Cardiorespiratory coupling in cetaceans; a physiological strategy to improve gas exchange?

[1,2,3*]Fahlman, A., [4]Miedler, S., [3]Marti-Bonmati, L., [5]Ferrero Fernandez, D., [5]Muñoz Caballero, P., [5]Arenarez, J., [6]Rocho-Levine, J., [7]Robeck, T., [8]Blawas, A.

[1] Global Diving Research, Inc. Ottawa, ON, K2J 5E8, Canada

[2] Research Department, Fundación Oceanogràfic de la Comunitat Valenciana, Gran Vía Marqués del Turia 19, 46005, Valencia, Spain

[3] Research Group on Biomedical Imaging (GIBI2[30]), Instituto de Investigación Sanitaria la Fe, 46026 Valencia. Spain

[4] Veterinary Cardiology, Plaza Mayor 7/10, 46120 Alboraya/Valencia/Spain
[5] Biology Department, Avanqua-Oceanográfic SL, Gran Vía Marqués del Turia 19, 46005, Valencia, Spain

[6] Dolphin Quest, Oahu, 5000 Kahala Ave, Honolulu, HI

[7]Sea World Orlando
[8] Nicholas School of the Environment, Duke University, Durham, NC, 27708 USA.

*Corresponding author: Phone: +34 654 492 427, e-mail: afahlman@whoi.edu

Abstract

In the current study we used transthoracic echocardiography to measure stroke volume (SV), heart rate (f_H), and cardiac output (CO) in adult bottlenose dolphins (*Tursiops truncatus*), a male beluga calf (*Delphinapterus leucas*, body mass [M_b] range: 151-175 kg), and an adult female false killer whale (*Pseudorca crassidens*, estimated M_b: 500-550 kg) housed in managed care. We also recorded continuous electrocardiogram (ECG) in the beluga, bottlenose dolphin, false killer whale, killer whale (*Orcinus orca*), and pilot whale (*Globicephala macrorhynchus*) to evaluate cardiorespiratory coupling while breathing spontaneously under voluntary control. The results show that cetaceans have a strong Respiratory Sinus Arrythmia (RSA), during which both f_H and SV vary within the interbreath interval, making average values dependent on the breathing frequency (f_R). The RSA-corrected f_H was lower for all cetaceans compared to similarly sized terrestrial mammals breathing continuously. As compared with terrestrial mammals, the RSA-corrected SV and CO were either lower or the same for the dolphin and false killer whale, while both were elevated in the beluga. When plotting f_R against f_H for an inactive mammal, cetaceans had a greater cardiac response to changes in f_R as compared with terrestrial mammals. We propose that these data indicate an important coupling between respiration and cardiac function that enhances gas exchange, and that this RSA is important to maximize gas exchange during surface intervals, similar to that reported in the elephant seal.

Summary Statement: Data from five species of cetaceans show a strong Respiratory Sinus Arrythmia, and an important cardiorespiratory coupling that enhances gas exchange.

Keywords Diving physiology, marine mammals, cardiac ultrasound, cetaceans, aortic flow, ejection fraction, dive response, respiratory sinus arrythmia

1. Introduction

Variation in heart rate (f_H) associated with breathing, often callled Respiratory Sinus Arrythmia (RSA), is seen in most vertebrates (Grossman and Taylor, 2007; Laske et al., 2010; Piccione et al., 2019; Zena et al., 2017). The RSA results in the acceleration of f_H during inspiration, and deceleration during expiration (Mortola et al., 2015). The physiological significance of RSA is debated (Hayano et al., 1996; Yasuma and Hayano, 2004), but it has been suggested that RSA reduces cardiac work and improves gas exchange by enhancing ventilation-perfusion matching (Ben-Tal et al., 2012; Ben-Tal et al., 2014; Yasuma and Hayano, 2004). In terrestrial mammals that breathe almost continuously, RSA is minimal (Piccione et al., 2019), but has been shown to vary with both breathing frequency (f_R) and tidal volume (V_T) (de Burgh Daly, 1986; Guillén-Mandujano and Carrasco-Sosa, 2014; Hirsch and Bishop, 1981). This variation in f_H with f_R and V_T may not be critically important for comparative studies on terrestrial mammals where breathing is more or less continuous and its effect on cardiac function may not cause large variation in average f_H. However, in animal that breathe apneustically, like marine mammals, variation in f_R may cause large variation in f_H, thereby confounding temporal averages of f_H.

In marine mammals, and specifically cetaceans, where breaths are separated by long respiratory pauses (Fahlman et al., 2017), significant changes in f_H have been observed following a breath (Andrews et al., 1997; Bickett et al., 2019; Castellini et al., 1994a; Castellini et al., 1994b; Cauture et al., 2019; Elmegaard et al., 2016; Elsner et al., 1966; Fahlman et al., 2019b; Harms et al., 2013; Houser et al., 2010; Kaczmarek et al., 2018; McDonald et al., 2018; Miedler et al., 2015; Noren et al., 2004; Noren et al., 2012; Reed et al., 2000; Williams et al., 1993). Variation in f_R is expected to have a large effect on average f_H, but despite this, most studies on cardiac function in marine mammals do not report f_R or the magnitude of RSA. For this reason, it has been suggested that studies that investigate cardiovascular changes with diving should estimate and report the RSA-corrected f_H for an inactive animal (Cauture et al., 2019; Fahlman et al., 2019b). However, RSA-correcting data does not provide a true estimate of the average f_H, stroke volume (SV) or cardiac output (CO) as it omits the dynamic period from the end of the breath until the instantaneous f_H (if_H) and SV (iSV) have stabilized after about 10-12 s (Cauture et al., 2019; Fahlman et al., 2017). Still, the RSA-correcteed f_H and SV provide a means to compare values between studies and species that are independent of f_R. Thus, a better understanding of the cardiorespiratory coupling between f_H and f_R is warranted and may provide improved understanding how marine mammals balance the trade off between gas exchange during apneustic periods with the periods of tachycardia while breathing.

Given the limited work that has been done on cardiorespiratory coupling, the confounding effect of f_R on f_H, the potential effect of RSA on gas exchange, and the possible common mechanism to the diving bradycardia, this study was aimed at providing comparative estimates of cardiorespiratory function in cetaceans. We measured semi-continuous f_H, SV, and calculated CO using transthoracic echocardiography immediately following the inhalation in the bottlenose dolphin (*Tursiops truncatus*). We hypothesized that changes in CO during the respiratory cycle would mainly be governed by changes in f_H as has been reported in other marine mammals during short apneas (Blix and Kjekshus, 1983; Blix et al., 1976; Elsner et al., 1964; Murdaugh et al., 1966). We also collected transthoracic echocardiography data from a false killer whale (*Pseudorca crassidens*) and a beluga (*Delphinapterus leucas*) while breathing at the surface at rest. These data were used to test the hypothesis that cardiac parameters in cetaceans at rest are allometrically similar to terrestrial mammals. In addition, we also included previously published continuous if_H from the bottlenose dolphin, with new data collected from the false killer whale, beluga, killer whale (*Orcinus orca*), and pilot whale (*Globicephala macrorhynchus*) to better define how f_H is modulated by breathing. These data were used to test the hypothesis that the relationship between f_R and f_H for inactive animals are similar in cetaceans and terrestrial mammals.

2. Material and methods

2.1. Animals

Ultrasound continuous flow doppler ultrasound was used to measure f_H and SV at the level of the left ventricular outflow tract from 1 adult female false killer whale housed at Sea Life Park (Hawaii-USA, January 2017), and one juvenile male beluga calf at the Oceanogràfic (Valencia-Spain, March/April 2017) (Table 1). In addition, we included previously published cardiovascular and/or respiratory data from 10 adult male and 3 adult female Atlantic bottlenose dolphins (Fahlman et al., 2019a; Fahlman et al., 2019b). The animal ID, sex, body mass (M_b), year of birth (known or estimated), and Aortic Valve Orifice diameter are summarized in Table 1. For the false killer whale, the M_b was estimated from length and girth, while for all other animals M_b was measured.

We used previously published electrocardiogram (ECG) data for up to 10 minutes in the bottlenose dolphin, and we added recently measured ECG from the juvenile beluga, and the adult false killer whale, and from adult the pilot whales (n=3) and killer whales (n=3, Sea World – Orlando) while the animals were breathing at the surface (Table 1).

The study protocols were accepted at each facility, as well as by the Animal Care and Welfare Committee at the Oceanogràfic (OCE-17-16, amendments OCE-29-18 and OCE-3-19i), and the Bureau of Medicine (BUMED, NRD-1015).

2.2. Experimental trials

All experiments were performed using operant conditioning as previously detailed (Fahlman et al., 2019a; Fahlman et al., 2019b). Participation by each individual was voluntary, and the animals were not restrained and could refuse to participate or withdraw at any point during the experimental trial without any negative consequences. None of the animals participating in this study were fasted. Each experiment (trial) consisted of an animal floating stationary in the water in left lateral recumbency with the blow-hole out of the water, allowing ultrasound probe placement over the left thoracic wall to find the left ventricle. In a few dolphins (n = 8), the false killer whale (n=1), the beluga (n=2), the pilot whale (n=3), and the killer whale (n=3) we also collected continuous ECG by placing 3 custom electrodes on the ventral surface (see below).

2.3. Ultrasound Acquisition of Heart Rate and Stroke Volume

The ultrasound machine (Vivid-I or Vivid-IQ, General Electric), with a 1-3 MHz phased array probe, was used to obtain left ventricular blood flow and velocities in continuous movie recordings up to 16 s in duration (Fahlman et al., 2019b; Miedler et al., 2015). The f_H was estimated from the flow traces, and the SV calculated from the velocity time integral of the systolic blood flow at the level of the left ventricular outflow tract multiplied with the cross-sectional area of the aortic valve orifice (the radius of the aortic valve orifice squared multiplied with π). The velocity time integral was evaluated using continuous flow doppler in flow direction from the left ventricle into the aorta, and an angel correction was used to measure the flow parallel to its path. The aortic diameter at the level of valve insertion at the different cardiac phases was measured to confirm that it was constant for all flow-rates. The CO was estimated as: $CO = f_H \times SV$.

2.4. Electrocardiogram Acquisition of Continuous Heart Rate

An ECG was recorded using three gold-plated electrodes mounted inside a silicone suction cup connected to a custom-built data recorder (UUB/1-ECGb, UFI,Morro Bay, CA, United States). The suctions cups were filled with conducting gel (Ten20 Conductive Neurodiagnostic Electrode paste, Waver and Company, Aurora, CO, USA) before being placed on the ventral side in a triangle or in a line down the sternum (Bickett et al., 2019; Cauture et al., 2019). The cetacean was instructed to turn ventral side up, the suction cups placed on the skin and once a clear ECG signal was detected, the animal rolled over to dorsal side up. Occasionally, the ECG signal was lost as the animal rolled and the procedure was repeated until a good signal was recorded when the cetacean was in a still position with the dorsal side up (Bickett et al., 2019; Cauture et al., 2019). The ECG was recorded at 400 Hz using a data acquisition system (Powerlab 8/35, ADInstruments, Colorado Springs, CO, United States), and displayed on a computer running LabChart (v. 8.1.13, ADInstruments, Colorado Springs, CO, United States). Initially, the electrodes were adjusted to assure a clear ECG trace. Next, the animal was allowed to breathe spontaneously for up to 10 minutes and the timing of each breath recorded.

The ECG was analyzed using the heart rate detection software in LabChart which automatically detected the R-peaks, using the following settings; typical QRS width = 80 ms, R-waves = 300 ms, pre-P baseline = 120 ms, maximum PR = 240 ms, maximum RT = 400 ms. Following the automatic detection, the R-peaks were manually verified and the if_H determined from the time between R-R peaks. Noisy sections, or possible beats that did not contain a clear R-peak were removed.

2.5. Data Assessment and Statistical Analysis

We compared the data for inactive animals within and between individuals. The relationship between a dependent variable (f_H, SV, and CO) and the experimental covariate, M_b, was analyzed using linear-mixed effects models (lme, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, version 3.3.3, 2016). We $\log_{10}$-transformed the variables to generate linear functions that could be used with the lme function in R. The individual animal was treated as a random effect, which accounted for the correlation between repeated measurements on the same individual (Littell et al., 1998). Normality for all models was confirmed by the qqnorm plot. Best models of remaining variables were chosen by the log-likelihood (LL) ratio test. Acceptance of significance was set to the $P < 0.05$ level, while $0.05 < P < 0.1$ was considered a trend. Data are presented as the mean ± standard deviation, unless otherwise stated.

3. Results

3.1. Cardiorespiratory dynamics

We measured f_H and SV, and calculated CO in the bottlenose dolphin, false killer whale, and juvenile beluga using transthoracic echocardiography. The changes in if_H and iSV associated with respiration are shown in Figure 1 for the bottlenose dolphin. Similar dynamic changes in if_H and iSV were seen in both the false killer whale and juvenile beluga whale. A representative figure of continuous if_H show that 5 species experienced a pronounced RSA, where the maximum if_H occurred approximately 3-5 s following the breath, and then slowly decayed towards a stable if_H (Fig. 2). This stable value we define as the RSA-corrected f_H for an inactive animal.

To better define the changes in cardiovascular function associated with breathing, we determined if_H and iSV in 4 dolphins where it was possible to visualize the heart during all phases of the respiratory cycle. The measurements began from the end of the inhalation and for the next 16 s, the maximal duration possible to record continuous data using this ultrasound machine. The data from 4 bottlenose dolphins are shown in Fig. 3, where both if_H (Fig. 3A) and iSV (Fig. 3B) increased, and then exponentially decayed towards the values for an inactive animal approximately 12-20 s following the breath (Fig. 2). The data were $\log_{10}$-transformed, and the exponential decay function for if_H (beats • min^{-1}) was; $\log_{10}(\mathrm{i}f_H) = 1.912 - 0.172 \times \log_{10}(\mathrm{time})$ ($\chi^2 = 148$, $P < 0.01$, Fig. 3A), where time is in seconds. The same relationship for iSV (ml) was; $\log_{10}(\mathrm{iSV}) = 2.402 - 0.090 \times \log_{10}(\mathrm{time})$ ($\chi^2 = 131$, $P < 0.01$, Fig. 3B). There was a significant correlation within each individual between the if_H and iSV following the breath (Fig. 3C, $\mathrm{iSV} = 112.6 + 1.719\ \mathrm{i}f_H$, $\chi^2 = 78$, $P < 0.01$).

3.2. Heart rate (f_H), stroke volume (SV) and cardiac output (CO) during rest

For an analysis between animals and species, we used the RSA-corrected f_H, SV and CO for an inactive animal. This was done by extracting the average f_H, SV, and CO at least 12-20 s following a spontaneous breath, and after the f_H and SV had been observed to stabilize for 5-7 s following the breath (Figs. 1-3)(Fahlman et al., 2019b; Miedler et al., 2015). For each animal and measurement, a minimum of at least 3 repeated measurements of f_H and SV were made and averaged. Figure 4 shows the average RSA-corrected f_H, SV and CO for the bottlenose dolphins (*Tt*), beluga calf (*Dl*) and adult false killer whale (*Pc*). Also shown on this figure are the predicted f_H, SV, and CO for a 170 kg and 520 kg terrestrial mammal (Bishop, 1997; Seymour and Blaylock, 2000; Stahl, 1967). For these data, the f_H was significantly higher, and SV and CO significantly lower in the dolphin as compared with both the similar sized beluga and the heavier false killer whale. The average mass-specific f_H (sf_H), was significantly higher for the dolphins (One sample t-test, P < 0.01, t-value=15.8. df=10) as compared with the false killer whale [sf_H; *Tt*: 0.265 ± 0.044 beats • min^{-1} • kg^{-1}; *Pc*: 0.056 ± 0.010 beats • min^{-1} • kg^{-1}; *Dl*: 0.236 ± 0.038 beats • min^{-1} • kg^{-1}], while both mass-specific SV [sSV; *Tt*: 1.07 ± 0.24 ml • kg^{-1}; *Pc*: 1.22 ± 0.24 ml • kg^{-1}; *Dl*: 3.06 ± 0.21 ml • kg^{-1}] and CO [sCO, *Tt*: 47.1 ± 10.5 ml • min^{-1} • kg^{-1}; *Pc*: 38.2 ± 14.8 ml • min^{-1} • kg^{-1}; *Dl*: 116.6 ± 17.3 ml • min^{-1} • kg^{-1}], were significantly higher for the beluga calf as compared with the dolphins (sSV, P < 0.01, t-value=27.5. df=10; sCO, P < 0.01, t-value=21.8. df=10).

The diameter of the aortic valve orifice of the false killer whale (t-value: 73.5, P < 0.01), and beluga calf (t-value: 40.8, Table 1) were both significantly larger as compared with the average aortic diameter from the bottlenose dolphins (mean ± S.D.: 3.3 ± 0.2 cm, P < 0.01), but there was no difference in aortic valve orifice diameter with M_b within the dolphins (F-value$_{1,9}$: 0.2, P > 0.6, Table 1).

3.3. Estimating average f_H, SV and CO for a range of f_R's

From Fig. 3, the average RSA-corrected f_H and SV (from around 12-16 s) of an inactive animal would be approximately 48 beats • min^{-1} and 192 ml, respectively, resulting in a CO of about 9.3 l • min^{-1}. Using the regression equations for Fig. 3A and B (section 3.1) for the un-corrected f_H and SV for an inactive dolphin, the average f_H and SV for an f_R of 2 breaths • min^{-1} would be approximately 54 beats • min^{-1} and 203 ml, respectively (this is the average f_H and SV from 0-30 s in Fig. 3). The same calculation for an f_R of 6 breaths • min^{-1} result in an average f_H and SV of 65 beats • min^{-1} and 223 ml, respectively, with a CO of 14.5 l • min^{-1}. In comparison, the average f_H for an inactive animal over a 10 minute period at the surface in the bottlenose dolphin, beluga and false killer whale were 65 ± 9 beats • min^{-1} (dolphin f_R: 4.1 ± 1.2 breaths • min^{-1} [range: 2.8-6.0]), 44 beats • min^{-1} (beluga

f_R:1.9 ± 1.5 breaths · min^{-1}) and 65 beats • min^{-1} (false killer whale f_R: 5.4 ±3 .3 breaths • min^{-1}), respectively.

To provide a comparison with an inactive terrestrial mammal with an M_b of 170 kg, the predicted f_R would be 14 breaths • min^{-1} (Stahl, 1967). Using this f_R and the regression results in Fig. 3, the average f_H and SV would be approximately 74 beats • min^{-1}, and 240 ml, respectively.

3.4. Relationship between breathing frequency and heart rate

The average f_R and f_H for the continuous f_H (ECG) from the inactive bottlenose dolphin, the false killer whale, the juvenile beluga, the pilot whale, and the killer whale are plotted in Fig. 5. In the plot, we also included published data for the harbor porpoise (*Phocoena phocoena*), the adult beluga (*Delphinapterus leucas*), and gray whale (*Eschrichtius robustus*) (Bickett et al., 2019; Elmegaard et al., 2019; Ponganis and Kooyman, 1999; Wahrenbrock et al., 1974). The regression for the cetaceans (Cetacean) were: $f_H = 22.0 + 13.8 f_R$ (Fig. 5). As a comparison we plotted the regression for the allometric estimate from terrestrial mammals (Terrestrial) for f_R and f_H (Stahl, 1967). While the allometric equations developed by Stahl (1967) were from rat to man (200 g to about 80 kg), we added points for the donkey (*Equus asinus*, M_b=165-190 kg) (Yousef and Dill, 1969), the Thoroughbred horse (*Equus ferus caballus*, M_b=448kg) (Kuwahara et al., 1996), the African elephant (*Loxodonta africana*), the Asian elephant (*Elephas maximus*) (M_b=1420-6000 kg) (Benedict and Lee, 1936; Honeyman et al., 1992; Olivares et al., 2019), and the hibernating American black bear (*Ursus americanus*) (Laske et al., 2010) (Fig. 5).

4. Discussion

This is the first study to report comparative cardiac (f_H, SV, and CO) measurements in inactive small and medium sized cetaceans. We show that both f_H and SV vary during the respiratory cycle in the bottlenose dolphin, false killer whale, and beluga, and for f_H in the killer whale and pilot whale. The data indicate that the RSA-corrected f_H for an inactive cetacean is lower in comparison to studies reporting a temporal average f_H, reflecting the importance of separating the effect of respiration on f_H for comparative purposes. Our data support that changes in CO are caused by dual changes in f_H and SV, but the former changes relatively more for a given change in CO. Of special interest is the higher mass-specific SV and CO in the beluga calf as compared to the similar sized dolphins and the larger false killer whale, possibly reflecting increased metabolic demand due to active growth. A sensitivity analysis was done using the temporal responses in if_H and iSV to evaluate the variation in resting f_H with varying f_R. In addition, we show that in cetaceans the RSA appears to follow a similar time course and that f_H respond more strongly to variation in f_R in cetaceans as compared with terrestrial mammals. We propose that RSA may be physiologically important to enhance gas exchange matching during the surface interval to minimize recovery duration (Andrews et al., 2000; Fahlman et al., 2018; Fedak et al., 1988). The data presented in the current study provide valuable comparisons within and between species that are important to understand physiological limitations in cetaceans. Furthermore, these data highlight the significance of access to animals under managed care that provide physiological measurements under voluntary conditions.

4.1. Changes in CO during RSA is made by both variation in f_H and SV

In past studies, changes in CO during maximal exercise in land mammals appeared to be mainly regulated by an increase in f_H (Evans and Rose, 1988; Taylor et al., 1987). Similar results have been reported in pinnipeds during voluntary and forced breath-holds, but there have also been instances where SV changed substantially both during static breath-holds and active submerged swimming (Blix and Kjekshus, 1983; Blix et al., 1976; Elsner et al., 1964; Murdaugh et al., 1966; Ponganis et al., 2006; Ponganis et al., 1991; Ponganis et al., 1990). Recently considerable changes in SV were reported in voluntarily participating bottlenose dolphins following exercise, or during and following static breath-holds (Fahlman et al., 2019b; Miedler et al., 2015). Immediately following a brief bout of high intensity exercise, the RSA-corrected if_H increased by 104% and iSV by 63% (Miedler et al., 2015). During a static breath-hold, the average RSA corrected iSV decreased by between 1-21% while the average RSA-corrected if_H decreased by 18% (Fahlman et al., 2019b). During recovery the RSA-corrected iSV and if_H increased by as much as 34% and 117%, respectively. Thus, similar to the studies in terrestrial and marine mammals, the changes in RSA-corrected CO in the bottlenose dolphin appear to be influenced more by changes in f_H, but there are also changes in SV, both during static diving and exercise (Fahlman et al., 2019b). In the current study, the variation in if_H and iSV changed predictably following and between breaths, and a 56% decrease in if_H (from 90 beats • min^{-1} to 40 beats • min^{-1}) caused a 32% decrease in iSV (Fig. 3C).

4.2. Correctly defining the f_H of an inactive marine mammal is crucial to assess the magnitude of the dive response

Studies have focused on assessing the magnitude of the dive response as the difference between the f_H during the dive, and a value for an inactive animal at the surface (Andrews et al., 1997; McDonald et al., 2018; Noren et al., 2004; Noren et al., 2012). In marine mammals, the aquatic breathing pattern, and the cardiorespiratory coupling between f_H and f_R complicates the ability to estimate a “normal” f_H for an inactive animal at the surface (Andrews et al., 1997; Cauture et al., 2019; Fahlman et al., 2019b; Fahlman et al., 2017). Because of this confounding effect of f_R, we propose that studies looking at the cardiovascular changes associated with diving have to account for this cardiorespiratory coupling. This is not a new suggestion, and previous studies have also expressed the importance to clearly define a normal f_H as this would affect conclusions whether animals experience a true bradycardia during a breath-hold (Belkin, 1964; Kooyman, 1985). In past studies, when using a more restrictive definition for normal $f_{H,}$ it was shown that the fresh water river cooter did not experience a diving bradycardia, while the Weddell seal only experience a diving bradycardia during dives > 8 min (Belkin, 1964; Kooyman, 1985). Thus, in species with substantial variation in f_H and SV with each breath, it is important to account for the confounding effect of RSA. Strictly defined or controlled conditions are common when measuring energy use, where, for example, the basal metabolic rate in homeotherms is measured under standard conditions (Kleiber, 1947; Pedersen et al., 2020; Rosen and Trites, 2013; Worthy et al., 2013). When an animal is not post-prandial but the metabolic measurements are done with the animal inactive, the measurements are commonly referred to as resting metabolic rate. Comparative studies on cardiac function, e.g. f_H and SV, on the other hand, generally lack strictly defined conditions that allow a direct comparison. For example, it is often not reported whether the study subjects were post-prandial (Fahlman et al., 2019b; Jones et al., 1973; Kaczmarek et al., 2018; Noren et al., 2004), even though this has been shown to alter the metabolic rate and cardiac function (Young et al., 2011). Similarly, many studies investigating cardiac function either in marine or terrestrial mammals do not report f_R, despite its known effect on f_H (Cauture et al., 2019; de Burgh Daly, 1986; Guillén-Mandujano and Carrasco-Sosa, 2014). While this may not be critically important for studies on animals that breathe more or less continuous, even terrestrial mammals experience considerable variation in f_H when breathing intermittently (Laske et al., 2010). In marine mammals, and specifically cetaceans, where breaths are separated by long respiratory pauses and significant changes in both f_H and SV have been observed following a breath (Figs. 1-3), but how much variation in average f_H does changes in f_R cause?

As an example of the impact of f_R on f_H, the average f_H (± s.d.) for the false killer whale was 48 ±7 beats • min^{-1} during a period when the f_R was 1 breath • min^{-1}, as compared with 70 ± 9 beats • min^{-1} when f_R was 4 breaths • min^{-1}. In the bottlenose dolphin, the if_H varies from around 80-100 beats • min^{-1} immediately following an inspiration, to between 35-50 beats • min^{-1} between breaths (Fig. 3 and see Fig. 1 in Cauture et al., 2019). Consequently, the f_R significantly affects the f_H for an inactive animal when measuring the number of beats over a pre-determined duration, and is especially dependent on where in the breathing cycle f_H is measured. In previous studies in the bottlenose dolphin, where the effect of f_R was not evaluated or considered, the f_H for an inactive animal was between 78-105 beats • min^{-1} (Noren et al., 2004; Noren et al., 2012; Williams et al., 1993; Williams et al., 2015). From these comparisons it may be prudent to suggest that the measurement conditions, such as fasted or fed, and f_R should be reported. In addition, the conditions for f_H for an inactive animal need to be clearly defined to allow comparisons between studies and species (Belkin, 1964; Kooyman, 1985). This may be particularly important in studies that attempt to evaluate the evolutionary significance of the dive response, and those used to compare cardiac measurements across species, especially marine and terrestrial, as the results may be affected by the f_R. By RSA-correction, comparisons within and between species and activity states (inactive, swimming, diving) could be made and be independent of f_R (Cauture et al., 2019; Fahlman et al., 2019b; Miedler et al., 2015).

4.3. Evaluation of the limitations of RSA-corrected values

From the data presented here and in previous studies (Andrews et al., 1997; Bickett et al., 2019; Cauture et al., 2019; Fahlman et al., 2019b; Harms et al., 2013; Kaczmarek et al., 2018; McDonald et al., 2018; Miedler et al., 2015), it appears that f_R can have a significant effect on measured cardiac function. While terrestrial mammals experience large changes in f_H when f_R is modified (Laske et al., 2010; Mortola et al., 2016), we are not aware of any study in terrestrial mammals that have attemped to remove the effect of f_R on f_H, making direct comparisons difficult. With increasing f_R, the RSA becomes less obvious as the average f_H during the interbreath interval remians elevated. The almost continuous breathing pattern in terrestrial mammals may be one reason why the effect of f_R on f_H has not been considered in comparative studies.

However, while RSA-correcting data may provide comparative cardiac indices, it does not provide a true estimate of the average f_H, SV or CO in an inactive cetacean as it omits the dynamic period from the end of the breath until the if_H and iSV have stabilized after about 12-20 s (Fig.1-3). Thus, the period of elevated if_H and iSV is not accounted for, and the estimated value would be lower than the value from continuous measurements. Using the temporal relationships between time, if_H and iSV (section 3.1) the average f_H and SV for different f_R's were estimated. For an f_R of 2 breaths • min^{-1}, the average f_H, SV, and CO would be 11% (e.g. 54 beats • min^{-1} as compared to 48 beats • min^{-1}, section 3.3), 6%, and 16% higher than the RSA-corrected value. At an f_R of 6 breats • min^{-1} the same values would be 25%, 14%, and 35% higher than the RSA-corrected value, respectively. For an f_R of a terrestrial mammal of 170 kg the average values would be similar to that predicted from a terrestrial mammal (Fig. 4). Thus, when acounting for the differences in f_R it appears that the average f_H and SV are similar between marine and terrestrial mammals of comparable body size. However, these calculations are done for the bottlenose dolphin, and clearly a better understanding of the cardiorespiratory coupling in mammals is important to correctly assess physiological differences without confounding effects between species..

4.4. Species differences in RSA-corrected f_H, SV and CO is not only due to size

While the RSA-corrected f_H and SV do not account for the values during the dynamic period, they do provide values that are not confounded by breathing and can therefore be used for comparative purposes between species. The data in Fig. 4 show that the RSA-corrected f_H was lower than that predicted from terrestrial mammals by between 34-51% for all species (Fig. 4). The RSA-corrected SV for an inactive animal was 94% greater in the juvenile beluga as compared with the predicted value from a 170 kg terrestrial mammal (Fig. 4, Bishop, 1997; Seymour and Blaylock, 2000; Stahl, 1967). In the dolphin, the RSA-corrected SV was 9% lower as compared with a similar sized terrestrial mammal (Fig. 4), while for the false killer whale, the RSA-corrected SV was almost the same as compared with a similar sized terrestrial mammal (Fig. 4) (Seymour and Blaylock, 2000). The RSA-corrected CO in the dolphin and false killer whale were both 34% and 33% lower, respectively, as compared with a similar sized terrestrial mammal (Bishop, 1997; Stahl, 1967). In the beluga, on the other hand, the RSA-corrected CO was 19% higher as compared with a terrestrial mammal of similar size, mainly due to the high SV. Interestingly, both the RSA-corrected resting SV and CO were considerably higher in the beluga calf as compared with the bottlenose dolphins of similar size (Fig. 4). As heart size correlates directly with M_b during ontogeny in smaller cetaceans (Mallette et al., 2016; McLellan et al., 2002), it is unlikely that these differences are casued by variation in heart size and body mass growth. However, the aortic valve orifice was greater in both the larger adult false killer whale and juvenile beluga as compared with the dolphins. Thus, aortic valve orifice diameter may be an important anatomical bottleneck in cardiovascular performance as its size appears to limit SV, and thereby CO. Anatomical data on aortic valve orifice diameter from different species, age classes, and sexes would be an interesting study to investigate how anatomy may limit cardiovascular performance.

4.6. Cardiorespiratory coupling in inactive marine and terrestrial mammals and its ecophysiological significance

To compare the cardiorespiratory coupling between species, we plotted f_R and f_H from the bottlenose dolphin, the false killer whale and juvenile beluga, the killer whale, and pilot whale. Reported data for the harbor porpoise, adult beluga and gray whale that were available from the literature were also included (Bickett et al., 2019; Elmegaard et al., 2019; Ponganis and Kooyman, 1999; Wahrenbrock et al., 1974). This regression provide an estimate how f_H varies with changes in f_R between cetaceans (Fig. 5). As a comparison we plotted f_R and f_H for terrestial mammals using allometric equations (Stahl, 1967). We also added data points for the donkey, Thoroughbred horse, and elephant as the allometric equations develop by Stahl (1967) did not account for animals larger than man. The resulting regression showed that the slope of the regresstion of f_R and f_H is much greater in the cetacean as compared with terrestrial mammals extrapolated from the allometric equations by Stahl (1967). This is a conservative estimate, as the addition of data points for the elephant, horse and donkey in the M_b range from 165-6000 kg suggest that the regression is even less steep. In other words, for each breath in a ceatacean there are almost 12 heart beats, while the same in a terrestrial mammal is 3 (Fig. 5). This difference is due to a large change in f_H for each breath in the cetacean, causing a greater RSA as compared with a terrestrial mammal. In the human, a breath casues a change in f_H of between 10-20 beats, while in in the bottlenose dolphin this variation is between 40-60 beats (Cauture et al., 2019; Mortola et al., 2016). The results here further supports this and show that f_H changes associated with f_R are greater in cetaceans (Fig. 5), the dynamic changes between breaths are consistent between cetacean species (Fig. 3), and affect both f_H and SV (Fig. 2). Past work has shown that the f_H f_R^{-1} is significantly greater not only in cetaceans, but also in a larger range of marine mammals (Mortola, 2015). It was suggesed that this is a non-respiratory adaptation to provide additional bouyancy. While this is an interesting suggestion, we propose that this greater cardiopulmonary coupling may be a physiological trait that help improve gas exchange. Interestingly, when overlyaing the data for the hibernating bear, although lower, it is closer to the line for the cetaceans as compared with the terrestrial mammals (Fig. 5). It was suggested that this extensive RSA in the hibernating bear would help conserve cardiac work while respiratory coupled tachycadia would help to maintain gas exchange and tissue perfusion (Laske et al., 2010). In the elephant seal, it was suggested that the differencecs in cardiorespiratory responses at sea versus on land could be a way to ensure rapid recovery rather than adequate gas exchange (Andrews et al., 2000). Thus, the steeper slope for cetaceans may indicate a greater cardiovascular response for each breath, which may help maximize gas exchange during short surface intervals.

In the northern elephant seal (*Mirounga angustirostris*), development of RSA appears to correlate postitively with the duration of an apneic event, and negatively with both apneic and eupneic f_H (Castellini et al., 1994b). It was also reported that the changes in f_H during diving at sea, during a sleep apnea on land, or with RSA were similar and it was suggested that these may all be regulated by a similar mechanism (Castellini et al., 1994a). In another study, the average f_H and f_R while at the surface at sea were considerably higher (f_H: 107±3 beats • min^{-1}; f_R: 22.0 ± 2.0 breaths • min^{-1}) as compared with that on land (f_H: 65± 8 beats • min^{-1}; f_R: 9.2±1.3 breaths • min^{-1}), but the mean apnea f_H were not different at sea or on land (Andrews et al., 2000). Thus, the higher at sea surface f_H may, at least in part, be confounded by a higher f_R as the animal returns to the surface to breath. In a study on harbour seals, it was noted that at times the seals performed what was termed “top-up” dives, where the seal spent a brief moment at the surface, only long enough to take a single breath, and then submerged again (Fedak et al., 1988). It was suggested that these top-up dives could be important circulatory events that helped improve gas exchange and reduce recovery time (Andrews et al., 1997; Fedak et al., 1988). A more recent theoretical study showed that the cardiac responses during the surface interval following shallow and deep diving in bottlenose dolphins is to maximize gas exchange, which helps to replenish more rapidly the O_2 stores and remove the CO_2 and thereby minimize the surface duration (Fahlman et al., 2018). Thus, the greater cardiovascular response to breathing in marine mammals may be a physiological trait that ensures rapid gas exchange and help reduce the surface interval during a foraging bout.

5. Conclusions

This is the first study to report comparative semi-continuous measurements of f_H, SV, and CO measurements, and continuous ECG in a range of small and medium sized odontocetes. We have shown that both if_H and iSV initially increase and then decreases exponentially following a breath, which agrees with data from static breath-holds and following high intensity exercise in the bottle nose dolphin (Fahlman et al., 2019b; Miedler et al., 2015). Of special interest is the higher mass-specific SV and CO in the beluga calf as compared to the similar sized dolphins and the larger false killer whale, possibly reflecting increased metabolic demand due to active growth. In addition, we show that in cetaceans f_H respond more strongly to variation in f_R as compared with terrestrial mammals, resulting in a greater RSA in the former. We propose that RSA may be an important to maximize gas exchange at the surface to minimize the time at the surface between dives. The data presented in the current study provide valuable comparisons within and between species that are important to understand physiological limitations in cetaceans.

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7. Competing interests

The authors declare no competing or financial interest.

8. Authors contributions

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

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10. Data Availability

The data used in this study are freely available upon request to afahlman@whoi.edu or can be found at the following link: https://osf.io/wdfmz.

11. List of abbreviations

CO: cardiac output, l • min^{-1}

-f_H: heart rate, calculated over a certain period of time, beats • min^{-1}

-f_R: breathing frequency, calculated over a certain period of time, breaths • min^{-1}

-if_H: instantaneous heart rate, calculated for each heart rate as 60 s divided by the period between R-peaks, beats • min^{-1}

-if_R: instantaneous breathing frequency, calculated for each breath as 60 s divided by the period between two breaths, breaths • min^{-1}

M_b: body mass, kg

SV: stroke volume, the volume of blood pumped out by the heart for each beat, ml beat^{-1}

V_T: tidal volume, the volume inhaled or exhaled during a breath

ECG: electrocardiogram

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Table 1. Morphometric details of participating animals.

Animal ID	Species	Sex	M_b (kg)	Birth year (Yr)	Aortic Valve Orifice diameter (cm)	f_R (breaths □ min^{-1})
Oo1	*Oo*	F	1904	2006		
Oo2	*Oo*	F	1974	2007		
Oo3	*Oo*	F	2375	1976		
Gm1	*Gm*	F	671	2011		
Gm2	*Gm*	F	741	2011		
Gm3	*Gm*	M	1122	2011		
KE	*Pc*	F	500-545	----	7.1	4.5±2.1
RNV18-00508	*Dl*	M	160 ±15 (162±20)	2016	5.4	1.9±1.5
Tt4529	*Tt*	F	159	1989	3.1	3.6±1.5
*Tt9772	*Tt*	M	164	1992	3.0	5.5±1.9
*Tt7601	*Tt*	M	182	2004	3.2	4.8±1.3
*Tt6511	*Tt*	M	140	2013	3.2	6.0±2.7
*Tt8725	*Tt*	F	161	2003	3.4	7.0±3.1
*Tt4560	*Tt*	M	151	2006	3.4	6.8±3.2
*Tt5550	*Tt*	F	146	2006	---	NA
*83H1	*Tt*	M	140	2008	3.4	4.6±2.5
*9FL3	*Tt*	M	235	1997	3.2	4±3.8
*9ON6	*Tt*	M	184	2000	3.4	4.4±3.8

*01L5	*Tt*	M	155	1985	3.2	5±4.2
*63H4	*Tt*	M	171	1991	---	5.7±4.5
*6JK5	*Tt*	M	207	1995	3.6	4.8±2.5
*99L7	*Tt*	M	178	1994	---	4.7±3.1

Animal id, species (*Orcinus orca-Oo, Globicephala macrorhynchus-Gm, Pseudorca crassidens-Pc, Delphinapterus leucas –Dl; Tursiops truncatus-Tt*) sex (F-female, M-male), body mass (M_b, kg), approximate year of birth for wild caught animals or year born for animals born under human care, aortic valve orifice diameter, and breathing frequency (f_R). *Previously published data (Fahlman et al., 2019b).

Figures

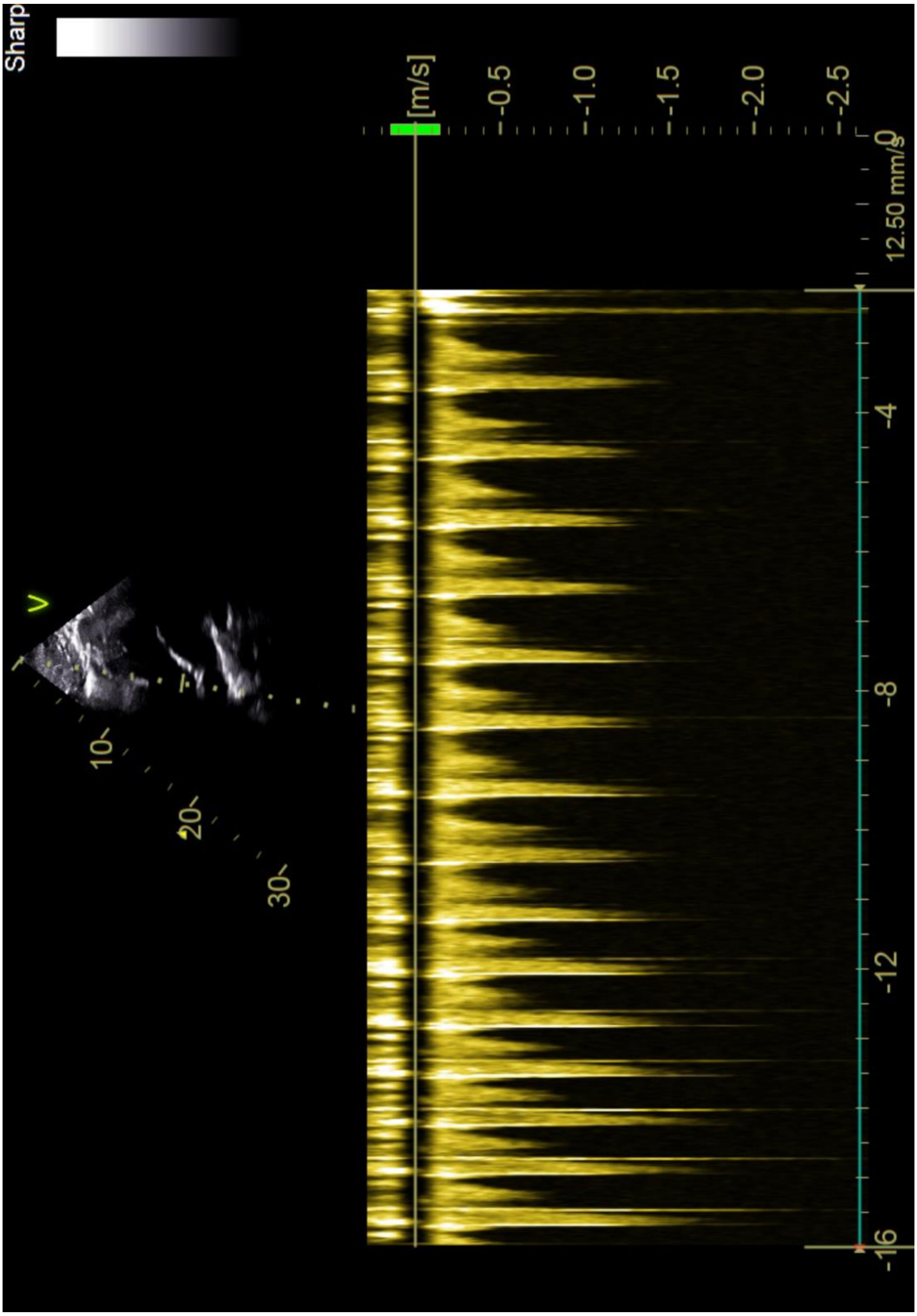


Figure 1. Trans-thoracic echocardiography immediately following a breath in a bottlenose dolphin, showing the Doppler flow profile at the left ventricular outflow tract using continuous flow Doppler. The flow begins as the aortic valve opens, which increases the flow velocity up to its maximum peak velocity, followed by a continuous decrease until the aortic valve closure. The figure shows how heart rate (duration between peaks) and stroke volume (area under each curve) changes throughout the interbreath interval.

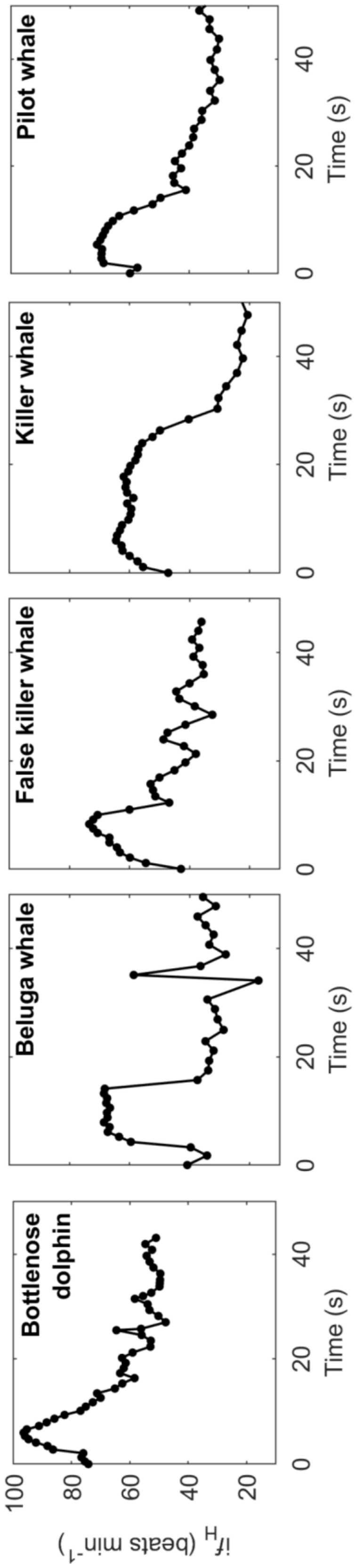
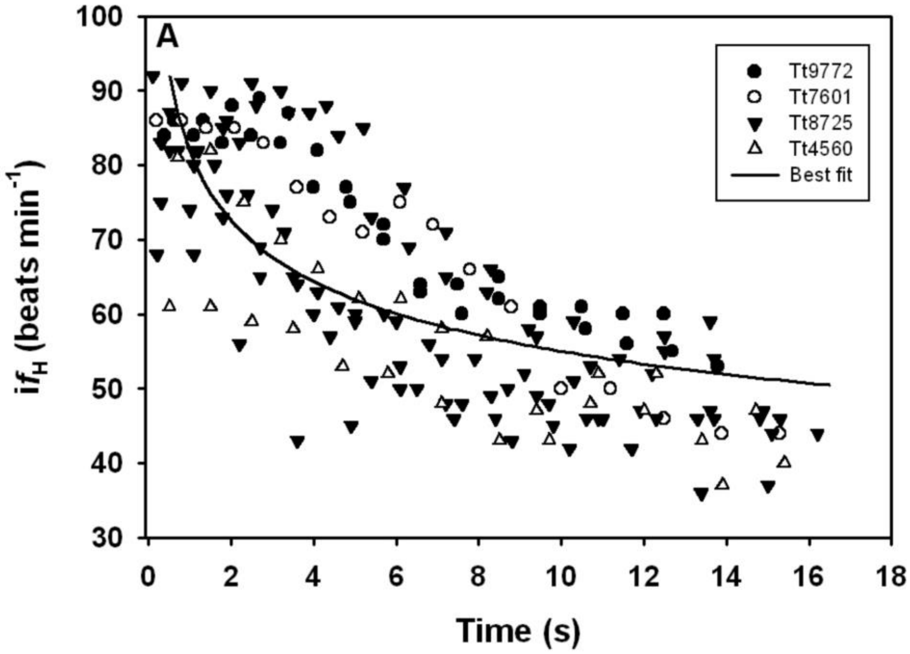
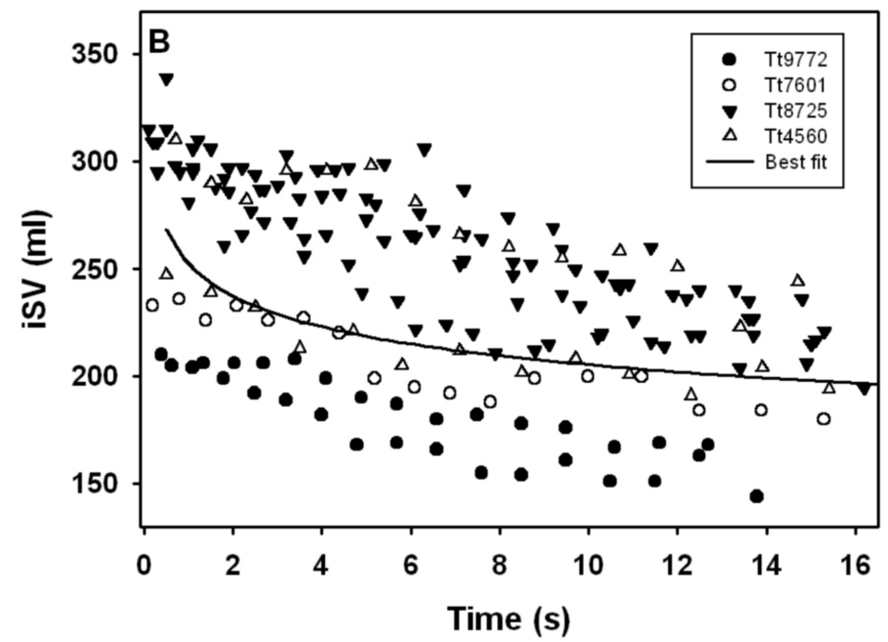


Figure 2. Representative instantaneous heart rate (if_H) against time following a breath in a bottlenose dolphin, a beluga calf, an adult false killer whale, and adult killer whale and an adult pilot whale**.**


A
Tt9772
Tt7601
Tt8725
Tt4560
Best fit
if_H (beats min^{-1})
100
90
80
70
60
50
40
30
0
2
4
6
8
10
12
14
16
18
Time (s)


B
Tt9772
Tt7601
Tt8725
Tt4560
Best fit
iSV (ml)
350
300
250
200
150
0
2
4
6
8
10
12
14
16
Time (s)

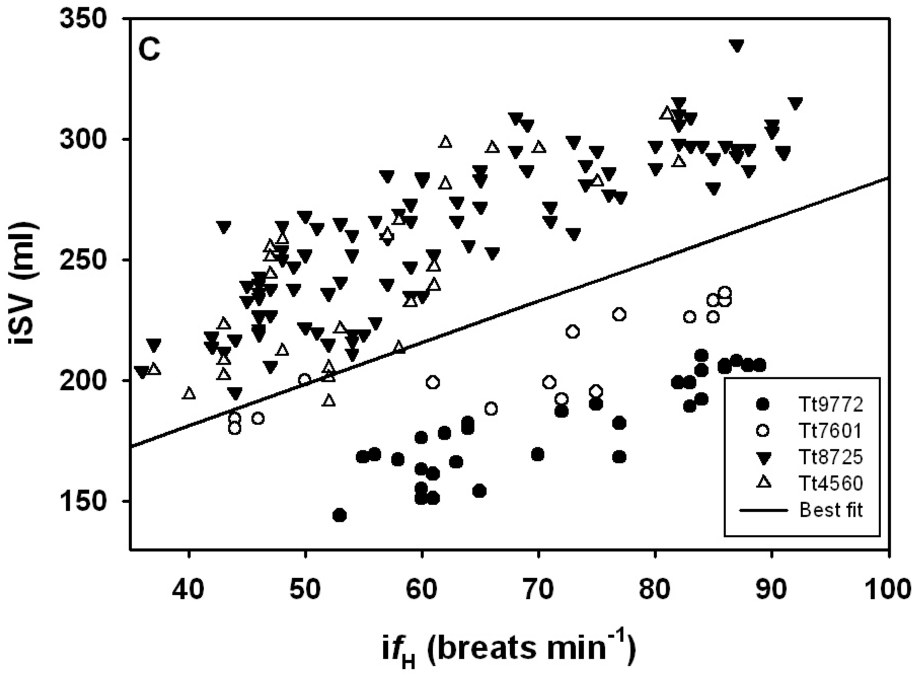


Figure 3. Scatter plot showing the change in instantaneous A) heart rate (if_H) B) and stroke volume (iSV) immediately following a breath (time 0 is end of inhalation) in 4 bottlenose dolphins, and C) the correlation between if_H and iSV. Both if_H and iSV increased following the end of the inhalation of a breath and then decayed exponentially (solid line in panels A and B and see results for details). The solid line in C is the best fit regression used to predict iSV from if_H.

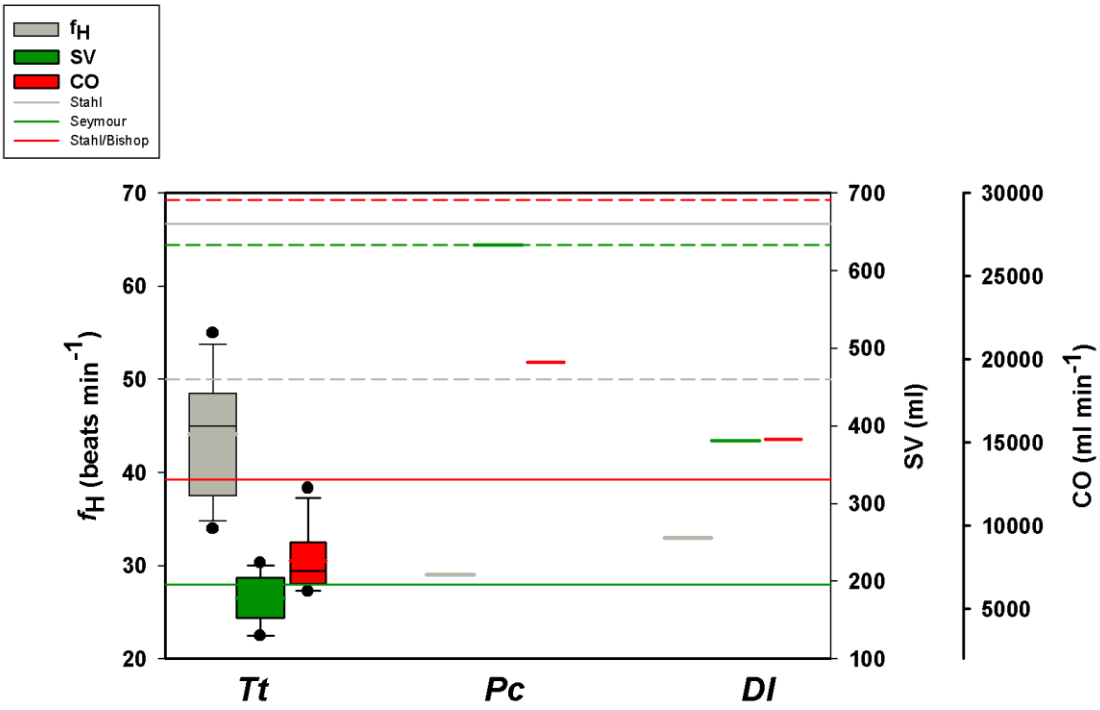


Figure 4. Box plots showing average heart rate (f_H, n=13 cetaceans with 11 dolphins), stroke volume (SV, n=11, cetaceans with 9 dolphins), and cardiac output (CO, n = 11) during rest in adult bottlenose dolphins (*Tursiops truncatus*, *Tt*), a beluga calf (*Delphinapterus leucas*, *Dl*), and an adult false killer whale (*Pseudorca crassidens*, *Pc*). Also shown on this figure are the predicted f_H (gray), SV (green) and CO (red) horizontal lines for a 170 kg (solid line) and 520 kg (broken line) terrestrial mammal (Bishop, 1997; Seymour and Blaylock, 2000; Stahl, 1967). As only 1 individual beluga and false killer whale was measured, the average values for these species are shown as solid horizontal lines without error bars.

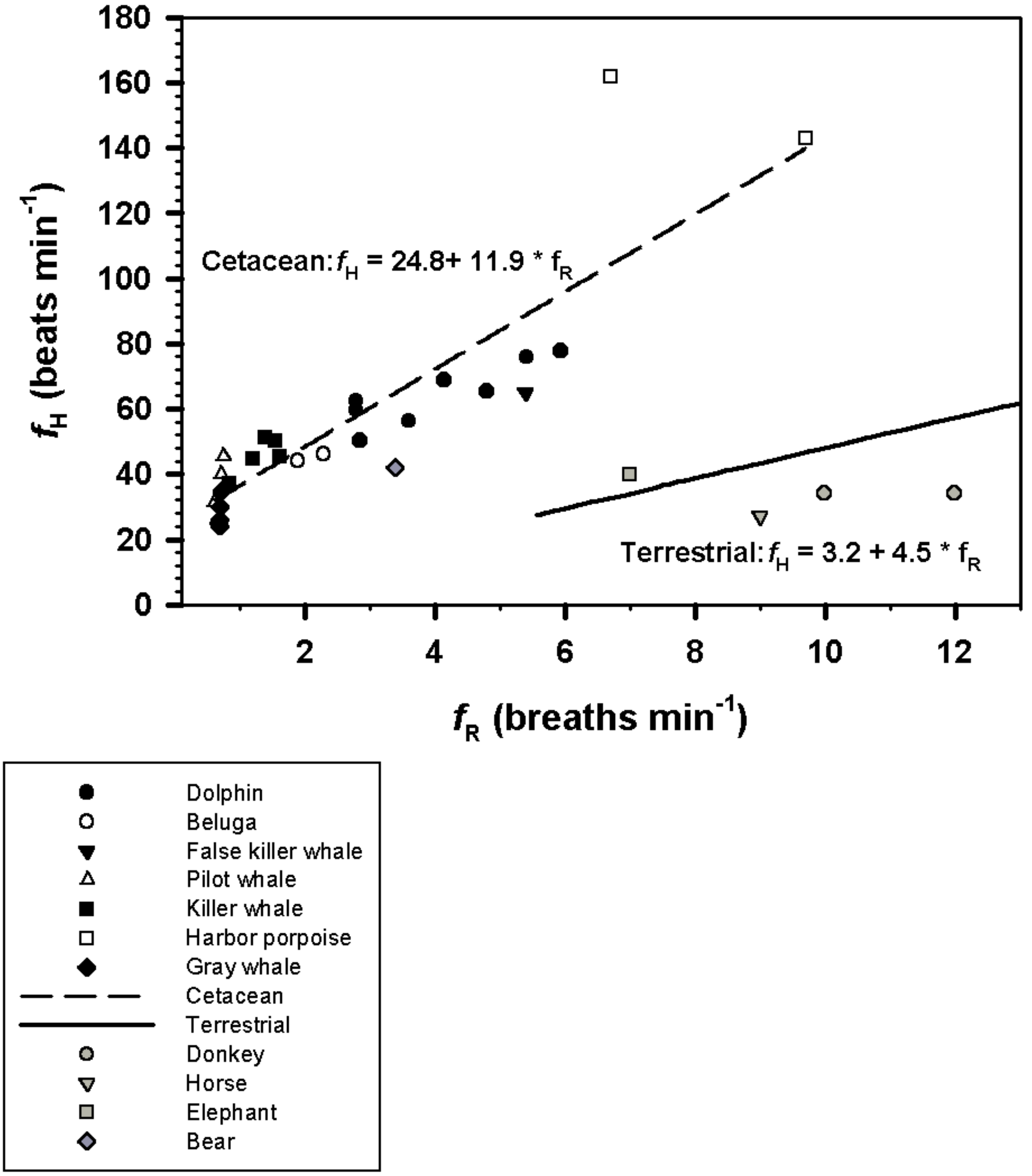


Figure 5. Scatter plot showing heart rate (f_H, beats • min^{-1}), and breathing frequency (f_R, breaths • min^{-1}) for different cetaceans (bottlenose dolphins [*Tursiops truncatus*], beluga [*Delphinapterus leucas*], false killer whale [*Pseudorca crassidens*], pilot whale [*Globicephala macrorhynchus*).], killer whale [*Orcinus orca*], harbor porpoise [*Phocoena phocoena*], gray whale [*Eschrichtius robustus*]) (Bickett et al., 2019; Elmegaard et al., 2019; Ponganis and Kooyman, 1999; Wahrenbrock et al., 1974). The regression lines for the cetacean data was: $f_H = 24.8 + 11.9\ f_R$, $r^2 = 0.82$). The regression for f_H ($f_H = 241\ M_b^{-0.25}$) and

f_R (f_R=53.5 $M_b^{-0.26}$) for terrestrial mammals was created from the allometric relationships presented in (Stahl, 1967) measured from rat to human (0.2-80kg). The regression line is extrapolated for values beyond this body mass range and start from 200 kg up to 6000 kg. Gray points are for the donkey (165-190 kg) (Yousef and Dill, 1969), the Thoroughbred horse (*Equus ferus caballus*, 448kg) (Kuwahara et al., 1996), and the African (*Loxodonta africana*) and Asian elephant (*Elephas maximus*, 1420-6000kg) (Benedict and Lee, 1936; Honeyman et al., 1992; Olivares et al., 2019). The data for the hibernating American black bear (*Ursus americanus*) was digitally extracted (Laske et al., 2010) to show comparative data in the same range of f_R's as cetaceans.