accounted for the variation in V_T, which is known to affect RSA (Cauture et al., 2019; Hirsch and Bishop, 1981). Similarly, because this study was only conducted under resting conditions, we cannot predict the impact of exercise or extended diving behavior on surface RSA or its relationship to submerged breath-holds. After an extended dive, where the dolphin remains actively stroking during the bottom portion and during the ascent of the dive, RSA may be disrupted by the requirements to recover quickly at the surface, as in elephant seals (Andrews et al., 1997). However, this study aimed only to establish resting surface f_H patterns in comparison to submersion bradycardia, considering the importance of surface f_H in underlying O_2 management requirements during gas exchange and the possibility of overlapping mechanisms regulating surface and diving f_H. The results of this work contribute to our understanding of the factors that influence fine-scale changes in f_H in marine mammals, which is critical for estimating the physiological thresholds on diving behavior.

Table 3. f_H and RSA statistics for surface IBIs>30 s and submerged breath-hold trials during which submersion bradycardia was observed

Trial type	Mean if_H (beats min^{-1})	Maximum if_H (beats min^{-1})	Minimum if_H (beats min^{-1})	RSA (%)	RSA (s)
Surface (IBI>30 s)	58.0±11.8 (*n*=50)	85.8±13.1 (*n*=50)	38.7±10.6 (*n*=50)	67.8±23.2 (*n*=14)	0.66±0.26 (*n*=14)
Submerged (*n*=5)	50.7±8.3	94.8±24.0	36.4±9.0	116.2±44.5	1.1±0.52
Statistics					
Trial type	χ^2(1, *n*=55)=6.1 ***P*=0.01**	χ^2(1, *n*=55)=1.5 *P*=0.21	χ^2(1, *n*=55)=2.8 *P*=0.10	χ^2(1, *n*=19)=10.4 ***P*<0.001**	χ^2(1, *n*=19)=8.3 ***P*<0.001**
Intercept	χ^2(1, *n*=55) =71.0 ***P*<0.0001**	χ^2(1, *n*=55)=211.7 ***P*<0.0001**	χ^2(1, *n*=55)=48.5 ***P*<0.0001**	χ^2(1, *n*=19)=86.0 ***P*<0.0001**	χ^2(1, *n*=19)=51.2 ***P*<0.0001**

Submerged data exclude two trials with 63H4. Trial type (surface versus submerged) was the fixed effect and animal ID was the random effect in a mixed effects ANOVA. For parameters that showed no significant difference between fasted and non-fasted surface trials for IBIs>30 s, fasted and non-fasted data were pooled (based on Table 1). For parameters that showed a significant difference between fasted and non-fasted trials, only non-fasted trials were compared with the submerged breath-holds. Bold indicates significance.

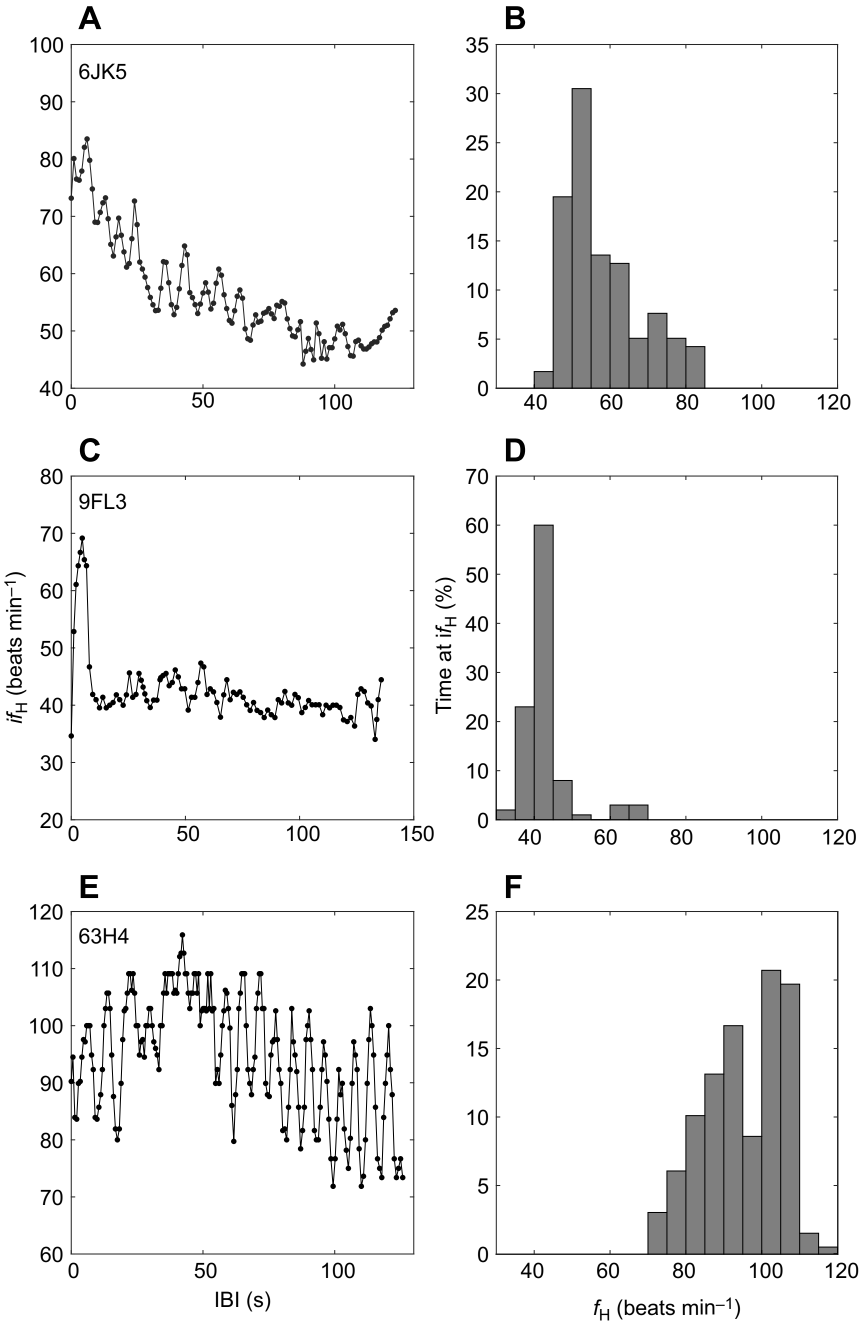

Fig. 5. f_H patterns during submerged breath-holds. if_H responses (A,C,E) and distribution of if_H (B,D,F) for three submerged breath-hold trials.

RSA and f_H are correlated with IBI

Although the patterns we observed in this study have previously been observed in many marine mammal species, the potential relationship between RSA and apnea has been studied in pinnipeds, but not cetaceans, which are fully aquatic, obligate divers (Castellini et al., 1994b). In contrast to those during diving, circulatory adjustments during surface intervals should increase perfusion to maximize gas exchange and minimize time spent during an otherwise unproductive state (Fahlman et al., 2020b, 2018; Fedak et al., 1988). For example, the rapid increase in if_H of the RSA may be important in generating an increase in peripheral perfusion to quickly replenish muscle and blood O_2 stores as a clustering of heart beats has been shown to improve O_2 uptake and reduce the physiological dead space to V_T ratio in humans (Arieli and Farhi, 1985; Yasuma and Hayano, 2004). We found that the maximum if_H following inhalation did not vary with IBI, although it was variable and could have been influenced by V_T (Cauture et al., 2019). This result indicates that, under resting conditions at the surface, the increase in f_H during the RSA is not modulated in relation to the upcoming IBI duration and could instead be a function of other respiratory variables (i.e. V_T). The data did, however, show a positive correlation between RSA and IBI, which is likely secondary to a negative correlation between mean and minimum if_H and IBI (Fig. 2). This result agrees with work in harbor seals breathing spontaneously on land where there was an observed oscillation in f_H between 120 and 70 beats min^{-1} for long IBIs, and high V_T, but for short IBIs, this oscillation was reduced (Påsche and Krog, 1980).

We also investigated the potential effect of the duration of the previous IBI on maximum if_H of the IBI, given that IBI was not a significant predictor of maximum if_H. Based on the observed f_H patterns, we expect that the previous IBI could have an effect on maximum if_H of an IBI in two ways: the duration of the previous IBI could determine the 'initial value' from which f_H varies during the next IBI; and the V_T of a breath, which may be correlated with the duration of the previous IBI and the corresponding degree of lung inflation, could result in differential changes in f_H. We found that there was a significant negative relationship between the duration of the previous IBI and the maximum if_H of the IBI, suggesting that following a long IBI, maximum if_H of the next IBI is lower and therefore may start from a lower initial value than that following short IBIs (Table S3, Fig. S1). Additionally, we found that there was a significant positive relationship between the duration of the previous IBI and the difference between maximum if_H of the IBI and the minimum if_H of the previous IBI. A possible explanation for this pattern is that following a long IBI there may be a greater need for gas exchange such that the animal compensates with a larger V_T of the breath and therefore the effect of lung inflation may be greater following long IBIs than short IBIs. Together, however, these patterns indicate that a higher degree of tachycardia that may result from a larger V_T associated with longer IBIs may not compensate for the low initial value of the f_H at the beginning of that IBI because of the preceding long IBI. These results suggest that the previous IBI may influence f_H patterns during a given IBI and that changes in f_H during breathing may be useful to estimate lung function, as previous studies have suggested (Cauture et al., 2019).

Given the relationships between RSA, f_H and IBI, we suggest that RSA in bottlenose dolphins is driven by a brief period of tachycardia associated with lung inflation whose effect is slowed and eventually eliminated as the IBI increases. Past studies in harbor seals have indicated an association between lung inflation and intermittent tachycardia followed by an exponential decay in f_H (Angell-James et al., 1981). If this is the case in bottlenose dolphins, an interesting question arises as to whether the tachycardia of breathing or the lower, stable f_H seen during IBIs>30 s reflects the 'normal', intrinsic f_H of the dolphin, the latter having been suggested for seals and manatees (Castellini et al., 1994a; Castellini and Zenteno-Savin, 1997; Gallivan et al., 1986). There is further support for this suggestion from cardiac output measurements during apneas in seals that match allometric predictions, as well as measurements of f_H in killer whales during stationary breath-holds that agree with allometric predictions for resting f_H (Bickett et al., 2019; Ponganis et al., 2006). Then, the tachycardia associated with breathing may be a response to improve gas exchange and not a 'normal' rate, an idea that has previously been proposed in studies in other taxa (Castellini et al., 1994a; Castellini and Zenteno-Savin, 1997; Fedak et al., 1988; Gallivan et al., 1986). Alternatively, this pattern could suggest that the tachycardia associated with breathing is the intrinsic f_H of the dolphin, which has previously been assessed during anesthesia with nitrous oxide to be approximately 100–120 beats min^{-1}, and that the response to apnea overrides this normal rate (Ridgway and McCormick, 1967). This would be similar to the accentuated antagonism proposed in other diving marine mammals where the diving f_H is largely regulated by vagal tone (Elliott, 2002). In addition to the lack of RSA at high f_R, in humans RSA has also been observed to be f_R independent at low f_R (long IBIs), where the degree of RSA does not continue to increase during an extended breath-hold or IBI, and this 'corner' frequency is correlated with resting f_H (Hirsch and Bishop, 1981). In this case, the normal rate in a dolphin may be a reduced f_H, or bradycardia, that is maintained in the absence of breathing, which is closer to the average diving f_H. For marine mammals, we propose that the IBI and the corresponding mean f_H where an IBI independence of RSA is observed may be of interest to define resting f_H more clearly in the dolphin for comparison with diving f_H (Fahlman et al., 2019; Kooyman, 1985).

Minimum if_H of RSA during long IBIs is comparable to bradycardia of submerged breath-holds

The minimum if_H of the RSA during long IBIs did not differ from the minimum if_H observed during submersion bradycardia associated with the breath-hold trials. Several individual dolphins consistently exhibited if_H at or below 35 beats min^{-1} during extended IBIs at the surface. This is comparable to the 24–35 beats min^{-1} measured in previous studies following both active and static breath-holds (Fahlman et al., 2020a, 2019; Noren et al., 2012, 2004; Ridgway, 1972). Similar observations have been made in California sea lions, hooded seals and emperor penguins, where minimum f_H observed between breaths on land or during resting in water was comparable to the minimum f_H observed during some periods of diving, particularly during dives below the ADL in studies of emperor penguins and California sea lions (Castellini et al., 1994a; Lin et al., 1972; McDonald and Ponganis, 2014; Meir et al., 2008; Påsche and Krog, 1980). In emperor penguins and sea lions, it was found that minimum f_H associated with RSA was only comparable to that of minimum f_H during diving for dives shorter than the species ADL (McDonald and Ponganis, 2014; Meir et al., 2008). The researchers suggested that for dives shorter than the ADL, bradycardia during diving could be regulated by a similar mechanism of cardiorespiratory control to that which drives RSA and that further reduction in f_H only occurs during dives past the ADL. Our data agree with this finding given that the 2 min breath-holds should be well within the calculated ADL of 6.5 min and the measured ADL of approximately 4 min of bottlenose dolphins (Fahlman et al., 2018; Williams et al., 1999).

Additional arrhythmias present during breathing and submerged breath-holds

In addition to RSA, we identified two other arrhythmic patterns in f_H measurements that may be indicative of additional cardiovascular controls on f_H at the surface. The first was a faster oscillation with an approximate frequency of 0.1 Hz within the gradual decrease in if_H of the RSA (see Fig. 3A). It is worth noting that this pattern was observed during most IBIs, and has also been observed in past studies in bottlenose dolphins (Cauture et al., 2019; Fahlman et al., 2020a). Although the mechanism for these oscillations is not immediately clear, possibilities may include local fluctuations in blood pressure or the influence of respiratory drive. The second arrhythmic pattern we observed during a single IBI was a series of paired heart beats that resulted in a higher frequency oscillation at approximately 0.3 Hz. The R–R interval between paired beats was reduced, resulting in a high if_H followed by an extended R–R interval until the next set of paired beats, resulting in a low if_H (Fig. 4). This pattern was only observed during one IBI, but a similar arrhythmia has been observed in some instances in resting killer whales, pilot whales and belugas (Bickett et al., 2019; Fahlman et al., 2020b), in wild gray seals at a frequency of approximately 0.1 Hz (Thompson and Fedak, 1993), and in elite free divers (Ferrigno et al., 1991). High-frequency changes in the R–R interval in pinnipeds have been reported across multiple species, during routine diving, resting and sleeping behaviors (Andrews et al., 1997; Ponganis et al., 2017). The pathological potential of these arrhythmias is debated. Some researchers have suggested that the sympathetic stimulation associated with activity, which acts to elevate f_H, conflicts with parasympathetic stimulation that regulates diving bradycardia, leading to cardiac anomalies and making diving marine mammals such as bottlenose dolphins, Weddell seals and narwhals more susceptible to injury (Davis and Williams, 2012; Williams et al., 2017, 2015a,b). In contrast, some studies have suggested that when both sympathetic and parasympathetic stimulation are highest, the variability in f_H is minimal and that benign arrhythmias are common in marine mammals (Ponganis et al., 2017). In the current study and from past observations, a range of if_H changes both while breathing spontaneously and while performing surface breath-holds are common in dolphins, killer whale, pilot whale and beluga (Fahlman et al., 2020a,b).

Compared with the surface f_H values, the patterns observed during breath-holds were more variable. This variation may be indicative of the presence of multiple factors contributing to f_H control during submersion. There were three distinct f_H modulation patterns seen during breath-hold trials: (1) a gradual decrease in f_H, like an extended RSA (Fig. 5A), (2) a rapid decrease in f_H, like submersion bradycardia (Fig. 5C), and (3) highly oscillatory and elevated f_H (Fig. 5E). The first pattern, an extended RSA-like modulation, was observed in two individual breath-hold trials, with minimum if_H around 35 and 45 beats min^{-1} (Fig. 5A). This result could suggest that f_H control during these breath-holds was under a similar mechanism to that which governs RSA at the surface and may also indicate that RSA becomes f_R independent at low breathing frequencies, as has been shown in humans (Hirsch and Bishop, 1981). The second, more rapid reduction in f_H was seen during three of the breath-hold trials (Fig. 5C). In these trials, f_H increased following the breath and then decreased rapidly before leveling off for the remainder of the breath-hold. This rapid bradycardia-like pattern could indicate a conservative response to performing a submerged breath-hold of an unknown duration where anticipation, or conditioning, alters the physiological response (Blix, 2018; Fahlman et al., 2020a; Thompson and Fedak, 1993). The final pattern, a f_H that was highly oscillatory and elevated during the entire breath-hold, was observed in one animal during two breath-holds on separate days (Fig. 5E). Across both breath-hold trials this animal displayed a mean if_H of 97.5±11.9 beats min^{-1} and a minimum if_H of 67.6±6.0 beats min^{-1}, both which are elevated compared to all other animals. For this dolphin, minimal f_H changes, e.g. RSA, were also seen during surface trials. It is possible that these differences indicate an animal that is not fully relaxed during the trials, resulting in a comparatively higher f_H during both the surface IBI and while submerged. Alternatively, it may be additional evidence of conditioned control of if_H as has been shown in both harbor porpoises, California sea lions, and bottlenose dolphins (Elmegaard et al., 2016; Fahlman et al., 2020a; Kaczmarek et al., 2018), where the dolphin is aware that it can terminate the breath-hold at any time and, for a relatively short apnea, does not require cardiovascular adjustment. These three distinct f_H responses during breath-holds are further evidence that the dive response is not a reflex and that dolphins, like other marine mammals, exhibit extensive cardiovascular plasticity.

IBI is shorter and f_H is elevated under non-fasted conditions

These data also provide an opportunity to investigate whether there are differences in cardiorespiratory control during fasted versus non-fasted surface trials that could relate to expected differences in metabolic requirements associated with feeding. We examined the relationship between surface f_H parameters and IBI duration and found several significant differences between data collected under fasted and non-fasted conditions. Generally, non-fasted trials showed increased f_H, and had a greater proportion of short IBIs and a smaller proportion of long IBIs compared with fasted trials (Table 1; Table S1). We expect an increase in metabolic overhead, or specific dynamic action (SDA), associated with digestion to result in a higher rate of O_2 consumption when an animal is non-fasted (Rosen et al., 2007). This increased metabolic demand could result in a number of cardiorespiratory changes to increase O_2 delivery, such as increased alveolar respiration, e.g. increased breathing frequency, or V_T, and/or increased cardiac output or arterial/venous difference. Given that shorter IBIs are associated with higher mean if_H and the dolphins demonstrated an increased f_R during non-fasted trials, our results support the possibility of dolphins increasing their alveolar ventilation through an increase in f_R. Increased f_R would also result in increased cardiac output due to the effect of f_R on both f_H and stroke volume (Cauture et al., 2019; Fahlman et al., 2020b). Both would help to increase delivery of O_2 to support the increased metabolic requirements of digestion, and thus these data suggest that cardiorespiratory function of bottlenose dolphins is responsive to metabolic changes. In gray seals it has been shown that rapid breathing and high f_H are utilized during periods associated with food processing and it was suggested that these changes may increase blood flow to splanchnic organs involved in digestion (Sparling et al., 2007).

Despite the regression analyses revealing no differences between fasted and non-fasted f_H and RSA when controlling for IBI, binned analyses suggested significant differences between some fasted and non-fasted f_H and RSA metrics within an IBI category. Although for long IBIs (IBIs>30 s), mean if_H and minimum if_H were not significantly different between fasted and non-fasted trials (Table 1), for IBIs<30 s there was a significant difference in both mean if_H and minimum if_H for fasted versus non-fasted trials. We found that both mean if_H and minimum if_H were higher during non-fasted as compared with fasted IBIs<30 s, likely reflecting differences in average IBI and a shorter duration of the stabilized minimum if_H that is associated with a shorter IBI. There was not a clear association between fasted state and maximum if_H, or either index of RSA; however, for long IBIs, RSA was greater for fasted than non-fasted trials. These results further suggest the importance of considering f_R when interpreting f_H as even when f_H and RSA metrics were compared within selective bins, we still observed significant differences due to the relative difference in IBIs within each bin.

Interestingly, the mean f_H of all combined fasted surface trials closely agreed with the allometric prediction of 64.2 beats min^{-1} for the average body mass of the animals that participated in the fasted trials (Table S1) (Stahl, 1966). This suggests that the allometric relationship provided by Stahl (1966) may be extrapolated to bottlenose dolphins under fasted, resting conditions. In contrast, the mean f_H for all combined non-fasted trials was 22% greater than the predicted resting f_H, highlighting the importance of specifying the conditions under which any f_H data were collected, as has previously been suggested (Fahlman et al., 2020b). Similar findings have resulted from f_H measurements in killer whales and pilot whales (Bickett, 2017), where the resting f_H under non-fasted conditions was 42% and 39% higher, respectively, than would be predicted by Stahl's (1966) allometric relationship. However, this same study (Bickett, 2017) found that the resting f_H of a beluga under non-fasted conditions was 18% lower than that predicted allometrically. Clearly, there are important differences in cardiovascular patterns in fasted versus non-fasted animals and, like metabolic rate, it may be the case that f_H is only comparable if measured under standardized conditions (Kleiber, 1932). Then, given that data collection conditions are standardized, the elevated f_H during non-fasted compared with fasted trials may indicate the potential for f_H to be used as a proxy for changes in metabolic rate in marine mammals (Fahlman et al., 2004; Green et al., 2007; Henderson and Prince, 1914).

Conclusions

Overall, our work shows that bottlenose dolphins exhibit RSA and that it predictably affects f_H during surface breathing. Minimum if_H during long IBIs is comparable to diving f_H reported for bottlenose dolphins in the literature and in our submerged breath-hold trials. We suggest that the bradycardia observed during diving, particularly in dives below the ADL, may partly be an extension of normal RSA to a long duration IBI. We observed that RSA was positively correlated with IBI and suggest that this relationship is secondary to a decrease in both minimum if_{H} and mean if_{H} during the IBI. The mean f_{H} during fasted trials agreed with the allometric prediction for resting f_{H} of a bottlenose dolphin, while mean f_{H} during non-fasted trials was higher. This suggests that f_{H} may fluctuate in relation to short-term metabolic requirements and highlights the importance of reporting the conditions under which any f_{H} data are collected such that they can be accurately compared with other datasets. Although there are other factors known to influence the progression of bradycardia, we believe that a component of the change in f_{H} associated with diving is not an adaptation specifically for diving but instead is a response to apnea in an intermittently breathing mammal. Additionally, RSA may provide a new method for determining resting f_{H} of an intermittent breather. These results demonstrate the importance of RSA in influencing f_{H} variability and emphasize the need to understand differing controls on f_{H} during surface and diving behavior in bottlenose dolphins. Investigations of f_{H} in free-ranging animals to understand how diving behavior alters surface f_{H} will be informative in further understanding the controls on fine-scale changes in f_{H} in cetaceans.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
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Data availability
Data are available from GitHub: https://github.com/ashleyblawas/Blawas_RSAindolphins

Supplementary information
Supplementary information available online at https://jeb.biologists.org/lookup/doi/10.1242/jeb.234096.supplemental

References

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

Angell-James, J. E., Elsner, R. and Daly, M. D. B. (1981). Lung inflation: effects on heart rate, respiration, and vagal afferent activity in seals. *Am. J. Physiol. Heart Circ. Physiol.* **240**, H190-H198. doi:10.1152/ajpheart.1981.240.2.H190

Arieli, R. and Farhi, L. E. (1985). Gas exchange in tidally ventilated and non-steadily perfused lung model. *Respir. Physiol.* **60**, 295-309. doi:10.1016/0034-5687(85)90059-3

Bickett, N. J. (2017). *The Electrocardiogram and Heart Rate in Three Mid-sized Whale Species*. University of California at San Diego.

Bickett, N. J., Tift, M. S., Leger, J. S. and Ponganis, P. J. (2019). Heart rates, heart rate profiles, and electrocardiograms in three killer whales, a beluga, and a pilot whale: An exploratory investigation. *Mar. Mamm. Sci.* **35**, 1112-1132. doi:10.1111/mms.12578

Blix, A. S. (2018). Adaptations to deep and prolonged diving in phocid seals. *J. Exp. Biol.* **221**, jeb182972. doi:10.1242/jeb.182972

Block, B. A. (2005). Physiological ecology in the 21st century: advancements in biologging science. *Integr. Comp. Biol.* **45**, 305-320. doi:10.1093/icb/45.2.305

Castellini, M. A. and Zenteno-Savin, T. (1997). Heart rate scaling with body mass in pinnipeds. *Mar. Mamm. Sci.* **13**, 149-155. doi:10.1111/j.1748-7692.1997.tb00620.x

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. (1994a). Patterns of respiration and heart rate during wakefulness and sleep in elephant seal pups. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **266**, R863-R869. doi:10.1152/ajpregu.1994.266.3.R863

Castellini, M. A., Rea, L. D., Sanders, J. L., Castellini, J. M. and Zenteno-Savin, T. (1994b). Developmental changes in cardiorespiratory patterns of sleep-associated apnea in northern elephant seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **267**, R1294-R1301. doi:10.1152/ajpregu.1994.267.5.R1294

Cauture, F., Sterba-Boatwright, B., Rocho-Levine, J., Harms, C., Miedler, S. and Fahlman, A. (2019). Using respiratory sinus arrhythmia to estimate inspired tidal volume in the Bottlenose Dolphin (Tursiops truncatus). *Front. Physiol.* **10**. doi:10.3389/fphys.2019.00128

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. doi:10.1007/s00359-012-0731-4

Elliott, N. M. (2002). *Pharmacological Blockade of the Cardiovascular Dive Response: Effects on Heart rate and Diving Behaviour in the Harbour Seal (Phoca vitulina)*. University of British Columbia. https://doi.org/10.14288/1.0090203.

Elmegaard, S. L., Johnson, M., Madsen, P. T. and McDonald, B. I. (2016). Cognitive control of heart rate in diving harbor porpoises. *Curr. Biol.* **26**, R1175-R1176. doi:10.1016/j.cub.2016.10.020

Elsner, R., Kenney, D. W. and Burgess, K. (1966). Diving bradycardia in the trained dolphin. *Nature* **212**, 407-408. doi:10.1038/212407a0

Fahlman, A., Handrich, Y., Woakes, A. J., Bost, C.-A., Holder, R., Duchamp, C. and Butler, P. J. (2004). Effect of fasting on the $\dot{V}O_2$ - f_H relationship in king penguins, Aptenodytes patagonicus. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R870-R877. doi:10.1152/ajpregu.00651.2003

Fahlman, A., Loring, S. H., Levine, G., Rocho-Levine, J., Austin, T. and Brodsky, M. (2015). Lung mechanics and pulmonary function testing in cetaceans. *J. Exp. Biol.* **218**, 2030-2038. doi:10.1242/jeb.119149

Fahlman, A., Jensen, F. H., Tyack, P. L. and Wells, R. S. (2018). Modeling tissue and blood gas kinetics in coastal and offshore common bottlenose dolphins, Tursiops truncatus. *Front. Physiol.* **9**. doi:10.3389/fphys.2018.00838

Fahlman, A., Miedler, S., Rocho-Levine, J., Jabois, A., Arenarez, J., Marti-Bonmati, L., García-Párraga, D. and Cauture, F. (2019). Re-evaluating the significance of the dive response during voluntary surface apneas in the bottlenose dolphin, Tursiops truncatus. *Sci. Rep.* **9**, 8613. doi:10.1038/s41598-019-45064-8

Fahlman, A., Cozzi, B., Manley, M., Jabas, S., Malik, M., Blawas, A. and Janik, V. (2020a). Conditioned variation in heart rate during static breath-holds in the bottlenose dolphin (Tursiops truncatus). *Front. Physiol.* **11**. doi:10.3389/fphys.2020.604018

Fahlman, A., Miedler, S., Marti-Bonmati, L., Fernandez, D. F., Caballero, P. M., Arenarez, J., Rocho-Levine, J., Robeck, T. and Blawas, A. (2020b). Cardiorespiratory coupling in cetaceans; a physiological strategy to improve gas exchange? *J. Exp. Biol.* **223**, jeb226365. doi:10.1242/jeb.226365

Fedak, M. A., Pullen, M. R. and Kanwisher, J. (1988). Circulatory responses of seals to periodic breathing: heart rate and breathing during exercise and diving in the laboratory and open sea. *Can. J. Zool.* **66**, 53-60. doi:10.1139/z88-007

Ferrigno, M., Grassi, B., Ferretti, G., Costa, M., Marconi, C., Cerretelli, P. and Lundgren, C. (1991). Electrocardiogram during deep breath-hold dives by elite divers. *Undersea Biomed. Res.* **18**, 81-91.

Gallivan, G. J., Kanwisher, J. W. and Best, R. C. (1986). Heart rates and gas exchange in the Amazonian Manatee (Trichechus inunguis) in relation to diving. *J. Comp. Physiol. B* **156**, 415-423. doi:10.1007/BF01101104

Green, J. A., Halsey, L. G., Butler, P. J. and Holder, R. L. (2007). Estimating the rate of oxygen consumption during submersion from the heart rate of diving animals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R2028-R2038. doi:10.1152/ajpregu.00691.2006

Hamlin, R. L., Ridgway, S. H. and Gilmartin, W. G. (1972). Electrocardiogram of pinnipeds. *Am. J. Vet. Res.* **33**, 867-875.

Henderson, Y. and Prince, A. L. (1914). The oxygen pulse and the systolic discharge. *Am. J. Physiol. Legacy Content* **35**, 106-115. doi:10.1152/ajplegacy.1914.35.1.106

Hicks, J. L., O'Hara Hines, R. J., Schreer, J. F. and Hammill, M. O. (2004). Correlation of depth and heart rate in harbour seal pups. *Can. J. Zool.* **82**, 1147-1156. doi:10.1139/z04-087

Hirsch, J. A. and Bishop, B. (1981). Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *Am. J. Physiol. Heart Circ. Physiol.* **241**, H620-H629. doi:10.1152/ajpheart.1981.241.4.H620

Irving, L. (1939). Respiration in diving mammals. *Physiol. Rev.* **19**, 112-134. doi:10.1152/physrev.1939.19.1.112

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. doi:10.1086/physzool.36.1.30152734

Kaczmarek, J., Reichmuth, C., McDonald, B. I., Kristensen, J. H., Larson, J., Johansson, F., Sullivan, J. L. and Madsen, P. T. (2018). Drivers of the dive response in pinnipeds; apnea, submergence or temperature? *J. Exp. Biol.* **221**, jeb176545. doi:10.1242/jeb.176545

Kanwisher, J. W. and Ridgway, S. H. (1983). The physiological ecology of whales and porpoises. *Sci. Am.* **248**, 110-120. doi:10.1038/scientificamerican0683-110

Kastelein, R. A. and Meijler, F. L. (1989). Respiratory arrhythmia in the hearts of harbour porpoises (Phocoena phocoena). *Aquat. Mamm.* **15**, 57-63. doi:10.1578/am.39.4.2013.389

Kleiber, M. (1932). Body size and metabolism. *Hilgardia* **6**, 315-353. doi:10.3733/hilg.v06n11p315

Kooyman, G. L. (1985). Physiology without restraint in diving mammals. *Mar. Mamm. Sci.* **1**, 166-178. doi:10.1111/j.1748-7692.1985.tb00004.x

Le Boeuf, B. J., Crocker, D. E., Grayson, J., Gedamke, J., Webb, P. M., Blackwell, S. B. and Costa, D. P. (2000). Respiration and heart rate at the surface between dives in northern elephant seals. *J. Exp. Biol.* **203**, 3265-3274.

Lewis, G. F., Furman, S. A., McCool, M. F. and Porges, S. W. (2012). Statistical strategies to quantify respiratory sinus arrhythmia: are commonly used metrics equivalent? *Biol. Psychol.* **89**, 349-364. doi:10.1016/j.biopsycho.2011.11.009

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. doi:10.1152/ajplegacy.1972.222.2.260

Lyamin, O. I., Korneva, S. M., Rozhnov, V. V. and Mukhametov, L. M. (2016). Cardiorespiratory responses to acoustic noise in belugas. In *The Effects of Noise on Aquatic Life II, Advances in Experimental Medicine and Biology* (ed. A. N. Popper and A. Hawkins), pp. 665-672. New York, NY: Springer.

McDonald, B. I. and Ponganis, P. J. (2014). Deep-diving sea lions exhibit extreme bradycardia in long-duration dives. *J. Exp. Biol.* **217**, 1525-1534. doi:10.1242/jeb.098558

McDonald, B. I., Johnson, M. and Madsen, P. T. (2018). Dive heart rate in harbour porpoises is influenced by exercise and expectations. *J. Exp. Biol.* **221**, jeb168740. doi:10.1242/jeb.168740

McDonald, B. I., Tift, M. S., Hückstädt, L. A., Jeffko, M. and Ponganis, P. J. (2020). Stroke effort and relative lung volume influence heart rate in diving sea lions. *J. Exp. Biol.* **223**, jeb214163. doi:10.1242/jeb.214163

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. doi:10.1242/jeb.013235

Mortola, J. P., Marghescu, D. and Siegrist-Johnstone, R. (2016). Thinking about breathing: effects on respiratory sinus arrhythmia. *Respir. Physiol. Neurobiol.* **223**, 28-36. doi:10.1016/j.resp.2015.12.004

Noren, S. R., Cuccurullo, V. and Williams, T. M. (2004). The development of diving bradycardia in bottlenose dolphins (*Tursiops truncatus*). *J. Comp. Physiol. B* **174**, 139-147. doi:10.1007/s00360-003-0398-9

Noren, S. R., Kendall, T., Cuccurullo, V. and Williams, T. M. (2012). The dive response redefined: underwater behavior influences cardiac variability in freely diving dolphins. *J. Exp. Biol.* **215**, 2735-2741. doi:10.1242/jeb.069583

Påsche, A. and Krog, J. (1980). Heart rate in resting seals on land and in water. *Comp. Biochem. Physiol. A Physiol.* **67**, 77-83. doi:10.1016/0300-9629(80)90410-7

Ponganis, P. J. (2007). Bio-logging of physiological parameters in higher marine vertebrates. *Deep Sea Research Part II: Top. Stud. Oceanogr.* **54**, 183-192. doi:10.1016/j.dsr2.2006.11.009

Ponganis, P. J. and Kooyman, G. L. (1999). Heart rate and electrocardiogram characteristics of a young California Gray Whale (Eschrichtius Robustus). *Mar. Mamm. Sci.* **15**, 1198-1207. doi:10.1111/j.1748-7692.1999.tb00885.x

Ponganis, P. J., Stockard, T. K., Levenson, D. H., Berg, L. and Baranov, E. A. (2006). Cardiac output and muscle blood flow during rest-associated apneas of elephant seals. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **144**, 105-111. doi:10.1016/j.cbpa.2006.02.009

Ponganis, P. J., McDonald, B. I., Tift, M. S. and Williams, C. L. (2017). Heart rate regulation in diving sea lions: the vagus nerve rules. *J. Exp. Biol.* **220**, 1372-1381. doi:10.1242/jeb.146779

Ridgway, S. (1972). Homeostasis in the aquatic environment. In *Mammals of the Sea* (ed. S. H. Ridgway), pp. 590-747. Springfield, IL: Thomas.

Ridgway, S. (1986). Diving by cetaceans. In *Diving in Animals and Man* (ed. A. O. Brubakk and J. W. Kanwisher), pp. 33-62. Trondheim, Norway: The Royal Norwegian Society of Science and Letters.

Ridgway, S. H., Carder, D. A. and Clark, W. (1975). Conditioned bradycardia in the sea lion Zalophus californianus. *Nature* **256**, 37-38. doi:10.1038/256037a0

Ridgway, S. H. and McCormick, J. G. (1967). Anesthetization of porpoises for major surgery. *Science.* **158**, 510-512. doi:10.1126/science.158.3800.510

Rosen, D. A. S., Winship, A. J. and Hoopes, L. A. (2007). Thermal and digestive constraints to foraging behaviour in marine mammals. *Phil. Trans. R. Soc. B* **362**, 2151-2168. doi:10.1098/rstb.2007.2108

Scholander, P. F. (1940). *Experimental Investigations on the Respiratory Function in Diving Mammals and Birds*. Hvalradets Skrifter.

Scholander, P. F. (1963). The master switch of life. *Sci. Am.* **209**, 92-107. doi:10.1038/scientificamerican1263-92

Sparling, C. E., Fedak, M. A. and Thompson, D. (2007). Eat now, pay later? Evidence of deferred food-processing costs in diving seals. *Biol. Lett.* **3**, 94-98. doi:10.1098/rsbl.2006.0566

Stahl, W. R. (1966). Scaling of respiratory variables in mammals. *J. Appl. Physiol.* **22**, 453-460. doi:10.1152/jappl.1967.22.3.453

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

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.

Williams, T. M., Bengtson, P., Steller, D. L., Croll, D. A. and Davis, R. W. (2015a). The healthy heart: lessons from nature's elite athletes. *Physiology* **30**, 349-357. doi:10.1152/physiol.00017.2015

Williams, T. M., Fuiman, L. A., Kendall, T., Berry, P., Richter, B., Noren, S. R., Thometz, N., Shattock, M. J., Farrell, E., Stamper, A. M. et al. (2015b). Exercise at depth alters bradycardia and incidence of cardiac anomalies in deep-diving marine mammals. *Nat. Commun.* **6**, 6055. doi:10.1038/ncomms7055

Williams, T. M., Blackwell, S. B., Richter, B., Sinding, M.-H. S. and Heide-Jørgensen, M. P. (2017). Paradoxical escape responses by narwhals (*Monodon monoceros*). *Science* **358**, 1328-1331. doi:10.1126/science.aao2740

Wilson, A. D. M., Wikelski, M., Wilson, R. P. and Cooke, S. J. (2015). Utility of biological sensor tags in animal conservation: utility of Sensor Tags. *Conserv. Biol.* **29**, 1065-1075. doi:10.1111/cobi.12486

Yasuma, F. and Hayano, J. (2004). Respiratory sinus arrhythmia. *Chest* **125**, 683-690. doi:10.1378/chest.125.2.683