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RESEARCH ARTICLE

Evaluating cardiac physiology through echocardiography in bottlenose dolphins: using stroke volume and cardiac output to estimate systolic left ventricular function during rest and following exercise

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ABSTRACT

Heart-rate (f_H) changes during diving and exercise are well documented for marine mammals, but changes in stroke volume (SV) and cardiac output (CO) are much less known. We hypothesized that both SV and CO are also modified following intense exercise. Using transthoracic ultrasound Doppler at the level of the aortic valve, we compared blood flow velocities in the left ventricle and cardiac frequencies during rest and at 1, 3 and 4 min after a bout of exercise in 13 adult bottlenose dolphins (*Tursiops truncatus*, six male and seven female, body mass range 143–212 kg). Aortic cross-sectional area and ventricle blood velocity at the aortic valve were used to calculate SV, which together with f_H provided estimates of left CO at rest and following exercise. f_H and SV stabilized approximately 4–7 s following the post-respiratory tachycardia, so only data after the f_H had stabilized were used for analysis and comparison. There were significant increases in f_H, SV and CO associated with each breath. At rest, f_H, SV and CO were uncorrelated with body mass, and averaged 41±9 beats min^{-1}, 136±19 ml and 5514±1182 l min^{-1}, respectively. One minute following high intensity exercise, the cardiac variables had increased by 104±43%, 63±11% and 234±84%, respectively. All variables remained significantly elevated in all animals for at least 4 min after the exercise. These baseline values provide the first data on SV and CO in awake and unrestrained cetaceans in water.

INTRODUCTION

The volume of blood leaving the left heart (the cardiac output, CO) is a product of the volume of blood ejected per heart beat (stroke volume, SV) and the heart rate (f_H). In many species, elevated O_2 demand causes a greater change in f_H than in SV (Barger et al., 1956; Grubb et al., 1983; Grubb, 1982; Ponganis et al., 1990), which has led some to suggest that f_H may be a good approximation of CO. However, several factors affect SV, and the relative increase from rest to maximal exercise is almost 3-fold for a range of different mammals (Bishop, 1997).

Marine mammals combine surface breathing with long periods underwater holding their breath. This results in a physiological conflict as exercise causes increased O_2 demand, while breath-hold diving results in O_2 limitation (Ponganis, 2011). Few studies have measured CO in live marine mammals, and most of these have been made in pinnipeds (Blix et al., 1983, 1976; Elsner et al., 1964; Murdaugh et al., 1966; Ponganis et al., 1991, 1990; Sinnett et al., 1978; Zapol et al., 1979; Sommer et al., 1968). Although it has been recognized that stress significantly affects physiological responses and probably affects any extrapolations to normal cardiac function, only three of these studies were performed in unrestrained and/or free-swimming animals (Elsner et al., 1964; Ponganis et al., 1991, 1990). Both the harbor seal (*Phoca vitulina*) and California sea lion (*Zalophus californianus*) had a metabolic scope at near-maximal exercise level, about 9 times the resting level, suggesting that the animals were exercising at near-maximal levels (Ponganis et al., 1991, 1990). In sea lions, SV and f_H were not reported (Ponganis et al., 1991), but CO and f_H in seals increased while SV decreased as the animal was swimming at the surface (Ponganis et al., 1990). A similar increase in CO was observed during exercise in the submerged harbor seal, while SV also increased slightly (Ponganis et al., 1990). In exercising bottlenose dolphins, a linear increase in f_H was observed with increasing metabolic rate (Williams et al., 1993), but SV was not measured. Given the conflicting effects of O_2 delivery during exercise and submersion, determining the cardiovascular responses is important to understand physiological function in cetaceans.

In cetaceans, no published data exist on cardiac contractile responses during forced or voluntary dives, with most studies being largely focused on cardiac frequency (Houser et al., 2010; Noren et al., 2004). One study measured CO in anesthetized bottlenose dolphins (Sommer et al., 1968), but the experimental artifacts of mechanical ventilation under general anesthesia and restraint out of water may have altered normal physiological function, so these data may have to be viewed with caution when compared with awake individuals.

Non-invasive cardiac transthoracic echocardiography is an established method to determine CO in humans and animals (Bouchard et al., 1987; Quiñones et al., 2002). This method has been attempted in bottlenose dolphins with limited success because of anatomical difficulties (Miedler et al., 2008). The inflated lung limits the view of the heart and, as cetaceans have a respiratory pause following inspiration (Fahlman et al., 2015; Kooyman and Cornell, 1981), their respiratory pattern makes it difficult to find appropriate acoustic echocardiographic windows. Thus, current estimates of CO in cetaceans, and how they may be modified during diving, are based on estimated values from pinnipeds and terrestrial species. These direct extrapolations could significantly bias our understanding of the physiology of cetaceans and the results of gas dynamics modeling regarding diving performance.

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The aim of this study was to provide estimates of f_H, SV and CO in the bottlenose dolphin, both at rest and following a short bout of intense exercise. We hypothesized that both f_H and SV change following intense exercise. Bottlenose dolphin species-specific data provided in this preliminary study will enhance our ability to more accurately understand the physiological limitations and gas exchange dynamics in cetacean diving physiology.

MATERIALS AND METHODS
Animals
Continuous flow Doppler ultrasound was used to measure f_H and SV at the aortic tract level during 32 rest and 31 post-exercise sessions from six adult male and six adult female Atlantic bottlenose dolphins [*Tursiops truncatus truncatus* (Montagu 1821)] and one female Black Sea bottlenose dolphin [*Tursiops truncatus ponticus* (Barabasch-Nikiforov 1940)] ranging from 6 to over 30 years old, housed at the Oceanografic, Valencia, Spain, between 2009 and 2011 (Table 1). While the study spanned 3 years, repeated measurements on the same animal were not separated by more than 1 year. The name, body mass and age (known or estimated) of the animals at the time of the study are summarized in Table 1.

Animal activity
We assumed that animal fitness would correlate with the daily activity level of each individual. Therefore, dolphins were divided into two groups: (1) animals in the high-activity group (H, Table 1) performed at least four different high-intensity training sessions per day over at least 1 year, while (2) animals in the low-activity group consisted mainly of breeding females that did not participate in daily high-intensity sessions during the year previous to the study (L, Table 1).

Experimental trials
All experiments were performed using operant conditioning and participation by the dolphins was voluntary (the animals were not restrained and could refuse to participate or withdraw at any point during the experimental trial). Each experiment consisted of an animal staying stationary in the water in left lateral recumbence, allowing us to find the left ventricular outflow tract by ultrasound probe placement.

To evaluate the effect of exercise, the dolphin was asked by the trainer to perform a minimum of four and up to six non-stop sequences of maximum intensity exercise, e.g. tail walks, fast speed swim and high jumps. The trainer continued to signal for additional behaviors until the dolphin showed initial signs of fatigue as assessed by an experienced trainer and veterinarian, based on animal attitude and the depth and frequency of respiration. The average duration of an exercise series was 1.5 min. The animal was then returned immediately to the trainer and was placed in position for ultrasound examination. The time taken to locate the aortic tract differed between animals, but always occurred within the first minute post-exercise. Once located, three repeated measurements of f_H, aortic diameter and ventricular flow were made and registered at 1, 3 and 4 min following the exercise. Because of the significant tachycardia associated with respiration, three repeated measurements of f_H and ventricular flow were made for each session. Ultrasound Doppler data were obtained at least 7 s after a breath, when the cardiac frequency had stabilized. Initial assessment indicated that both f_H and SV increased significantly following respiration effort (Fig. 1).

Ultrasound data acquisition
A Vivid-i (General Electric) ultrasound machine with a 2.5–3.5 MHz probe was used to obtain and register left ventricle outflow tract velocity (Fig. 2). f_H was estimated from the flow traces and SV was calculated from the integrated blood flow velocity times and the aortic cross-sectional area based on the aortic diameter at the level of the aortic valve. Accurate flow measurement requires the Doppler beam to be parallel to the flow of blood and the aortic annulus diameter to remain unchanged during systole (Quiñones et al., 2002). We measured the aortic diameter at the level of the insertion of the valve (valve annulus) at the different cardiac phases to confirm that the annulus diameter did not change during the different phases of the systole and for different flow rates. The aortic cross-sectional area was confirmed to be circular, and assumed to be representative of the cross-sectional area of the outflow tract where the blood flow was measured. SV was estimated as $SV=r^2\pi VTI$, where r is the radius of the aortic cross-sectional area and VTI is the velocity time integral at the same location. CO was then calculated as the product of f_H and SV, $CO=f_H \times SV$.

Data assessment and statistical analysis
The relationships between dependent variables (CO, SV and f_H) and experimental covariates (animal condition: pre-exercise and 1, 3 and 4 min post-exercise; trial number; body mass; exercise group: high or low intensity) were analyzed using linear-mixed effects models (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, version 3.1.0, 2014). 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). Initially, a univariate analysis on each independent variable was performed; only those variables with a P-value <0.10 (Wald's tests) were considered in a multivariate analysis. The best models of the remaining variables were chosen by the Akaike information criterion (AIC) against the null model (AIC_{null}), and significant parameters assessed by the t-value between the estimate and its standard error. Acceptance of significance was set to the $P<0.05$ level, and $0.05<P<0.1$ was considered a trend. Data are presented as the mean±s.d., unless otherwise stated.

RESULTS
f_H, SV and CO during rest
Neither body mass nor basal activity level warranted inclusion in the model (generalized linear model with animal as a random factor), and the average resting f_H (41±9 beats min^{-1}, $P>0.1$), SV (136±19 ml, $P>0.5$) and CO (5514±1182 ml min^{-1}, $P=0.09$) were not significantly different between animals (Table 1). The average mass-specific f_H, SV and CO were 0.237±0.067 beats min^{-1} kg^{-1}, 0.788±0.135 ml kg^{-1} and 32.2±9.2 ml min^{-1} kg^{-1}, respectively.

f_H, SV and CO during recovery from exercise
A trial consisted of ultrasound evaluation pre- and post-exercise (see Materials and methods) and trials were repeated up to three times for some animals on different days (Table 1). For f_H, the best model to describe the effect of exercise included exercise status [AIC=966, AIC_{null}=1105, $P<0.05$, $f_H=40+(42×1 min)+(14×3 min)+(9×4 min)$, where 1 min, 3 min and 4 min are 0, except for the specified time. For example, the estimated f_H 1 min following exercise would be $f_H=40+(42×1)+(14×0)+(9×0)$], while body mass ($M_b$; AIC=1105, $P>0.1$), activity level (AIC=1106, $P>0.4$) and trial number (AIC=1107, $P>0.7$) did not warrant inclusion. A Tukey multiple comparison test showed that exercise significantly increased f_H at 1, 3 and 4 min post-exercise (Fig. 3A; Z-value all <−2.19, $P<0.05$).

The best model to describe the effect of exercise on SV included exercise status [pre-exercise/rest, and 1, 3 and 4 min post-exercise, AIC=1160, AIC_{null}=1296, $P<0.05$, $SV=135+(88×1 min)+(35×3 min)+(15×4 min)$, where 1 min, 3 min and 4 min are 0, except for the specified time], while M_b (AIC=1298, $P>0.5$), activity level (AIC=1298, $P>0.5$) and trial number (AIC=1298, $P>0.6$) did not warrant inclusion. A Tukey multiple comparison test showed that exercise significantly increased SV (Fig. 3B) at 1, 3 and 4 min post-exercise (Z-value all <−2.68, $P<0.01$).

The best model to describe the effect of exercise on CO included exercise status [AIC=2360, AIC_{null}=2518, $P<0.05$, $CO=5309+(13,087×1 min)+(3923×3 min)+(1948×4 min)$, where 1 min, 3 min and 4 min are 0, except for the specified time], while M_b (AIC=2517, $P>0.1$), activity level (AIC=2520, $P>0.8$) and trial number (AIC=2519, $P>0.7$) did not warrant inclusion. A Tukey multiple comparison test showed that exercise significantly increased CO post-exercise (Fig. 3C; Z-value all <−2.65, $P<0.01$).

Table 1. Animal ID, body mass, sex, age, exercise group, number of repeated tests, aortic radius, and resting heart rate, stroke volume and cardiac output of the study animals

Animal ID	Body mass (kg)	Sex	Age (years)	Exercise group	N	Aortic radius (cm)	$f_{H,rest}$ (beats min^{-1})	SV$_{rest}$ (ml)	CO$_{rest}$ (ml min^{-1})
Billie	164	F	30–35	L	1	1.60	47	134	6298
Kiara	200±5	F	30–35	L	3	1.45	35±4	104±7	3645±637
Damm*	180±5	M	10–12	H	3	1.60	34±3	155±17	5258±685
Elly	165±11	F	25–30	L	2	1.55	33±4	138±3	4560±679
Grisel	196±11	F	30–35	L	3	1.55	51±3	134±2	6806±421
India	168	F	25–30	H	1	1.50	40	127	5080
Lazo	169±4	M	15–20	H	3	1.50	42±6	118±8	4895±642
Laura	174±2	F	30–35	L	3	1.60	42±18	159±15	6853±3605
Lito	143±1	M	10–15	H	3	1.55	53±5	128±6	6782±378
Loreto	163±7	M	15–20	H	3	1.55	33±6	140±5	4578±797
Luiso	212	M	15–20	H	1	1.55	38	121	4598
Nika	189±2	F	30–35	H	3	1.95	27±4	177±16	4841±797
Rocky*	151±2	M	12–14	H	3	1.55	54±1	138±5	7482±332
Grand mean	175±20	–	–	–		1.57±0.12	41±9	136±19	5514±1182

Age is the approximate age at the time of study of wild-caught animals or the actual age calculated from the birth year for animals born under human care. Exercise group refers to the high-intensity (H) and low-intensity (L) groups. N is the number of repeated tests. $f_{H,rest}$ is resting heart rate, SV$_{rest}$ is resting stroke volume and CO$_{rest}$ is resting cardiac output. F, female; M, male.
*Animals born under human care.

DISCUSSION

This is the first study, to our knowledge, that provides data on SV and CO in awake, unrestrained bottlenose dolphins during rest and following exercise in water. Our data show that bottlenose dolphins

Fig. 1. Transthoracic ultrasound data during rest . Data showing f_H and aortic flow during rest for a period (A) in between breaths or (B) immediately following a breath.

adjust both f_H and SV to regulate CO. The variation in M_b between animals did not significantly affect our results. This study demonstrates that just a short intense exercise bout increased both f_H and SV, and thereby CO, for up to 4 min following the exercise bout. There were no significant differences in resting or post-exercise cardiac variables between dolphins in the two groups that routinely performed either high or low exercise. The relative increase was greater in f_H than in SV, resulting in an average 234% (range 145–387%) increase in CO at 1 min after exercise. Our results provide novel data on the cardiac physiology of cetaceans during rest and following exercise, and also provide baseline clinical information for diagnostic purposes that will help improve health assessment for both animals under human care and free-ranging cetaceans. Furthermore, cardiovascular physiology in cetaceans is important to better understand gas management during diving, field metabolic rate and marine mammal ecophysiology.

Numerous studies have measured f_H during restraint, semi-restraint, and voluntary diving and swimming in a range of breath-hold diving vertebrates (Andrews et al., 1997; Berkson, 1967; Blix et al., 1983; Folkow et al., 1967; Halsey et al., 2007; Hindle et al., 2010; Jones et al., 1973; Jones and Holeton, 1972; Noren et al., 2004; Ponganis et al., 1997, 1991, 1990; Scholander, 1940; Williams et al., 1993), and while there are marked differences in cardiac responses in forced versus voluntary/natural diving, most results indicate a significant diving bradycardia. In the few studies that have measured SV (Blix et al., 1983, 1976; Elsner et al., 1964; Folkow et al., 1967; Jones and Holeton, 1972; Murdaugh et al., 1966; Ponganis et al., 1991, 1990; Sinnett et al., 1978; Zapol et al., 1979), there appears to be disagreement whether SV changes during diving. If SV remains constant, CO can be estimated from f_H alone. This assumption has been made in the estimation of metabolic rate from f_H (Butler et al., 2004). In the current study, we found a marked increase in both f_H and SV following exercise (Fig. 3A,B). The relationship between exercise and submersion is complex; at high levels of exercise, the response during submersion is opposite to that during low-intensity exercise (Williams et al., 1993). Our data clearly show that both f_H and SV increase to meet elevated demands for O_2 delivery after intense exercise while breathing at the surface, which agrees with previous work on f_H in dolphins during intense exercise (Williams et al., 1993).
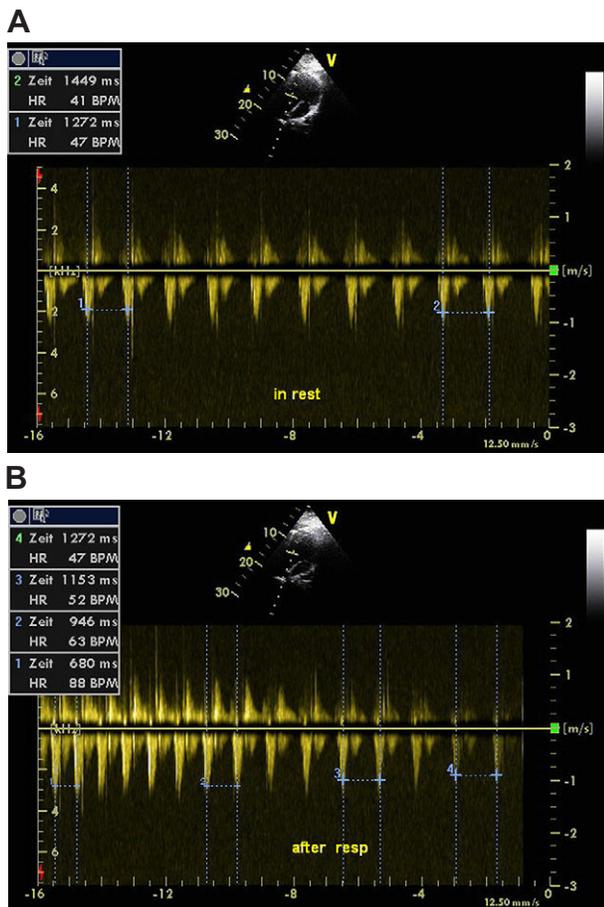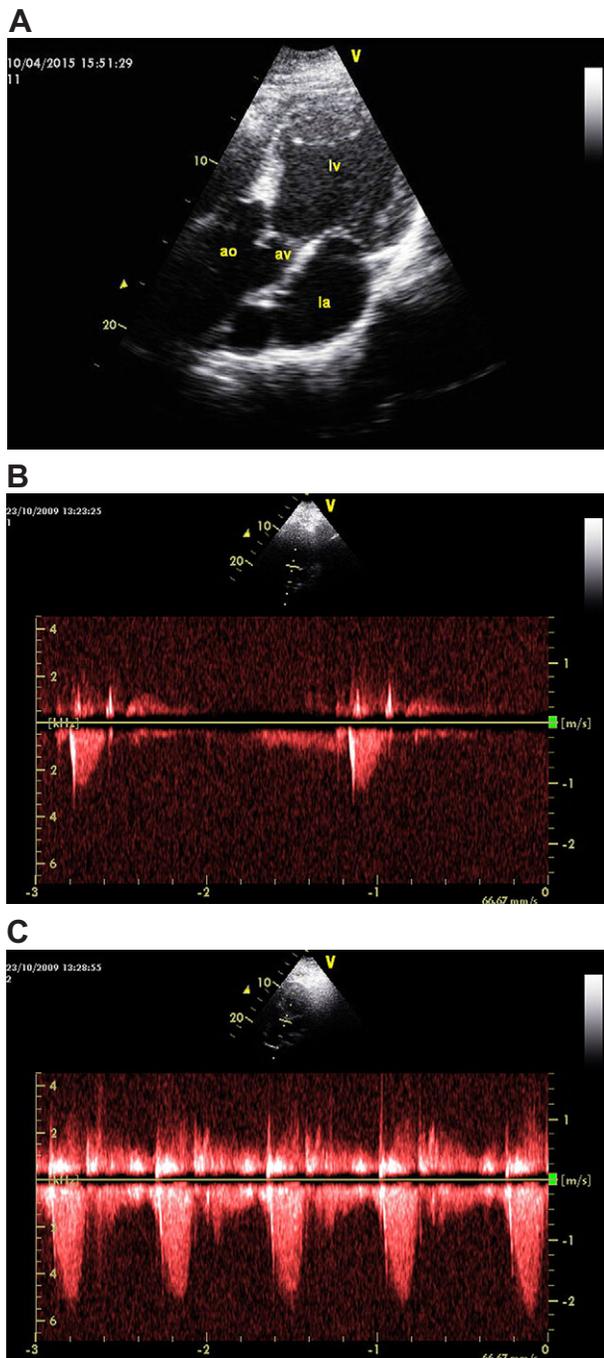
Fig. 2. Transthoracic ultrasound data before and after exercise. (A) Two-dimensional axis view of the dolphin heart showing the aorta (ao), aortic valve (av), left atrium (la) and left ventricle (lv). (B) Aortic continuous flow ultrasound during rest [stroke volume (SV) 122 ml, cardiac output (CO) 4400 ml min^{-1}] and (C) 1 min after exercise (SV 240 ml, CO 22,490 ml min^{-1}), both measured at the second half of the inter-breath period.

Fig. 3. Change in heart rate (f_{H}), SV and CO at 1, 3 and 4 min following a short intense exercise series. Data are expressed as the percentage change from pre-exercise (rest) levels (Table 1). All data up until 4 min after exercise were significantly different from pre-exercise levels ($P<0.05$; see Results).

The increase in f_{H} and the time to recovery (the Foster test) following intense exercise is a standard test in human exercise physiology to assess cardiovascular fitness (Maud and Foster, 2006). We hypothesized that dolphins that were involved in multiple daily sessions of high-intense exercise would have improved fitness and would therefore recover faster. However, our data do not support this hypothesis, as there were no differences in the temporal recovery rates between the two groups. It is possible that the animals
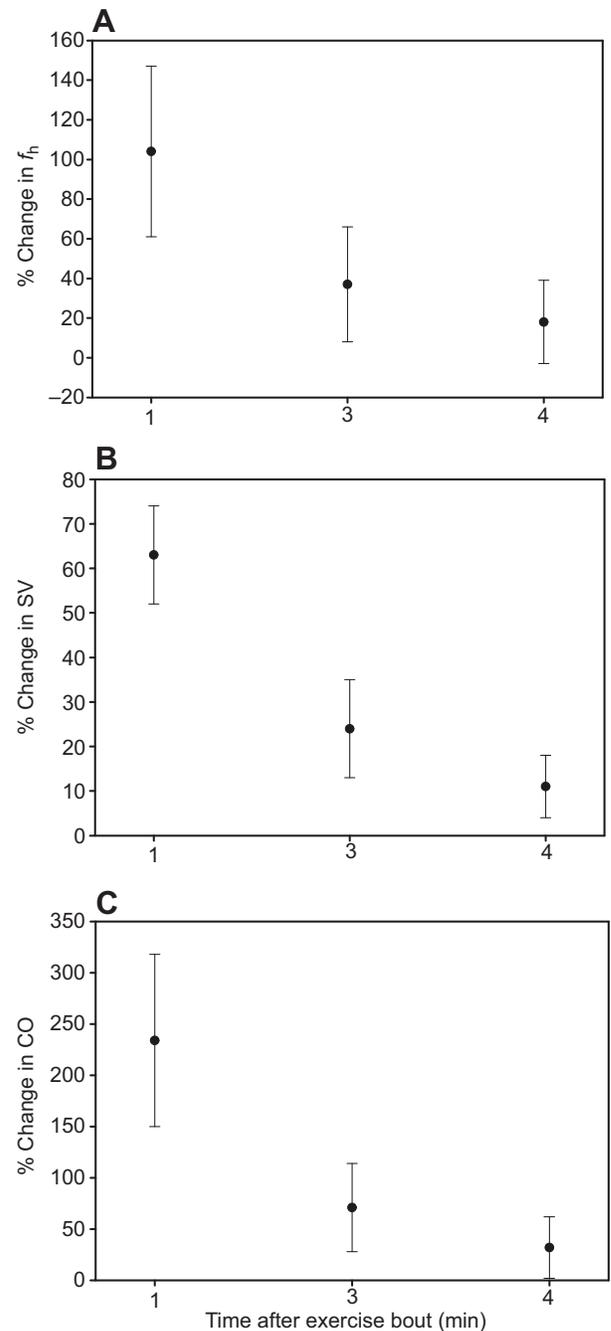
exerted themselves to varying levels and probably not to maximal exercise tolerance. In previous studies, a variety of methods have been used to look at the effect of exercise on dolphin energetics and cardiovascular responses (van der Hoop et al., 2014; Williams et al., 1993; Yazdi et al., 1999). In one study, the dolphins were trained to swim against a static load, which is similar to the tests done on humans (Williams et al., 1993). In two other studies, the dolphins were trained to swim a pre-determined course, but were allowed to select the speed (van der Hoop et al., 2014; Yazdi et al., 1999). In the current study, the exercise bout was achieved based on the experience of each animal and using a minimum of four and a

maximum of six high-intensity behaviors (tail walks, fast swims or high jumps) without time to recover in between. Direct comparisons are difficult to make as this method is more subjective and the dolphins most probably do not reach maximal exercise levels. However, the average relative increase in f_H (103±43%; for range, see Fig. 3A) immediately following exercise was similar to the increase seen during high-intensity exercise in a previous study (Williams et al., 1993). Submaximal exercise levels could also explain the high within-animal variability in the relative increase in CO from rest and after the first minute following exercise (Fig. 3C; range 145–339%). Alternatively, there may be no differences between animals in the two exercise groups as dolphins in both groups may participate in behaviors and activities that enhance fitness in between sessions.

Another important limitation for the current study is that f_H, SV and CO may have decreased significantly during the first minute before ultrasound measurements. Based on immediate examination (10 s post-exercise) in certain individuals with highly accessible cardiac acoustic windows, f_H, SV and CO increased by as much as 307%, 294% and 727%, respectively (S.M. and D.G.-P., unpublished data). Thus, considerably higher levels of f_H, SV and CO are likely in bottlenose dolphins. However, because of the complexity of properly finding the appropriate transthoracic acoustic window and the aortic tract, 1 min was the maximum time to guarantee taking accurate measurements in all individuals coming back to position after exercising.

Interestingly, for the dolphins in the current study, SV increased by an average of 62%, 1 min following a short intense bout of exercise at the surface. In the harbor seal, SV appeared to initially increase with increasing workload, but then decreased at the highest exercise levels (see fig. 5A,B in Ponganis et al., 1990). An initial increase in SV followed by a plateau as the animals approach maximal effort has been observed in a range of vertebrates, including humans (Barger et al., 1956; Carlsten and Grimby, 1966; Grubb et al., 1983; Rushmer, 1959). This finding may actually indicate that the dolphins did not exercise at maximal effort as the increase in SV was >40% for all animals (range 44–81%). Still, this increase in CO is similar to the allometric estimate in terrestrial mammals of 300% from rest to maximal exercise (Bishop, 1997). Although more standardized exercise criteria are required to evaluate fitness, and the influence of exercise and post-exercise recovery, e.g. $\dot{V}_{O_2,max}$ etc., our data provide some interesting estimates of cardiovascular plasticity in cetaceans. Two animals in this study were born under human care while the remaining 11 animals were founders originally from the wild (Table 1). While this is a limited sample size, there were no obvious differences in the cardiac performance of these two animals and the founders. From a health care perspective, more standardized and more demanding exercise tests may help assess physical and cardiovascular fitness in animals under human care. The present study provides relevant information for both basic cetacean physiology and future accurate modeling as well as clinical information for diagnostic purposes contributing to better care of animals.

Our resting f_H was significantly lower (41±8 beats min^{-1}) than values from previous studies in the awake, unrestrained (60–105 beats min^{-1}; Houser et al., 2010; Noren et al., 2004; Williams et al., 1993) or anesthetized bottlenose dolphin (84–140 beats min^{-1}; Sommer et al., 1968). The most likely explanation is our conservative approach in estimating f_H and SV exclusively after cardiac frequency had stabilized in between breaths. Higher average readings would have been obtained if the f_H was estimated over the whole inter-breath cycle (Fig. 1). The resting

mass-specific SV and CO values in the dolphins were considerably lower than those measured in the harbor seal (P. vitulina, mass-specific SV=2.9 ml kg^{-1} and CO=263 ml min^{-1} kg^{-1}; Ponganis et al., 1990) and California sea lion (Z. californianus, mass-specific SV=2.0 ml kg^{-1} and CO=160 ml min^{-1} kg^{-1}; Ponganis et al., 1991) using thermodilution techniques. Comparative values for mass-specific SV and CO in anesthetized dolphins placed out of water on a table were lower than those in the seal or sea lion (mass-specific SV=0.4–0.8 ml kg^{-1} and CO=47–105 ml min^{-1} kg^{-1}; Sommer et al., 1968), while mass-specific SV of these anesthetized dolphins was similar to our values (Table 1). Our average SV, f_H and CO for an average 175 kg dolphin were also lower than those estimated from allometric relationships in terrestrial mammals ranging in size from 0.333 to 446 kg (Bishop, 1997; SV=389 ml beat^{-1}, f_H=66 beats min^{-1}, CO=21,856 ml min^{-1}). The differences in cardiac variables between our measurements and those in anesthetized dolphins placed out of water highlight the importance of studying awake and unrestrained animals in their natural environment to provide data that will be relevant to understanding free-ranging animals. General anesthesia, mechanical ventilation, placement out of water and restraint could all affect hemodynamics and explain these differences. The differences in unrestrained and awake individuals could be explained by our decision to only measure cardiac variables at least 7 s following a breath, which was the time required for the cardiac frequency to stabilize following the respiratory tachycardia. While this may be a conservative approach, it reduces within and between individual and species differences. For example, cetaceans generally have a lower breathing frequency but even during voluntary breaths they respire quite forcefully (Fahlman et al., 2015). In pinnipeds, in contrast, breath durations are longer and respiratory flow rates are generally lower, with distinct flow limitations (Kerem et al., 1975; Kooyman et al., 1971). Thus, if respiration significantly alters cardiac function, resting f_H and SV should be compared during periods between breaths to minimize the impact of respiration on cardiac function. This would enhance the ability to compare data between species with distinctly different breathing patterns. Similarly, within-species comparisons would be made easier by reducing the variability caused by the changes that occur during breathing. Alternatively, future studies should attempt to assess the effect that respiration has on cardiac function. Concurrently measuring cardiac and respiratory physiology is difficult but possible with trained animals that participate voluntarily, which allows important aspects of physiology to be assessed with limited impact of stress. A better understanding of the effect of exercise and diving is important as it may provide novel methods to estimate field metabolic rate, a crucial component of bioenergetics models and for a better understanding of how these species may respond to anthropogenic changes to the environment.

Our data demonstrate considerable cardiac plasticity of the dolphin heart (up to 330% increase in CO, 60 s after finishing an average of 90 s intense exercise bout). The increase during maximal exercise or immediately after exercise may be significantly higher (over 700% increase in CO based on preliminary data). Thus, extreme and rapid cardiovascular changes may be crucial to help conserve O_2 during diving and rapidly replenish O_2 and remove CO_2 while at the surface. Thus, evolutionary pressure would enhance physiological mechanisms that would allow significant variation and refined control of CO under different environmental conditions, e.g. surface versus diving (Williams et al., 2015). When tightly managed, this dynamic behavior of the heart has several advantages. For example, minimizing blood flow to the muscle helps extend the aerobic dive

limit (Davis and Kanatous, 1999; Davis and Williams, 2012). The ability to alter both f_H and SV, as confirmed in this study, provides cardiovascular plasticity to regulate CO and thereby pulmonary and systemic perfusion. Disruption of these mechanisms could have severe consequences in terms of gas management, which may provide an explanation for recent reports of gas bubble disease in cetaceans and marine turtles exposed to different stressors while diving (Bernaldo de Quirós et al., 2012; Fahlman et al., 2014; Fernandez et al., 2005; García-Párraga et al., 2014; Hooker et al., 2012; Jepson et al., 2003). These data provide important information for gas dynamics models to help understand how anthropogenic disturbances may alter dive behavior and gas dynamics and eventually limit survival (Fahlman et al., 2009, 2006, 2014; Hooker et al., 2009). The data obtained here allow improved estimates of CO and will significantly improve model estimates. Thus, improved understanding of the cardiovascular responses of these animals during exercise, diving and stress will enhance ecophysiological knowledge and help with mitigation efforts.

In summary, this study provides the first simultaneous measurements of f_H and SV in cetaceans. As respiration significantly increases f_H and most probably SV (Figs 1 and 2), and as we were not able to assess the magnitude of each breath other than by sound, we chose to only analyze data during stable inter-breath periods. Our reported average f_H values are therefore slightly lower than previous reports, but we believe our results have lower variability and provide comparable values across individuals or species with varying respiratory effort. We report a significant increase in f_H, SV and CO, 1 min following a short bout of intense exercise that decreased exponentially with time towards the resting value but remained elevated for at least 4 min in all studied individuals. The post-exercise recovery time for f_H and SV exceeded 4 min, the maximum duration of measurements. Interestingly, neither animal performance nor training fitness seemed to affect the recovery phase. In future studies, we will assess how respiratory effort affects cardiac function and tease apart the contradictory effects of submergence and exercise.

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