RESEARCH ARTICLE

The dive response redefined: underwater behavior influences cardiac variability in freely diving dolphins

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SUMMARY

A hallmark of the dive response, bradycardia, promotes the conservation of onboard oxygen stores and enables marine mammals to submerge for prolonged periods. A paradox exists when marine mammals are foraging underwater because activity should promote an elevation in heart rate (f_H) to support increased metabolic demands. To assess the effect of the interaction between the diving response and underwater activity on f_H, we integrated interbeat f_H with behavioral observations of adult bottlenose dolphins diving and swimming along the coast of the Bahamas. As expected for the dive response, f_H while resting during submergence (40 ± 6 beats min^{-1}) was significantly lower than f_H while resting at the water surface (105 ± 8 beats min^{-1}). The maximum recorded f_H ($f_{H,max}$) was 128 ± 7 beats min^{-1}, and occurred during post-dive surface intervals. During submergence, the level of bradycardia was modified by activity. Behaviors such as simple head bobbing at depth increased f_H by 40% from submerged resting levels. Higher heart rates were observed for horizontal swimming at depth. Indeed, the dolphins operated at 37–58% of their $f_{H,max}$ while active at depth and approached 57–79% of their $f_{H,max}$ during anticipatory tachycardia as the animals glided to the surface. f_H was significantly correlated with stroke frequency (range=0–2.5 strokes s^{-1}, r=0.88, N=25 dives) and calculated swim speed (range=0–5.4 m s^{-1}, r=0.88, N=25 dives). We find that rather than a static reflex, the dive response is modulated by behavior and exercise in a predictable manner.

Key words: bradycardia, cetacean, dive response, diving, dolphin, exercise, marine mammal, tachycardia.

INTRODUCTION

The adaptations that enable marine mammals to prolong breath-hold durations while diving have intrigued comparative physiologists for nearly a century. In their original experiments with forcibly submerged animals, Scholander and colleagues (Scholander, 1940; Irving et al., 1941; Scholander et al., 1942) described the suite of physiological adjustments that occur during breath-hold across terrestrial and aquatic animals alike. Originally termed the 'diving reflex', the adjustments included a characteristic, pronounced slowing of the heart (bradycardia) and peripheral vasoconstriction that accompanied the cessation of breathing upon submergence. Over the years, the term 'dive reflex' has been replaced with 'dive response' to reflect the variability in physiological changes that have been observed across different types of dives (for a review, see Ponganis et al., 2003). These original landmark studies remain the cornerstone in our understanding of the cardiovascular adjustments required for conserving oxygen and prolonging the duration of submergence by air-breathing vertebrates. In general, the physiological response to submersion is qualitatively similar for aquatically adapted and terrestrial mammals, with diving-induced bradycardia considered to be the major mechanism to regulate blood oxygen depletion rate, thereby conserving oxygen for the brain and heart (Harrison and Tomlinson, 1960; Scholander, 1963; Van Citters et al., 1965; Ridgway et al., 1975; Davis and Kanatous, 1999; Alboni et al., 2011).

A complicating factor is that wild marine mammals routinely engage in a wide variety of behaviors while submerged that can include high-intensity activities, especially when foraging. When oxygen is readily available, heart rate is usually graded by exercise intensity to accommodate increased metabolic demands of working muscle. Such an exercise response has been demonstrated in both active terrestrial mammals (Mitchell, 1977) and marine mammals exercising on the water surface (Williams et al., 1993). However, when mammals are submerged, the heart rate response to exercise in general appears to be independent of the level of exertion (as defined by swim speed) for many species (Fedak et al., 1988; Ponganis et al., 1997; Hindle et al., 2010). Even humans, with few adaptations for an aquatic lifestyle, demonstrate a dive response that overrides the exercise response. For example, Smeland et al. (Smeland et al., 1984) found that final minimum heart rate levels were nearly identical for resting and exercising human subjects during face submergence. How marine-adapted mammals balance the physiological responses needed to simultaneously support seemingly conflicting diving and exercising cardiovascular responses is not readily understood (Castellini et al., 1985; Hochachka, 1986).

To date, it has been difficult to assess the adaptive changes in heart rate with different levels of underwater activity by marine mammals. This has been due in part to the difficulty of simultaneously monitoring behavioral and cardiovascular events across the natural range of physiological states when an animal dives.

The available studies suggest only modest changes in heart rate with exercise in marine mammals. For example, Williams et al. (Williams et al., 1993) reported that the heart rates of dolphins exercising near the water surface were not statistically different from resting levels when swim speed was less than or equivalent to $2.1\,\mathrm{m\,s^{-1}}$. Only when swimming speed was increased above cruising speeds to $2.9\,\mathrm{m\,s^{-1}}$ did the heart rates of the dolphins exhibit a significant exercise effect. Likewise, seals swimming at low speeds in flumes showed little change in submerged heart rate from resting levels (Fedak et al., 1988; Williams et al., 1991) and the bradycardia of sea lions was not consistently related to diving or swimming effort (Ponganis et al., 1997; Hindle et al., 2010).

Here we re-examine the relationship between heart rate and underwater behavior in a diving marine mammal to determine how diving heart rate may be modified during different levels of physical exertion as may occur during foraging or social interactions (Herzing, 1996). For the first time, heart rate patterns are linked to observations of discrete behaviors during open-water diving. The bottlenose dolphin (*Turiops truncatus*) served as a model species because of its trainability for performing a wide variety of activity levels. The results of this study indicate that the 'dive response' is altered by physical exertion. This is a novel finding for marine mammals. Except during the initial descent and final ascent portions of the dive, previous studies of heart rate in naturally diving marine mammals reported relatively stable heart rates during any one dive and heart rate appeared to be unaffected by physical exertion (for a review, see Butler and Jones 1997). Rather, we found that numerous factors associated with underwater behavior, including high levels of physical exertion, influence the magnitude of cardiac adjustment during periods of submergence. In contrast to previous studies examining relatively slow swimming speeds in pinnipeds and cetaceans, we found a significant correlation between swimming intensity and heart rate response during submergence, suggesting that the dive response is indeed altered by the exercise response in a marine mammal.

MATERIALS AND METHODS
Animals

Three adult bottlenose dolphins [*Tursiops truncatus* (Montagu 1821)] (Table 1) housed at The Dolphin Experience (Freeport, Grand Bahama Island, The Bahamas) were trained over 6 months for the experimental protocols. The animals were maintained in large ($15\times15\times5\,\mathrm{m}$ deep) saltwater enclosures connected to the open ocean and fed a daily diet of capelin and herring supplemented with multi-vitamins (Sea Tabs, Pacific Research Laboratories, San Diego, CA, USA). Total body length and maximum girth (at the anterior dorsal fin insertion) were measured during the month of study (February 2009) and were used to estimate body masses using a dolphin-specific morphometric calculator (Messinger et al., 1999). This calculator was designed specifically for bottlenose dolphins, and utilizes gender, total body length, maximum girth and age of the dolphin to estimate body mass. Data used to parameterize the equation in the calculator were acquired from dolphins at four facilities including The Dolphin Experience. The mean estimated mass of the three study animals was $186\pm12\,\mathrm{kg}$ (Table 1). All experimental procedures were conducted in accordance with the Institutional Animal Use and Care Committees at the University of California at Santa Cruz, and permitted under National Marine Fisheries Service Marine Mammal Permit No. 984-1587-00.

Electrocardiograph instrumentation and experimental trials

The dolphins were trained to wear a neoprene vest that carried an IQmark Advanced Holter electrocardiograph (ECG) monitor (version 7.2, Midmark Diagnostics Group, Versailles, OH, USA) housed in a custom-designed waterproof box ($18\times8\times4.5\,\mathrm{cm}$; Backscatter Underwater and Video, Monterey, CA, USA). ECG waveform signals were received continuously through shielded wires connected to two suction cup electrodes (5 cm diameter with a 2 cm diameter silver plate electrode) that were attached to the dolphins. One cup was placed on the sternum along the ventral midline directly below the pectoral fin insertions, and the other was placed above the right scapula according to Williams et al. (Williams et al., 1993) and Noren et al. (Noren et al., 2004). Heart rate was recorded continuously throughout the experimental sessions once the electrodes and vest were positioned.

On experimental days, the dolphins followed a boat to the open ocean where they performed a series of trainer-directed behaviors. Test behaviors were of variable duration and included: (1) rest while submerged (Fig. 1A), (2) low-intensity activity while submerged (Fig. 1B), (3) horizontal submerged swimming (Fig. 1C), (4) vertical glide to the water surface (Fig. 1D) and (5) post-dive surface interval. Underwater trials took place at 15 m depth in the open ocean. After the animal dove, it stationed near the trainer and was then signaled to perform a discrete behavior at the ocean floor: rest, low-intensity activity (i.e. head bob or jaw pop) or horizontal submerged swimming during which the dolphin chose its speed. Behaviors were performed in a random order so that the animal could not anticipate the behavior to be performed. In only a few cases, a second discrete behavior was performed before the animal was signaled to return to the surface. In some instances the dolphin returned to the surface to breathe before the trainer's signal was given. Dolphins were rewarded with fish throughout the dive, and the quantity of the fish reward was similar across all behaviors. In addition to open-water trials, heart rate was measured during rest on the water surface in the animals' enclosures. This enabled us to obtain resting heart rates at the surface exclusive of the effects of recovery from swimming and diving.

Behavioral and locomotor monitoring

Throughout the underwater trials, behavior and locomotor movements of the dolphins were monitored visually and recorded by SCUBA divers with a handheld video recorder ($30\,\mathrm{frames\,s^{-1}}$; Sony Handicam, Sony Corporation, Tokyo, Japan) in an underwater housing (Backscatter Underwater and Video). Depending on the trial, three to four divers accompanied the dolphins to direct and monitor specific behaviors. Internal clocks for the heart rate instrumentation and video recorder were synchronized to correlate cardiac signals with specific behavioral events. Simultaneous monitoring allowed for the determination of the influence of submergence, activity and exercise intensity on instantaneous changes in heart rate. Stroke frequencies were analyzed by extracting video clips of the submerged swimming

Table 1. Gender and morphology for the adult bottlenose dolphins in this study

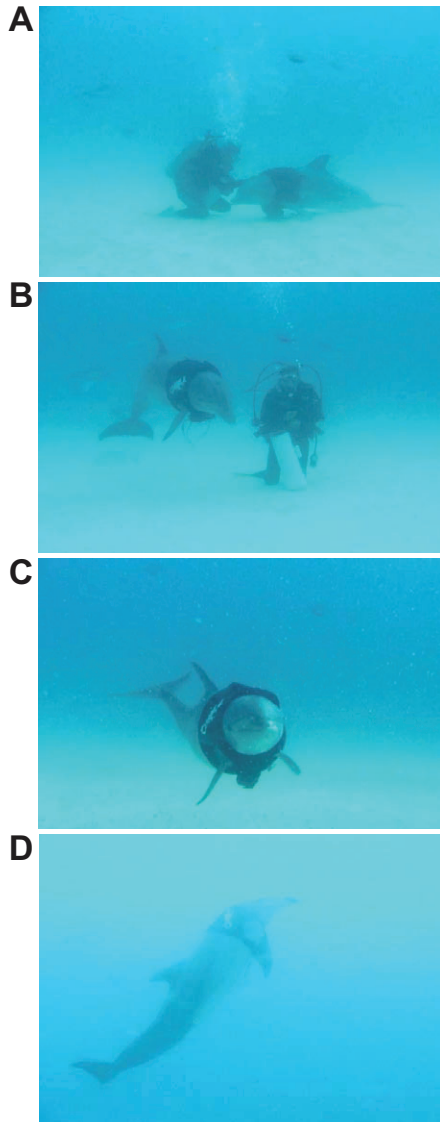| ID	Gender	Body length (cm)	Maximum girth (cm)	Estimated body mass (kg)
1M	Male	249	137	189
2M	Male	241	132	174
1F	Female	241	147	196

Fig. 1. Adult bottlenose dolphins diving to 15 m wearing the neoprene vest containing the electrocardiogram heart rate monitor. Photographs show (A) resting at depth, (B) low-intensity activity ('head bobbing'), (C) horizontal swimming at depth and (D) vertical approach to the water surface after completing a period of submergence at depth.

dolphins using digital video software (Pinnacle Studio 8, Pinnacle Systems, Mountain View, CA, USA). Videos were examined frame by frame to determine fluke stroke frequency, defined as the time it took the fluke to move one cycle from the highest point of vertical displacement and returning to that same point of displacement. The time clock for this software was set at 0.01 s. Swimming speed was calculated from stroke frequency using a previously determined equation for adult, non-reproductive bottlenose dolphins (Noren, 2008):

$$U = 2.09f_S + 0.13, \qquad (1)$$

where U is dolphin swim speed (m s^{-1}) and f_S is stroke frequency (strokes s^{-1}).

Heart rate analyses

The ECG waveform (Fig. 2) for all sessions was visually inspected to ensure that the instrumentation accurately determined interbeat

intervals in the absence of signal artifacts associated with muscle activity. The interbeat interval was then used to calculate instantaneous heart rate (beats min^{-1}). For diving tests, the instantaneous heart rate was plotted in relation to time into the trial and color coded according to the recorded behavioral state (Fig. 3). Within these trials, a sample was defined as the mean of all of the instantaneous heart rates associated with a discrete behavior. Delineations between consecutive samples were based on the animal changing its behavioral state as indicated in the video record. For behavioral samples that followed the descent to depth or preceded the ascent to the surface, instantaneous heart beat data were visually inspected to determine the inflection point that defined the beginning and end of the steady-state physiological period for that behavior. Heart beats prior to or after this segment represented transitional heart rates associated with breathing or anticipatory tachycardia, respectively, and were not included in the mean, following the procedures of Noren et al. (Noren et al., 2004). For resting trials at the surface in the animal's enclosure, the mean of the instantaneous heart rate was inclusive between two consecutive breaths.

Our primary interest was to quantify differences in heart rate associated with different behaviors during submergence. Each data point represents a unique sample (a distinct behavior), and each sample was of a slightly different duration and exercise intensity; therefore, measurements were considered to be independent and not repeated. Although we collected data from three individuals, the purpose of this study was not to examine individual variation. This approach enabled us to pool the data and to have a large enough sample size for statistical analyses. The approach of combining data across individuals when sample size is low follows the methods used by previous studies on diving heart rates in marine mammals and penguins (i.e. Noren et al., 2004; Meir et al., 2008). Nonetheless, because individual variation may weaken the resulting relationships, this pooled analytical approach reinforces the robustness of the conclusions (Meir et al., 2008).

The reported means for heart rate representing each behavioral state were the average of the samples across all three dolphins. Differences in heart rate across activity state were determined by one-way ANOVA in combination with a pairwise Tukey's test (multiple comparison procedure). The SegReg (www.waterlog.info) program was used to determine whether one or more linear regressions best described the relationship between observed stroke frequency and heart rate (and calculated swim speed and heart rate). Briefly, the SegReg program selects the best breakpoint and function type based on maximizing the statistical coefficient of explanation. Sigma Stat 2.03 (Systat Software, Chicago, IL, USA) was used for all other statistical procedures. Data are presented as means ± 1 s.d. Results were deemed significant at $P < 0.05$.

RESULTS

The three dolphins performed a total of 25 dives to a 15 m depth in the open ocean. The mean duration of the dives was 1.92±0.85 min (range=0.27–3.42 min), during which the dolphins performed one to two discrete behaviors of variable duration. We found that instantaneous heart rate of submerged dolphins varied with behavior. The minimum and maximum heart rates, which defined the range of heart rates for each dolphin, occurred during submerged rest at 15 m depth and during surface intervals following the dive, respectively (Figs 3, 4). These heart rate extremes for freely diving dolphins are in agreement with values measured previously for submerged dolphins resting in an enclosure [50±6 beats min^{-1} (Noren et al., 2004)] and for maximal exertion by trained dolphins pushing against a load cell at >129 kg [139±4 beats min^{-1} (Williams et al.,
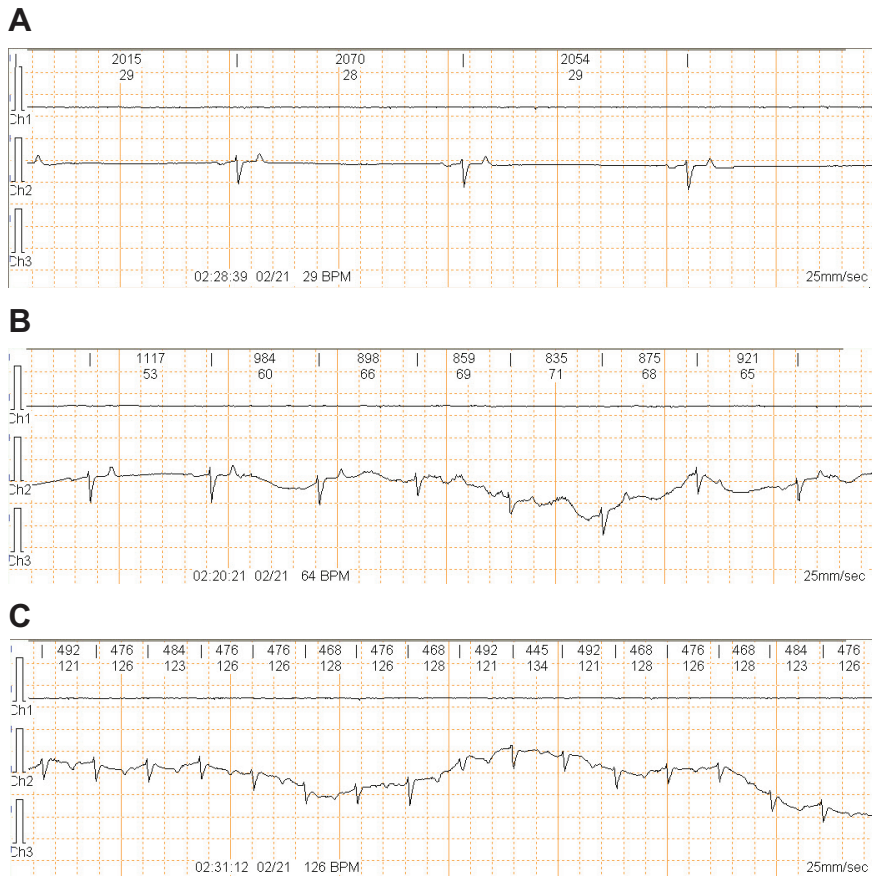
A

B

C

Fig. 2. Representative electrocardiogram (ECG) traces from an adult bottlenose dolphin. The ECGs represent three different behaviors: (A) rest at 15 m depth, (B) horizontal swimming at 1.7 strokes s⁻¹ at 15 m depth and (C) post-dive surface interval. Numbers along the top of the traces denote the interbeat interval in milliseconds; the row of numbers below is the calculated heart rate in beats per minute. Variability in the baseline in B and C is due to body and respiratory movements. Note that the specific ECG waveform was dependent on electrode placement and includes discernible P-wave, QRS complex and T-wave.

1993)]. In view of this, the present study appeared to elicit the range of heart rates expected for bottlenose dolphins.

Effect of submergence on heart rate

Variability in heart rate of the dolphins was related in part to location in the water column, particularly at 15 m depth *versus* the water surface (Figs 3, 4). Mean heart rate for all three subjects during submerged rest at 15 m (40±6 beats min⁻¹, N=15) for a mean duration of 85±51 s (range=14–160 s) was significantly lower than mean heart rate at the water surface, which reached 128±7 beats min⁻¹ (N=26) during the post-dive surface intervals. These surface intervals ranged in duration from 7 to 118 s (mean=50±29 s). Interestingly, heart rate during rest at the surface in the enclosures (105±8 beats min⁻¹, N=25), inclusive between two consecutive breaths, was 1.6 times greater than the predicted resting heart rate (65±1 beats min⁻¹) determined from the allometric regression for heart rate for terrestrial mammals in Stahl (Stahl, 1967). Submerged resting heart rate was within 62% of this predicted value. For the purpose of this study, we term heart rate during submerged rest as baseline heart rate ($f_{H,baseline}$) and heart rate at the water surface after diving as maximum heart rate ($f_{H,max}$).

Effect of submerged activity on heart rate

As would be expected from an exercise response, heart rate in submerged dolphins was related to activity type (sedentary behaviors *versus* swimming) and intensity level of the behaviors (Figs 3–5). During the open-water sessions, heart rate was significantly different across the behavioral categories ($F_{5,95}$=346.802, P<0.001). All results from the subsequent all pairwise multiple comparison were significant at P<0.05, with the exception that mean heart rate during submerged swimming and submerged low-intensity activity were

not deemed statistically different despite the observation that heart rate was greater in magnitude while swimming.

Indeed, underwater activity modified the level of bradycardia in a predictable manner. Low-intensity behaviors, such as head bobbing, jaw popping and various postural adjustments that lasted for a mean duration of 68±45 s (range=10–145 s), resulted in a 40% increase to 56±7 beats min⁻¹ (N=7) over submerged resting heart rate values ($f_{H,baseline}$ 40±6 beats min⁻¹, N=15). In comparison, horizontal submerged swimming that lasted for a shorter duration (mean=37±15 s, range=17–67 s) resulted in a 55% increase to 62±8 beats min⁻¹ (N=10) over $f_{H,baseline}$ (Fig. 4). In general, dolphins approached 37–58% of their $f_{H,max}$ while active at depth. The dolphins approached 57–79% of their $f_{H,max}$ as they glided to the water surface after submergence to 15 m, demonstrating an anticipatory tachycardia (Fig. 4).

We also found that submerged heart rate during open-water sessions was correlated with the level of physical exertion during horizontal swimming (denoted by stroke frequency and speed). Both of these relationships were best described by one linear regression (no breakpoint in the data was identified):

$$\bar{f}_H = 12.3 f_S + 40.6, \tag{2}$$

where $\bar{f}_H$ is mean heart rate (beats min⁻¹) and f_S is in strokes s⁻¹ (range=0–2.5 strokes s⁻¹, r=0.88, P<0.001, N=25). After converting stroke frequency into swim speed according to Eqn 1, this relationship is described by:

$$\bar{f}_H = 5.7 U + 40.5, \tag{3}$$

where mean heart rate ($\bar{f}_H$) is in beats min⁻¹ and swim speed (U) is in m s⁻¹ (range=0–5.4 m s⁻¹, r=0.88, P<0.001, N=25; Fig. 5).
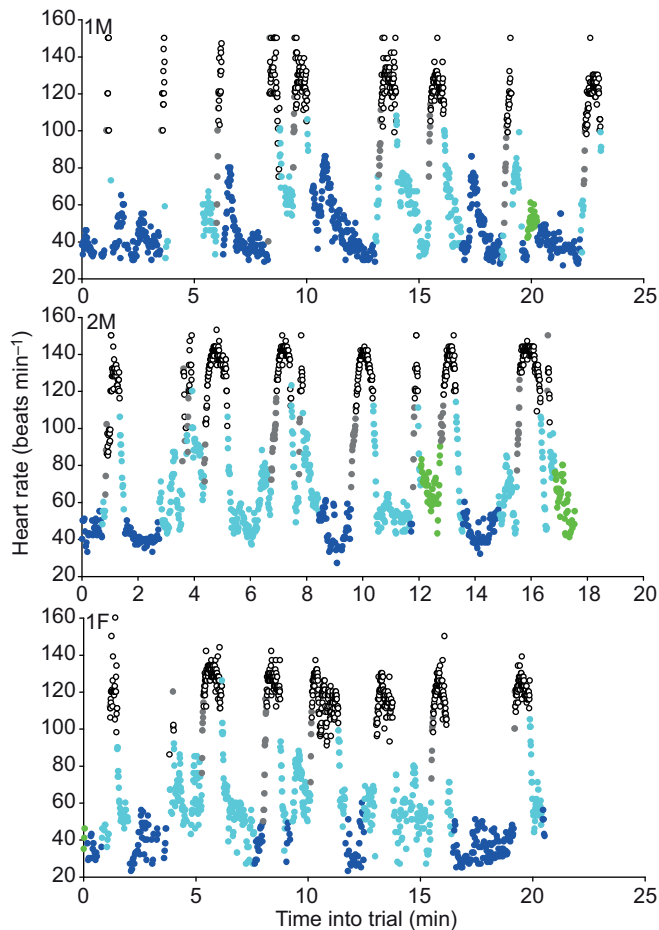
Fig. 3. Instantaneous heart rate for three adult bottlenose dolphins (1M, 2M and 1F) during diving bouts to 15 m depth. Each point represents an instantaneous heart beat for submerged rest (blue), submerged low-intensity activity (green), submerged horizontal swimming (cyan), vertical glide to the water surface (gray) and post-dive surface interval (white) behaviors plotted in relation to time into the trial.
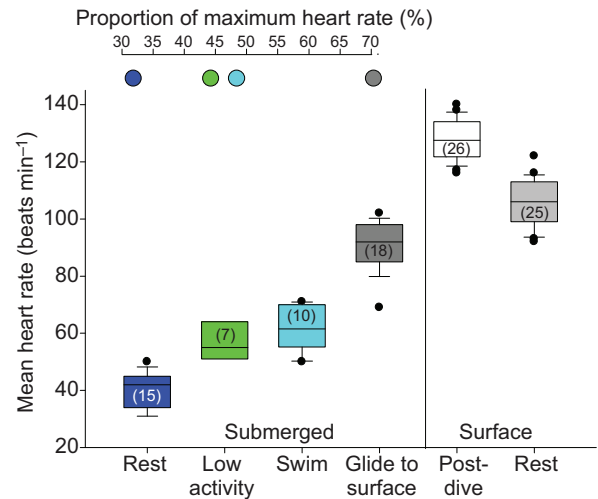

Fig. 4. Mean heart rate of adult bottlenose dolphins while submerged at 15 m and while at the water surface. Lower and upper edges of the box plots indicate the 25th and 75th percentiles, respectively. Error bars below and above each box show the 10th and 90th percentiles, respectively. Lines within the boxes denote the median, and circles show outliers. All behavioral categories were significantly different, with the exception of submerged low-intensity activity and submerged swim (see Results for statistics). The colored circles denote the proportion of maximum heart rate (measured during post-dive surface intervals) utilized for each behavioral category: submerged rest (blue), submerged low-intensity activity (green), submerged horizontal swimming (cyan) and vertical glide to the water surface (gray).

DISCUSSION

The present study demonstrates the variability in diving bradycardia that can occur with changes in behavior, particularly physical exertion, during submergence by a marine mammal. In freely diving bottlenose dolphins, heart rate cycled systematically as the animals descended and ascended (Fig. 3). Superimposed on this was a refinement in the level of bradycardia related to specific behaviors when at depth (Fig. 4). In particular, the exercise response was evident during submergence and appeared to override the dive response, especially as exercise intensity increased. In the case of swimming behaviors, heart rate was positively correlated to stroke frequency (range=0–2.5 strokes s^{-1}) and the corresponding swim speed (range=0–5.4 m s^{-1}; Fig. 5).

Interestingly, the pattern in heart rate showed elements of the surface swimming exercise response of dolphins. For bottlenose dolphins trained to swim next to a boat (Williams et al., 1993), heart rate was shown to vary little for speeds equal to or slower than the routine minimum cost of transport (2.1 m s^{-1}). Likewise, in the present study, the heart rate of a submerged dolphin swimming at approximately 1.2 m s^{-1} did not appear to differ from submerged resting levels, although the low sample size for slow swimming during submergence precluded a statistical analysis. However,

when swimming speeds exceeded 3.5 m s^{-1}, we observed a marked increase in heart rate that represented a substantial percentage (45–58%) of $f_{H,max}$. These higher swim speeds are faster than the minimum cost of transport speed (2.1 m s^{-1}) for surface-swimming dolphins (Williams et al., 1993) and exceed the energetically optimal speeds (averaging 2.0 m s^{-1}) observed during dives for a wide range of marine mammals (Videler and Nolet, 1990).

In view of these results, it may not be surprising that this exercise response has been overlooked when reviewing heart rate records retrieved from diving marine mammals. Whether for hydrodynamic or energetic reasons, deep-diving birds, pinnipeds and cetaceans tend to move through the water column at predictable, energetically efficient swim speeds (Costa et al., 1989; Fish and Hui, 1991; LeBoeuf et al., 1992; Davis et al., 1999; Watanuki et al., 2003; Watanuki et al., 2005; Miller et al., 2004; Tyack et al., 2006). This reliance on routine swimming speeds is similar to that observed for freely moving terrestrial mammals, which utilize a comparatively narrow range of routine running speeds near the middle of a much broader range of potential speeds (Wickler et al., 2001). Consequently, dolphins (Williams et al., 1993) and ponies (Hoyt and Taylor, 1981) traveling short distances as well as large migrating ungulates (Pennycuick, 1975) and whales (Mate and Urban-Rámirez, 2003) generally move over a relatively narrow range of preferred cost-efficient speeds.

This behavioral control over routine exercise levels likely contributed to the different conclusions for the relationship between heart rate, speed and stroke frequency reported for diving marine mammals by the present study and others. Although we found that heart rate and the level of physical exertion were correlated during horizontal submerged swimming by dolphins (Fig. 5), flume studies involving gray (*Halichoerus grypus*) and harbor (*Phoca vitulina*)
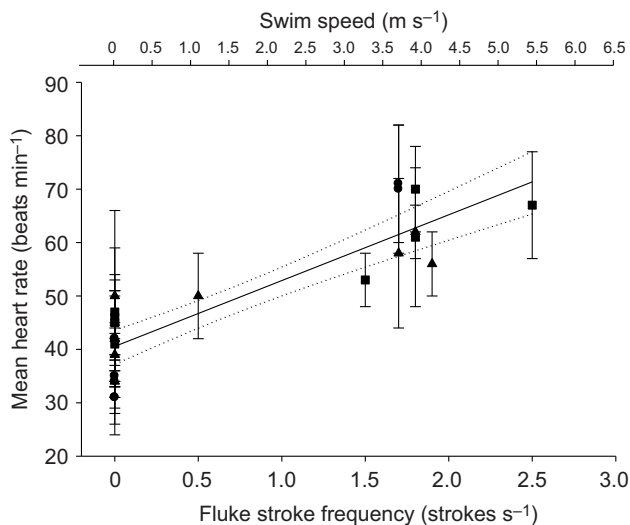
Fig. 5. Mean heart rate of adult bottlenose dolphins in relation to stroke frequency and horizontal swimming speed at 15 m depth. Mean ± 1 s.d. heart rates during specific exercise intensities for individual dolphins are represented by triangles (1M), squares (2M) and circles (1F). Heart rate data from all three dolphins were combined to construct the least squares linear regression, denoted by the solid line. Dashed lines show the 95% confidence intervals for the regression. See Results for equations and statistics.

seals showed little change in submerged heart rate at relatively slow swimming speeds (Fedak, 1986; Williams et al., 1991). Studies measuring the heart rate of freely diving marine mammals also showed conflicting results, which may be related to the range of speeds examined. Andrews et al. (Andrews et al., 1997) observed comparatively high heart rates for northern elephant seals (*Mirounga angustirostris*) diving on the continental shelf compared with the heart rates measured while the seals were off the shelf. Because elephant seals swim faster when they are on the continental shelf (Le Boeuf and Crocker, 1996), Andrews et al. (Andrews et al., 1997) suggested that activity level might have been one of the possible mechanisms for this alteration in bradycardia. However, this could not be confirmed because heart rate and swim speed were not simultaneously measured. In contrast, Hindle et al. (Hindle et al., 2010) measured stroke frequency, overall dynamic body acceleration (ODBA) and heart rate in trained, free-ranging Steller sea lions (*Eumetopias jubatus*). But the findings in the sea lion study were inconclusive because the authors demonstrated a correlation between ODBA and mean heart rate during shallow 10 m dives, but reported that ODBA was not correlated with mean heart rate during deep 40 m dives. Based on the tendency of marine mammals to move over a narrow range of optimal speeds to optimize the use of oxygen reserves during submergence, Hindle et al. (Hindle et al., 2010) may have been limited in their ability to detect an exercise response on heart rate during the deeper dives.

This is not to imply that swimming speed and, by inference, heart rate never change in the diving animal. Observed sustainable swim speeds for adult bottlenose dolphins are 3.1 m s^{-1} (Lang, 1975) and maximum swim speeds of 6.32 m s^{-1} (Noren et al., 2006) and 8.15 m s^{-1} (Rohr et al., 2002) have been routinely observed. These elevated speeds are undoubtedly important during periods of prey capture and predator avoidance. Indeed, Weddell seals (*Leptonychotes weddellii*) sprint at speeds exceeding two times routine levels when pursuing Antarctic silverfish 300 m below the sea ice (Davis et al., 1999), and have shown an exercise response

on diving bradycardia (Davis and Williams, 2012). Furthermore, a recent study on diving short-finned pilot whales (*Globicephala macrorhynchus*) named these animals the 'cheetahs of the seas' based on remarkable swimming sprint speeds of 3 to 9 m s^{-1} when actively foraging (Aguilar Soto et al., 2008). Although heart rate was not measured, if the physiology of this odontocete is similar to that of bottlenose dolphins, one might expect that diving pilot whales approach $f_{H,max}$ when pursuing fish at depth, a prediction worth exploring.

In addition to predictable effects due to behavior and activity level (Figs 4, 5), a closer examination of instantaneous heart rate in freely diving dolphins (Fig. 3) suggests that there may be another central nervous system (CNS) influence on heart rate (for a review, see Butler and Jones, 1997). 'Anticipation' of events, whether to prolong submergence or to approach the water surface, may have modified the magnitude of cardiovascular adjustment during submergence, particularly during the rest behavior. By videoing the underwater sessions of the instrumented dolphins, we found that the animals were capable of adjusting heart rate independent of body position, behavior or exercise. For example, the level of bradycardia of quiescent, submerged dolphins sometimes drifted as the duration of the breath-hold progressed, and these changes in heart rate were not associated with muscle movements (Fig. 3). The drift was in both directions, both decreasing and increasing heart rate. The instances of decreasing heart rate while resting at 15 m depth may have been associated with CNS control in 'anticipation' of the animals prolonging breath-hold, as previously described by Elsner et al. (Elsner et al., 1966). The examples of increasing heart rate while resting at depth may have corresponded with the 'anticipation' of the animals approaching the surface to breathe. Anticipatory tachycardia, a pronounced elevation in heart rate that occurs as animals approach the water surface after diving (Fig. 3A), has been attributed to the influence of the CNS (Kooyman, 1989) and it may actually begin before the animal physically starts the ascent. The influence of CNS control on heart rate in aquatic animals is not a novel idea. It has been demonstrated in animals during forced *versus* voluntarily submergence (Kooyman, 1989) and it can be conditioned through training as demonstrated in California sea lions (*Zalophus californianus*) (Ridgway et al., 1975). Thus, as found for humans (De Pascalis et al., 1991), instruction and biofeedback can play an important role in the voluntary control of heart rate in submerged marine mammals.

In summary, the cardiovascular profile associated with the dive response is commonly described as a marked decrease in heart rate on submergence, followed by a relatively invariant bradycardia at depth and an anticipatory tachycardia on ascent. Here we find that this response, in terms of heart rate, is flexible in diving dolphins. Such a variable dive response raises questions about the management of blood gases during submergence. Although there is a premium to conserve oxygen through bradycardia and an associated redistribution of blood flow (Scholander, 1940; Irving et al., 1941; Harrison and Tomlinson, 1960; Elsner, 1965; Elsner et al., 1966), heart rate varies with the intensity of underwater behaviors (Figs 4, 5), as does peripheral blood flow, as evident from changes in skin temperature and heat flow from the extremities of diving dolphins (Williams et al., 1999; Noren et al., 1999). Rather than a hindrance to diving, alterations in blood flow (as facilitated by alterations in heart rate) throughout submergence theoretically facilitate more effective unloading of endogenous oxygen stores by enabling the parallel depletion of the blood and muscle oxygen reserves (Davis and Kanatous, 1999). This may explain the unexpectedly high muscle oxygen saturation measured during diving in other marine

mammals (Hill et al., 1995). How these fluctuations in the dive response will affect the management of other blood gases, including carbon dioxide and nitrogen, that may impact the susceptibility to decompression syndromes remains to be answered (Hooker et al., 2012). Clearly, underwater behavior and activity level have a larger influence on heart rate during submergence than previously presumed.

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