

RESEARCH ARTICLE

Dive heart rate in harbour porpoises is influenced by exercise and expectations

Birgitte I. McDonald^{1,2,*}, Mark Johnson³ and Peter T. Madsen²

ABSTRACT

The dive response, a decrease in heart rate (f_H) and peripheral vasoconstriction, is the key mechanism allowing breath-hold divers to perform long-duration dives. This pronounced cardiovascular response to diving has been investigated intensely in pinnipeds, but comparatively little is known for cetaceans, in particular in ecologically relevant settings. Here, we studied the dive f_H response in one of the smallest cetaceans, the harbour porpoise (*Phocoena phocoena*). We used a novel multi-sensor data logger to record dive behaviour, f_H , ventilations and feeding events in three trained porpoises, providing the first evaluation of cetacean f_H regulation while performing a variety of natural behaviours, including prey capture. We predicted that tagged harbour porpoises would exhibit a decrease in f_H in all dives, but the degree of bradycardia would be influenced by dive duration and activity, i.e. the dive f_H response would be exercise modulated. In all dives, f_H decreased compared with surface rates by at least 50% (mean maximum surface $f_H=173$ beats min^{-1} , mean minimum dive $f_H=50$ beats min^{-1}); however, dive f_H was approximately 10 beats min^{-1} higher in active dives as a result of a slower decrease in f_H and more variable f_H during pursuit of prey. We show that porpoises exhibit the typical breath-hold diver bradycardia during aerobic dives and that the f_H response is modulated by exercise and dive duration; however, other variables such as expectations and individual differences are equally important in determining diving f_H .

KEY WORDS: Diving physiology, Bradycardia, Dive response, Cetacean, Heart rate regulation, Exercise

INTRODUCTION

Successful and efficient foraging is essential for an organism's survival and reproduction. Marine mammals face an extraordinary foraging challenge compared with their terrestrial relatives in that they must exercise to search, capture and consume prey while holding their breath during often long and deep dives. Understanding the physiological mechanisms that underlie their ability to perform such extended breath-holds while transiting and foraging is essential if we are to understand their role in the ecosystem and their ability to exploit prey resources in a changing world.

The dive capacity of breath-hold divers is dependent on both the available O_2 stores and the rate at which the O_2 stores are depleted. Heart rate (f_H), peripheral blood flow distribution and muscle

workload are the primary determinants of the rate and pattern of O_2 store use and ultimately breath-hold duration (Ponganis, 2015). Although recent advances in technology have greatly improved our understanding of O_2 management in pinnipeds (seals and sea lions) (Hindle et al., 2010; McDonald and Ponganis, 2013, 2014; Meir et al., 2009), much less is known about how cetaceans (whales and dolphins) perform extended breath-hold dives (Elsner et al., 1966; Houser et al., 2010; Noren et al., 2012; Williams et al., 2015, 1999). To address this knowledge gap, we investigated the relationship between fine-scale diving behaviour, including feeding events, and f_H in captive harbour porpoises, *Phocoena phocoena* (Linnaeus 1758).

Since the initial forced submersion studies (e.g. Scholander, 1940), decreased f_H has been considered central to the diving ability of breath-hold divers. Cardiovascular regulation is critical during diving because changes in f_H and blood flow affect not only blood O_2 uptake from the lung but also the rate and magnitude of blood O_2 delivery to tissues. During forced submersion, a severe bradycardia results in the isolation of muscle and peripheral organs from the blood flow, thereby slowing the depletion of blood O_2 , which is reserved for the heart and brain (Scholander et al., 1942; Zapol et al., 1979). More recent studies on trained and freely diving animals indicate that the dive response is dynamic, with declines in f_H of varying degrees depending on dive duration and depth, exercise and expectations (Davis and Williams, 2012; Elmegaard et al., 2016; McDonald and Ponganis, 2014; Noren et al., 2012; Thompson and Fedak, 1993; Williams et al., 2015).

The advent of miniaturized data loggers has greatly improved our capability to measure and hence understand how wild pinnipeds manage O_2 , but because of the difficulty of deploying physiological loggers on wild cetaceans, most of what we know is from captive studies, primarily with bottlenose dolphins. Dolphins trained to dive to depths greater than 60 m exhibit a diving f_H profile similar to profiles observed in diving seals and sea lions, decreasing from pre-dive values between 100 and 120 beats min^{-1} to lows of 20–30 beats min^{-1} , before increasing again as the dolphin starts its ascent (Houser et al., 2010; Williams et al., 1999). Williams et al. (1999) concluded that the dive response dominates the typical mammalian exercise response (increase in f_H and blood flow) because the degree of bradycardia observed in the diving dolphin was similar to values observed for shallow stationary dives (Elsner et al., 1966). However, more recently, a correlation between stroke rate and f_H has been documented in short shallow dives of trained bottlenose dolphins (Davis and Williams, 2012; Noren et al., 2012; Williams et al., 2015). The authors proposed that this is because marine mammals exhibit an exercise-modulated f_H response that maximizes the aerobic duration of a dive (Davis and Williams, 2012). Compared with bottlenose dolphins, a much less intense decline was seen in captive harbour porpoises during short shallow dives (Reed et al., 2000; Teilmann et al., 2006). Recently, porpoises were documented to alter the degree of bradycardia based on

¹Moss Landing Marine Laboratories, California State University, 8272 Moss Landing Road, Moss Landing, CA 95039, USA. ²Zoophysiology, Department of Bioscience, Aarhus University, 8000 Aarhus C, Denmark. ³Sea Mammal Research unit, University of St Andrews, St Andrews KY16 8LB, UK.

*Author for correspondence (gmcDonald@mml.calstate.edu)

 B.I.M., 0000-0001-5028-066X

anticipated breath-hold duration (Elmegaard et al., 2016), but nothing is known about how they regulate f_H in dives of different activity levels and durations.

From a diving physiology perspective, porpoises are interesting as they are among the smallest cetaceans and yet they live in low-temperature environments at high latitudes where elevated metabolic rates are needed to combat heat loss (Williams and Maresh, 2015). Wild porpoises meet these metabolic demands by high-rate foraging (Wisniewska et al., 2016). It is therefore essential to understand how they manage O_2 in these active foraging dives if we are to understand how their physiological capacity may limit their ability to deal with natural and anthropogenic disturbances to their environment. Pilot whales have been documented to sprint at speeds of 3–9 m s⁻¹ when actively foraging (Aguilar Soto et al., 2008), and it was hypothesized that during these feeding sprints, pilot whales may exhibit f_H approaching maximum levels ($f_{H,max}$) (Noren et al., 2012). Although porpoises do not exhibit such high speeds, they do increase activity during feeding events, allowing us to test this hypothesis with porpoises feeding on dead and live fish in captivity.

Here, we examined the dive response in three harbour porpoises using newly developed multi-sensor electrocardiogram (ECG) recording tags during stationary, active and prey capture dives. We investigated the relationship between f_H and activity to assess the potential level of muscle perfusion during dives with different activity levels. We hypothesized that: (1) porpoises will decrease f_H in all dives, but will only exhibit an extreme decrease in long-duration dives; (2) in more-typical short dives, harbour porpoises will show a positive relationship between f_H and workload/activity, suggesting they are perfusing muscle during diving; and (3) f_H will be elevated in prey capture sprints. Using a unique combination of controlled and free dives, we document the influence of both exercise and dive duration on the dive response. However, other factors such as individual differences and expectations are also important.

MATERIALS AND METHODS

Animals

Dive f_H was examined in three trained harbour porpoises (*Phocoena phocoena*) at the Fjord & Belt Aquarium (Kerteminde, Denmark) from March to June 2014. Two of the porpoises were female, Freja (17 years old, 158 cm) and Sif (10 years old, 160 cm), and the third porpoise, Eigil, was male (17 years old, 151 cm). The porpoises were incidentally captured in pound nets as yearlings, and have been housed in a large net pen (35×10×5 m L×W×D) at the Fjord & Belt Aquarium since capture. Sif was 6–8 months pregnant during the study. The porpoises were housed at the Fjord & Belt Aquarium under permits from the Ministry of Environment and Food of Denmark (SN 343/FY-0014 and 1996-3446-0021). The experimental procedure was approved by the Aarhus University IACUC committee and complied with recommendations of the Danish Council for Experiments on Animals.

Instrumentation

A modified Dtag 3 (ECG-Dtag3, 15.5×8.5×3 cm, 265 g), a multi-sensor archival tag, was attached ~5–10 cm behind the blowhole via four 5 cm silicone suction cups (Fig. 1A) (Johnson and Tyack, 2003). The ECG-Dtag 3 has two external silver chloride electrodes (10 mm) embedded in 5 cm suction cups that were placed rostral and caudal to the heart, close to the axis of the ventricle contraction to maximize the signal (Elmegaard et al., 2016). The ECG-Dtag3 recorded the differential potential between the electrodes relative to the ground in water sampling at 10 kHz (16-bit resolution and a 2-pole, 4 kHz anti-alias filter). Additionally, the tag recorded pressure

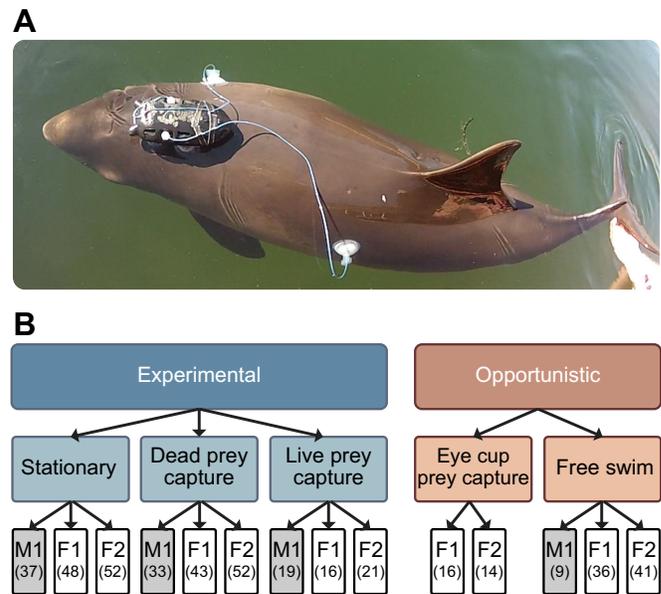


Fig. 1. Instrumentation and experimental protocol. (A) A harbour porpoise instrumented with the ECG-Dtag 3 with two external electrodes. (B) Diagram of the experimental design with sample sizes (in parentheses) for each dive type and porpoise. Grey indicates the male porpoise, white represents the two female porpoises.

and 3-axis acceleration at 2 kHz, and stereo sound (500 kHz sampling rate, 16 bit, 0.5–150 kHz bandwidth), allowing determination of activity, ventilations, echolocation behaviour and prey capture with precise synchrony to the f_H data.

Experimental protocol

f_H was collected using two protocols: (1) experimental and (2) opportunistic (Fig. 1B). For the experimental protocol, porpoises were trained to perform stationary and prey capture dives (with either dead or live fish) in a range of dive durations (~0.5–2.5 min) while instrumented with the ECG-Dtag3. Experimental sessions consisted of a block of approximately 5–7 dives of the same dive category (stationary, dead prey capture or live prey capture). Dive duration was randomly assigned to each dive before the session with a goal of two short duration (~30 s), two medium duration (~45–55 s) and two long duration (>60 s) dives per session. Before and after each experimental dive, the porpoise stationed for a minimum of 1 min at the surface with minimal activity to make sure the blood gases reached resting levels. This ensured that each dive was independent of the proceeding dive. The 1 min duration was selected after review of f_H and respiration rate data obtained during training sessions. All sessions were recorded with a GoPro mounted on the observer's head for validation of tag-collected behavioural data.

For stationary dives, porpoises stationed on a bite plate (Freja) or target (Sif, Eigil) at 1 m depth. Water movement in the sea pen varies with tide phase and so the target was oriented to minimize the effort required to stay on station. The porpoises remained on station until a whistle signalled that the behaviour was complete. Stationary dives were between ~30 and 70 s in duration. If dives were extended much beyond 60 s, the porpoise would return to the surface before the trainer recalled the animal. For the dead prey capture dives, a trainer was located at one end of pool with the porpoise and a 'feeder' was located at the opposite side (~20 m apart). When the trainer sent the porpoise on a trial, the feeder splashed and tossed a fish into the water (capelin or herring). Once the porpoise caught the

fish, another fish was tossed into the pool a few metres away, and this continued until the animal was recalled or, on longer dives, surfaced on its own. Dive duration was determined by varying the number of fish, i.e. 1 fish for short dives, 3–5 fish for medium duration dives, and for long duration dives, we provided fish until the animal surfaced. For the long duration dives, the 1 min surface interval started with the first breath after the porpoise returned to the trainer. The live prey capture dives were conducted exactly like the dead prey capture trials, except live black gobies (*Gobius niger* – a local species that the porpoises occasionally feed on when they enter the net pen) were used rather than dead fish.

In addition to the experimental sessions, we opportunistically collected data during (1) free swims between sessions and (2) training sessions where the porpoises had silicone eye cups placed over their eyes as they performed short to medium duration dead prey capture trials as described above (eye cup prey capture). The free swims allowed us to examine f_H during dives where the porpoises controlled the dive duration, activity and surface duration.

Data processing and analyses

All data processing and analyses were performed using custom-written scripts in MatLab (The MathWorks, Inc., Natick, MA, USA). Sound files were examined aurally and visually in 5 s windows using a spectrogram display (Hamming window, fast Fourier transform size 512, 75% overlap). Ventilations and feeding buzzes were identified (Deruiter et al., 2009; Wisniewska et al., 2016). Dives were determined from the ventilations: the start of the dive was the time of the last ventilation before the porpoise initiated the dive; the end of the dive was the first ventilation after the completion of the behaviour. This usually coincided with the porpoise returning to the trainer, but on longer dives, the end of dive ventilation often occurred before the porpoise was recalled to the trainer. In either case, the post-dive interval did not begin until the porpoise returned to the trainer. In addition to identifying dives, we also estimated bottom time. Because the dives were shallow and varied in depth as the porpoises chased fish, the bottom of the dive was not always clear from the dive profiles. Therefore, bottom time was estimated to begin 5 s after the dive start and end 5 s before dive end. This eliminates the periods of rapid decline and the anticipatory increase in f_H at the beginning and end of each dive, respectively.

ECG data were decimated to a sampling rate of 250 Hz, and filtered to remove excess noise [fir filter to remove 50 Hz noise, followed by Butterworth filters (0.1, 0.3)]. R-peaks were identified using a custom-written peak detector script and all records were visually inspected to correct for missing or spurious peaks. Instantaneous f_H was determined from the R–R peak intervals.

We calculated two activity indexes from the raw 3-axis acceleration data: (1) minimum specific acceleration (MSA), a measure of how much the total acceleration deviates from the gravity acceleration (Simon et al., 2012) – this is an underestimate of the specific acceleration generated by the animal and is calculated as the absolute value of the norm of acceleration minus the gravity acceleration; and (2) overall dynamic body acceleration (ODBA), another integrated measurement of body motion in three spatial dimensions, which is the norm of the high-pass-filtered acceleration (Wilson et al., 2006). MSA and ODBA were highly correlated (see General results). For this reason, only MSA was used to investigate the relationship between activity and f_H .

A suite of f_H variables was determined for each dive including: total number of heart beats during a dive (total heart beats), dive f_H (total heart beats/dive duration), initial and minimum instantaneous dive f_H , bottom f_H (heart beats during bottom of dive/bottom time

duration), decrease in instantaneous f_H in the first 5 s of dive (f_H at 5 s–initial f_H); and for experimental dives with a 1 min surface interval between dives, we calculated pre- and post-dive f_H (number of heart beats in the 30 s preceding or following the dive/0.5 min) and pre- and post-dive mode (most common instantaneous f_H within bin-sizes of ~ 5 beats min^{-1}). Additionally, for each dive we calculated average MSA (total MSA/dive duration).

To investigate the relationship between feeding behaviour and f_H , average instantaneous f_H and total MSA was calculated for a 10 s window centred at the start of every feeding buzz. For comparison, average instantaneous f_H and total MSA were also calculated for a 10 s window at the beginning of each feeding dive (10–20 s into the dive).

The relationship between MSA and ODBA was evaluated by performing correlation analyses. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to investigate the relationship between dive behaviour and f_H , with porpoise ID as a covariate to account for individual differences between porpoises (JMP, SAS, Cary, NC, USA). Differences in pre-dive f_H , dive f_H , minimum f_H , post-dive f_H , decline in f_H in the first 5 s of a dive, and average dive MSA between the dive types were investigated using two-way ANOVA, followed by *post hoc* Tukey tests. In all models, dive type (stationary, prey capture, etc.) and individual (porpoise ID) were fixed effects. The influence of feeding behaviour on activity and f_H was also investigated using two-way ANOVA to test for differences in f_H and activity between buzz and non-buzz segments. Dive types (dead versus live prey capture) were analysed separately. Feeding state (buzz/non-buzz) and porpoise ID were the fixed variables. The relationships between dive duration and MSA to dive f_H and minimum f_H were investigated using ANCOVA with porpoise ID as a covariate. Residuals of the final models were evaluated to assess model violations. No assumptions were violated.

RESULTS

General results

f_H and activity data were obtained from 437 dives from 3 porpoises (Fig. 1, Table 1). The porpoises exhibited a large range in f_H from over 200 beats min^{-1} at the surface down to 12 beats min^{-1} in the longest dive of 4 min (Fig. 2). Dive MSA was highly correlated with ODBA (Pearson $r=0.98$, $P>0.001$). We therefore used dive MSA for all analyses investigating the relationship between f_H and activity.

Surface f_H and sinus arrhythmia

Pre-dive surface f_H averaged 121 ± 22 beats min^{-1} , but there were significant differences between individuals, with Sif and Eigil having significantly higher f_H (Sif: 133 ± 16 beats min^{-1} , Eigil: 132 ± 17 beats min^{-1}) compared with that for Freja (104 ± 18 beats min^{-1}) (Table S1). Pre-dive f_H did not differ between stationary dives and dead prey capture dives, but was approximately 10 beats min^{-1} higher before live prey capture dives (Table 1). During the 1 min surface intervals, the porpoises often exhibited sinus arrhythmias with f_H decreasing from over 150 beats min^{-1} to values resembling f_H exhibited during dives; however, they did not show this decrease between every ventilation (Fig. 3A). During the free swim, we saw similar patterns and often if porpoises took two ventilations between dives, f_H stayed elevated between these ventilations (Fig. 3B).

Dive f_H and activity

Dive f_H was $51\pm 8\%$ lower than the pre-dive f_H (Fig. 2). Dive f_H profiles, in general, were U-shaped with a rapid decline, a relatively stable f_H near the bottom, and a rapid increase in f_H as the animal

Table 1. Heart rate (f_H) and activity (MSA) summary statistics for experimental and opportunistic dives and for each porpoise

	Duration (s)	Pre-dive f_H (beats min ⁻¹)	Initial f_H decline (beats min ⁻¹)	Dive f_H (beats min ⁻¹)	Minimum f_H (beats min ⁻¹)	Post-dive f_H (beats min ⁻¹)	Mean dive MSA (m s ⁻²)
Dive type							
Stationary ($n=137$)	48±14	118±20 ^a	87±26 ^a	60±10 ^a	42±9 ^a	118±25 ^a	0.39±0.11 ^a
Dead prey capture ($n=128$)	58±23	121±25 ^a	67±25 ^b	67±13 ^b	48±12 ^b	132±24 ^b	0.71±0.15 ^b
Live prey capture ($n=56$)	56±24	131±16 ^b	65±20 ^b	75±10 ^c	55±8 ^c	138±14 ^b	1.04±0.18 ^c
Free dives ($n=86$)	29±27		73±24 ^b	96±25 ^d	63±21 ^d		0.53±0.20 ^d
Eye cup prey capture ($n=30$)	54±16		89±30	51±12	29±8		0.82±0.08
Porpoise							
Eigil (M1)		132±17 ^a	71±23 ^a	79±10 ^a	59±9 ^a	147±18 ^a	0.68±0.27 ^a
Sif (F1)		133±16 ^a	79±28 ^a	77±23 ^a	51±18 ^b	130±20 ^b	0.62±0.27 ^a
Freja (F2)		104±18 ^b	75±27 ^a	60±18 ^b	40±12 ^c	110±20 ^c	0.60±0.24 ^a
Statistics (F statistic, P value)							
Dive type		$F_{2,312}=12.7$ $P<0.0001$	$F_{3,395}=18.8$ $P<0.0001$	$F_{3,395}=137.9$ $P<0.0001$	$F_{3,395}=87.8$ $P<0.0001$	$F_{2,312}=34.2$ $P<0.0001$	$F_{3,395}=292.9$ $P<0.0001$
Porpoise ID		$F_{2,312}=92.7$ $P<0.0001$	$F_{2,395}=0.9$ $P=0.41$	$F_{2,395}=117.1$ $P<0.0001$	$F_{2,395}=133.7$ $P<0.0001$	$F_{2,312}=95.0$ $P<0.0001$	$F_{2,395}=3.3$ $P=0.0375$
Dive type×porpoise ID		$F_{4,312}=6.5$ $P<0.0001$	$F_{6,395}=8.2$ $P<0.0001$	$F_{6,395}=15.7$ $P<0.0001$	$F_{6,395}=25.0$ $P<0.0001$	$F_{4,312}=7.0$ $P<0.0001$	$F_{6,395}=7.6$ $P<0.0001$

Data are means±s.d. Two-way ANOVA with dive type, porpoise ID and interaction term were performed, followed by *post hoc* Tukey's tests. Within each column, superscript letters indicate significant differences between dive types and porpoises. Effects tests results are presented below the summary data. MSA, minimum specific acceleration; M, male; F, female.

ascended (Fig. 2). Within the first 5 s of the dive, f_H decreased an average of 65–89 beats min⁻¹, depending on dive type (Table 1). Although all porpoises exhibited a similar f_H profile shape, they differed in surface and dive f_H (Fig. 4B; Fig. S1, Table S1).

To investigate the influence of activity and duration on f_H , porpoises performed dives of varying duration with significantly different activity levels as measured by the dive MSA (Fig. 4A, Table 1). Stationary dives had low activity (dive MSA=0.39±0.11 m s⁻²); activity increased approximately 80% during dead prey capture dives (dive MSA=0.71±0.15 m s⁻²) and an additional ~45% in live prey capture dives (dive MSA=1.04±0.18 m s⁻²). Dive and minimum f_H differed significantly across the experimental dive types, with the lowest f_H exhibited during stationary dives and the highest f_H in live prey capture dives (Figs 2 and 4, Table 1). Additionally, post-dive f_H following dead and live prey capture dives was significantly higher than that following stationary dives (Table 1).

Relationship between f_H and activity

Activity and dive duration both influenced f_H in the experimental dives when porpoises did not know how long they would be requested to dive [Table 2; $f_H=59.9+22.7(\text{MSA})-0.14(\text{duration})+(\text{ID correction: Eigil}=9.6, \text{Sif}=1.1, \text{Freja}=-10.7)$, $R^2=0.74$, $F_{4,316}=222.4$, $P<0.0001$]. Dive duration had a significant, but small, negative effect on dive f_H when controlling for MSA (Fig. 5A, Table 2), while activity had a positive effect on f_H (Fig. 5B, Table 2). The non-significant interaction terms (duration×ID and MSA×ID) indicate that the relationship of dive duration and activity to dive f_H was the same for all three porpoises (same slope), although they had significantly different intercepts. During free dives, when porpoises controlled activity level and dive duration, f_H decreased with increasing dive duration for all porpoises, while activity did not have an impact on dive f_H (Fig. 5C,D, Table 2). However, the significant duration×ID interaction term indicates that the relationship was not consistent between porpoises (Table 2). In prey capture dives when the porpoises had silicone eye cups placed over their eyes, dive and minimum f_H were lower than values during stationary dives, despite activity levels similar to prey capture dives without eye cups (Fig. 2, Table 1).

In addition to examining the relationship between f_H and activity at the level of the dive, we also investigated f_H during feeding buzzes, which are often associated with a burst of activity. The accelerometry data support the hypothesis that there is increased activity associated with a feeding event, with a 30–50% increase in total MSA during the 10 s window centred at the start of the buzz compared with that at a 10 s window at the beginning of the dive with no feeding events (Fig. 6, Table 3). Despite this increase in activity, there was a small (5%) or no increase in f_H (Fig. 6, Table 3). However, overall activity was higher during live prey capture dives, which was associated with higher f_H in live prey capture dives (Fig. 6).

DISCUSSION

Dive and surface f_H

Little is known about f_H management in cetaceans during ecologically relevant activities such as foraging because of the difficulty associated with obtaining such data from free-ranging animals. Here, we investigated f_H in captive harbour porpoises, a model species, as they performed a variety of dive types, including foraging dives. The U-shaped dive f_H profile exhibited by harbour porpoises is akin to f_H profiles observed in aerobic dives of seals, sea lions and captive dolphins (Davis and Williams, 2012; Hill et al., 1987; Hindle et al., 2010; McDonald and Ponganis, 2014; Noren et al., 2012; Thompson and Fedak, 1993). A previous study on f_H in harbour porpoises performing surface breath-holds documented a progressive development of bradycardia over the duration of the breath-hold (Reed et al., 2000). Reed et al. (2000) proposed that this was because harbour porpoises continue to use the lungs as an O₂ store while diving. Because harbour porpoises are relatively shallow divers in comparison to many other cetaceans, the air in the lungs (containing ~80% nitrogen) may be less of a liability than in deeper diving species. While lung O₂ stores may be an important source of O₂ in these shallow divers, in our study they exhibited a rapid decrease in f_H like other breath-hold divers, disproving the hypothesis that bradycardia onset is slow as a result of the importance of lung O₂ stores.

As we predicted, porpoises exhibited lower f_H in longer duration dives, as observed in freely diving seals, sea lions and penguins

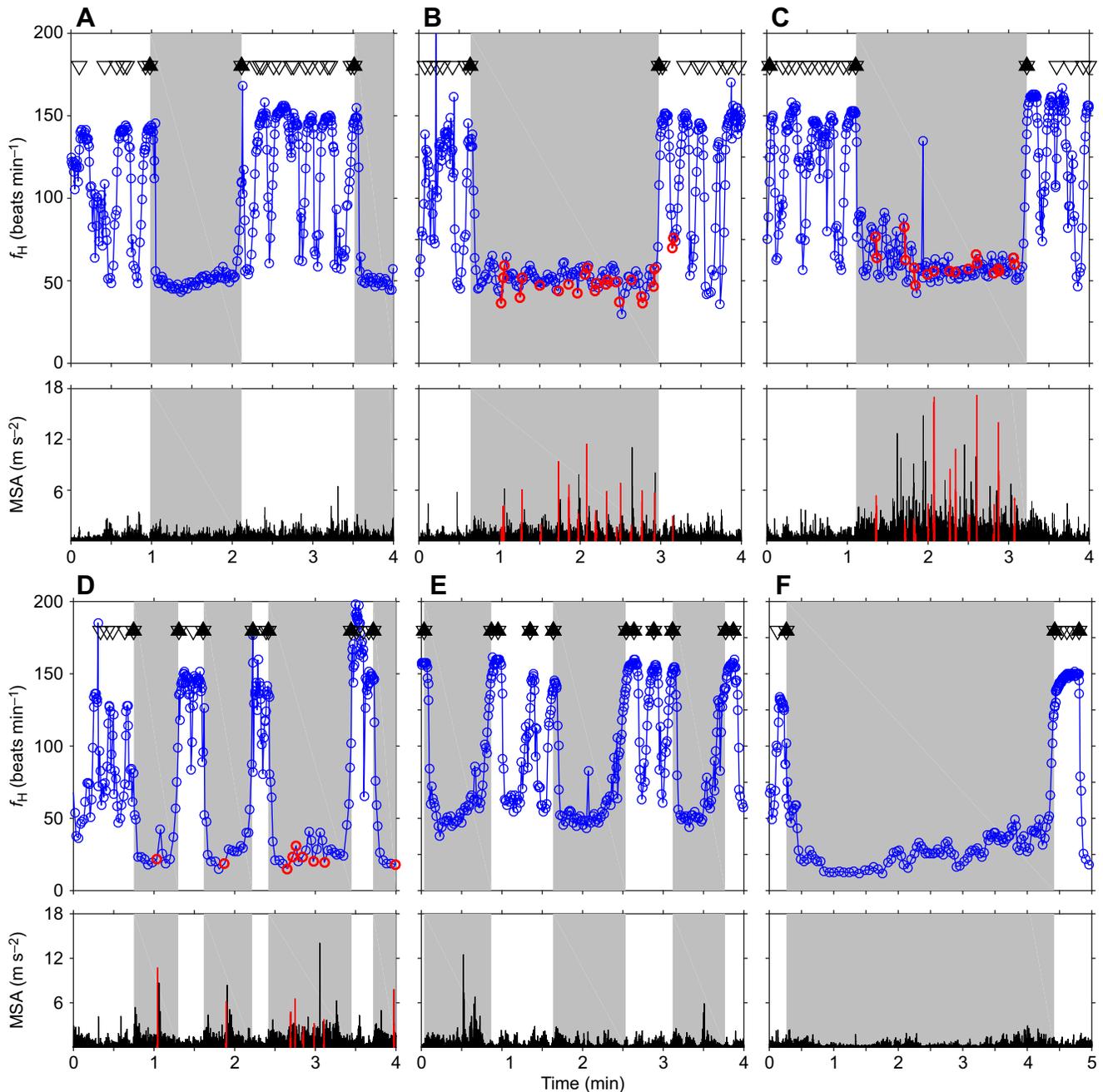


Fig. 2. Diving heart rate (f_H) profiles and minimum specific acceleration (MSA) from an adult female harbour porpoise (Freja: F2). (A–C) Examples of the systematically collected data: (A) a long stationary dive, (B) a dead prey capture dive, and (C) a live prey capture dive. (D–F) Examples of opportunistically collected data: (D) dead prey capture dives while the animal wears eye cups, (E) typical short free dives (not during a training session), and (F) the longest free dive recorded. Triangles indicate a breath (solid triangles indicate the breath before the start and after the end of a dive), grey shading indicates a dive, and red indicates when the porpoise produced a buzz.

(Hill et al., 1987; McDonald and Ponganis, 2014; Thompson and Fedak, 1993; Wright et al., 2014). The relationship was weak in experimental dives with randomized dive durations, probably because the porpoises could not predict dive duration. However, during free swims when porpoises controlled dive duration, there was a strong negative relationship between dive duration and f_H . During short free dives (<20 s), f_H only decreased to around 50–100 beats min^{-1} , while in the longest dive (4 min), f_H decreased to 12 beats min^{-1} , and stayed below 15 beats min^{-1} for over 1 min. We only observed one dive >2.5 min, and the level of bradycardia in this

4 min dive was lower than that typically observed in 4 min dives of California sea lions (McDonald and Ponganis, 2014) and most other pinnipeds (Fedak et al., 1988; Thompson and Fedak, 1993), indicating that although most dives of harbour porpoises are short and shallow, they are capable of planning and executing extreme bradycardia if needed to extend dive duration.

Although the f_H profile shape was similar to that of other breath-hold divers, the surface respiratory f_H documented here is the highest reported for any adult marine mammal (Boyd et al., 1999; McDonald and Ponganis, 2014; Noren et al., 2012; Thompson

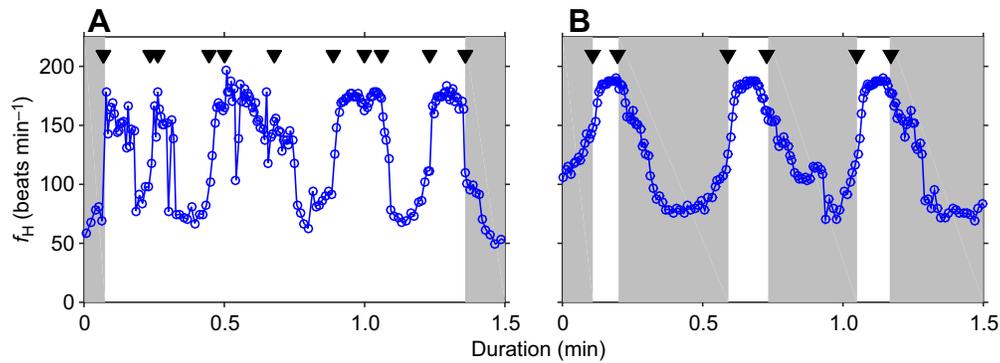


Fig. 3. Surface f_H and sinus arrhythmia. (A) The surface f_H between experimental dives is variable, but a clear respiratory sinus arrhythmia is not present in this case. Surface f_H may decrease to f_H observed during a dive between breaths, but often remains high between breaths as observed in seals, sea lions and dolphins (Andrews et al., 1997; Houser et al., 2010; McDonald and Ponganis, 2014). (B) In the short surface intervals exhibited during free dives, f_H remains elevated between breaths. Data from an adult female porpoise (Sif: F1). Grey shading indicates a dive.

and Fedak, 1993; Williams et al., 2015). Although mean f_H during surface resting varied between individuals, all three porpoises exhibited instantaneous f_H of over 200 beats min^{-1} before 8–23%

and after 8–41% of their dives. Mean maximum pre-dive f_H ranged from 156 to 191 beats min^{-1} . In comparison, bottlenose dolphin calves, juveniles and adults had a mean maximum surface f_H of ~ 120 – 130 beats min^{-1} (Noren et al., 2004) and adults had maximum exercising f_H of ~ 150 beats min^{-1} (Williams et al., 1993). Only juvenile sea lions, which are approximately 20–30 kg lighter than porpoises, have been reported with respiratory f_H as high as that of the porpoises in this study (Ponganis et al., 1997). Although it is inherently difficult to define the resting f_H in a cetacean that has irregular breathing and is diving continuously, from scaling relationships the predicted f_H for a 50 kg mammal is approximately 55 beats min^{-1} (White and Kearney, 2014). It is interesting to note that this is similar to the f_H found here for porpoises performing a stationary dive at 1 m depth, but such a comparison may be misleading, as it represents a mixture of a high metabolic rates (typical of marine mammals) countered by a dive response.

The combination of a high surface f_H and a moderate dive bradycardia supports the hypothesis that porpoises have high

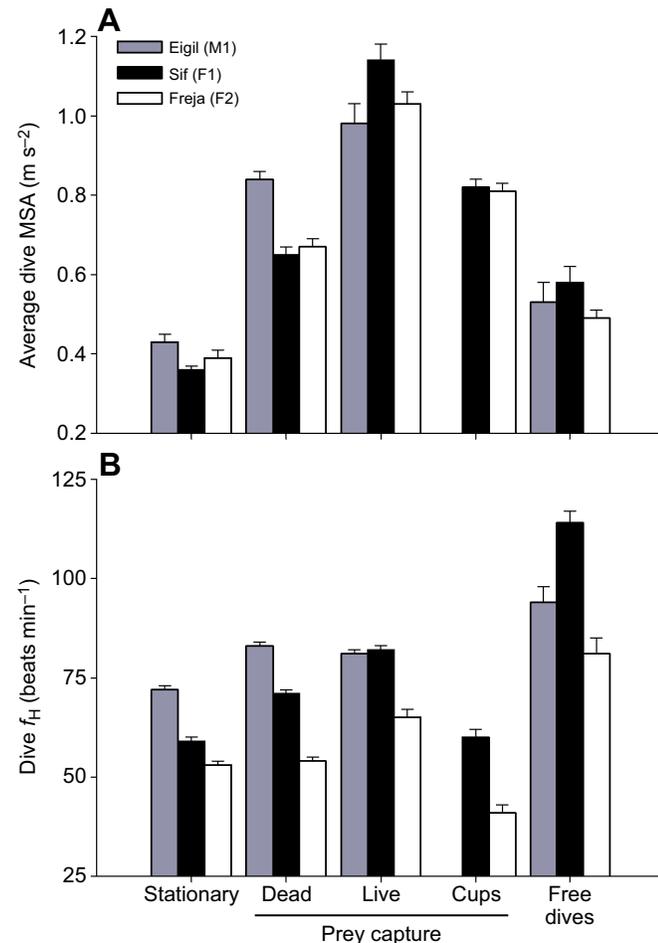


Fig. 4. Dive f_H and activity by dive type. (A) Activity (MSA) and (B) f_H increase from low values in stationary dives to high values in live prey capture dives in all three porpoises, but the degree of change differs (Table 1). f_H was lowest during prey capture dives with eye cups, despite activity levels being intermediate between those of dead and live prey capture dives (without eye cups). The highest dive f_H was observed in free dives. Data are means \pm s.e.m., 437 dives from 3 porpoises.

Table 2. Parameter estimates and confidence intervals for ANCOVA examining the relationship of dive duration and activity to f_H in experimental and free dives

	Estimate	Lower 95%	Upper 95%
Experimental dives ($n=321$)			
Intercept	59.9	57.5	62.4
Dive duration	-0.14	-0.18	-0.10
MSA	22.7	20.0	25.4
Eigil (M1)	9.6	8.5	10.7
Sif (F1)	1.1	0.1	2.1
Freja (F2)	-10.7	-11.7	-9.7
Free dives ($n=86$)			
Intercept	118.5	109.2	127.8
Dive duration	-0.90	-1.25	-0.56
Eigil (M1)	1.4	-6.2	9.1
Sif (F1)	6.8	-0.05	13.7
Freja (F2)	-8.3	-13.9	-2.7
Eigil \times (duration-28.7)	0.25	-0.27	0.78
Sif \times (duration-28.7)	-0.76	-1.32	-0.20
Freja \times (duration-28.7)	0.51	0.16	0.86

Interaction terms were not significant in the experimental dives model, indicating that although the porpoises had different f_H (intercepts), the relationship (slope) was the same for all three porpoises. In contrast, during the opportunistic dives the duration \times ID interaction term was significant. Although f_H decreased with increasing dive duration for all porpoises, the relationship (slope) was different for each porpoise. This is accounted for by the interaction term ID \times (duration-28.7). Bold indicates significance.

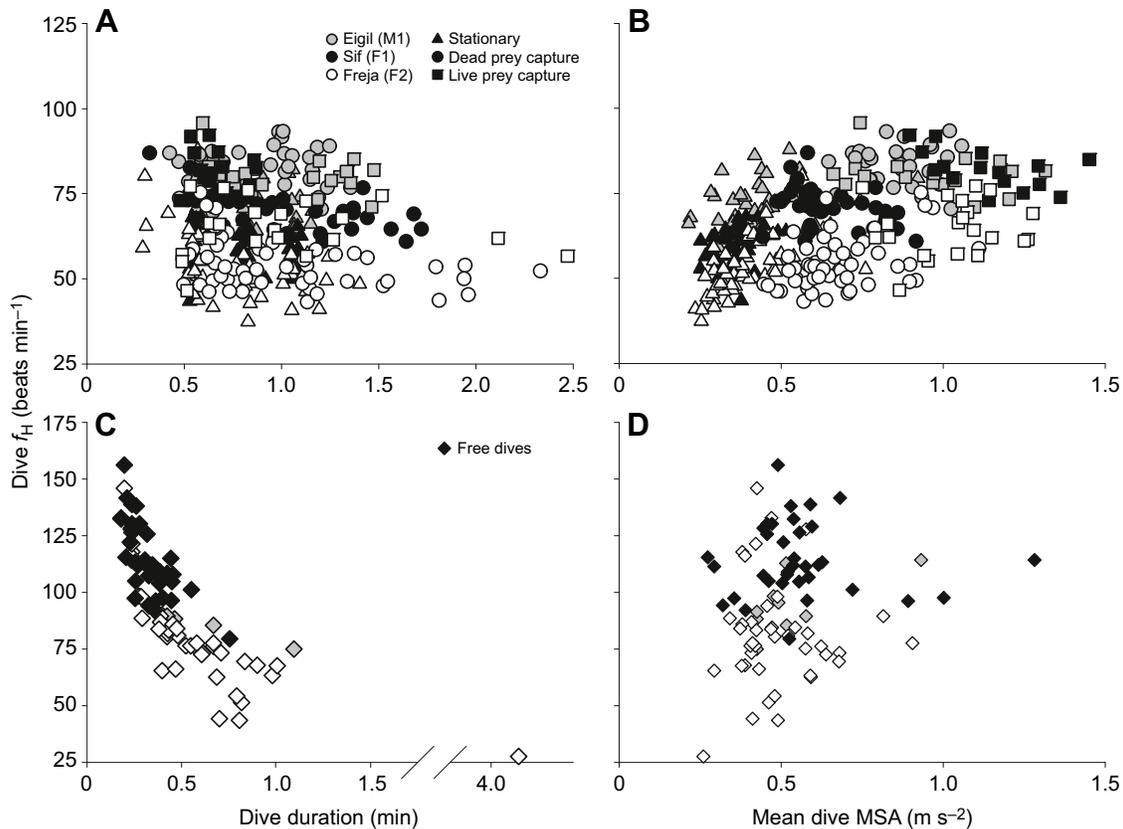


Fig. 5. Relationship of dive duration and activity (MSA) to dive f_H . (A,B) During experimental dives, there was a positive relationship between activity and dive f_H (B) and a small negative relationship between dive duration and dive f_H (A) [Table 2: $f_H = 59.9 + 22.7(\text{MSA}) - 0.14(\text{duration}) + (\text{ID correction})$, $R^2 = 0.74$]. (C,D) In free dives, there was a negative relationship between dive duration and dive f_H (C), but no relationship between activity and f_H (D) (Table 2). Data represent 407 dives from 3 porpoises.

metabolic rates which will limit their dive durations. The calculated aerobic dive limit (cADL) of porpoises has been estimated to be between 3 and 5.4 min, depending on the metabolic rate used in the calculation (Noren et al., 2014; Reed et al., 2000). Based on the high f_H observed in this study we predict that the true ADL will be on the lower end of that range. This is consistent with studies on wild porpoises that found that 94–95% of dives are <3 min in duration (Otani et al., 1998; Westgate et al., 1995).

Exercise modulated f_H response

Currently, one of the biggest knowledge gaps in the diving physiology of marine mammals relates to muscle perfusion during natural dives. Although this is difficult to measure directly, the role of muscle perfusion during diving is often evaluated by examining the relationship between f_H and workload (activity). Porpoises exhibited an exercise-modulated f_H response during experimental dives (Figs 4 and 5), as observed in Weddell seals and captive bottlenose dolphins (Davis and Williams, 2012; Noren et al., 2012; Williams et al., 2015). The positive relationship between activity (MSA) and f_H suggests that muscle perfusion increases as a function of muscle workload in the short aerobic dives. The exercise-modulated dive response has been proposed to be key to maximizing aerobic dive duration (Davis and Williams, 2012; Williams et al., 2015). The decrease in f_H during diving is needed to efficiently use blood and muscle O_2 stores (Davis and Kanatous, 1999); however, the degree of bradycardia can be adjusted throughout dives to balance the O_2 demands of exercising muscle (Davis and Williams, 2012). Although we found a relationship between activity and f_H for

porpoises, the relationship was much more subtle and variable than that observed in dolphins. Between stationary and active dives, there was only a ~25% increase in dive f_H despite an increase in activity, as measured by excess triaxial acceleration, of 160%. In comparison, there was a 39–57% increase in bottlenose dolphin f_H between stationary dives and horizontal swimming dives (Noren et al., 2012), and within dives, there was almost a direct relationship between stroke rate and f_H (Davis and Williams, 2012). The strong relationship between f_H and activity in dolphins led Noren et al. (2012) to propose that some cetaceans, like pilot whales, may exhibit high f_H during prey chases at depth (Aguilar Soto et al., 2008). Within the experimental limits of this study, we found no evidence for this in harbour porpoises. Although activity levels did transiently increase by 30–50% during a prey capture event, there was no increase in f_H associated with prey capture. Because porpoises only exhibited a moderate bradycardia in the prey capture dives, and are probably therefore maintaining some muscle blood flow, they may not need to increase f_H during the short bursts of activity. Alternatively, because of the suspected high metabolic rates, they may not increase f_H during the bursts of activity because they need to conserve O_2 for the brain and heart, even in these short dives.

Expectations and individual variability

The high variability in f_H associated with overall dive activity (but not individual prey captures) in experimental dives, but the lack of a similar relationship during free dives, suggests that, although porpoises do exhibit an exercise-modulated dive response at times, other factors are likely to be important in determining the level of

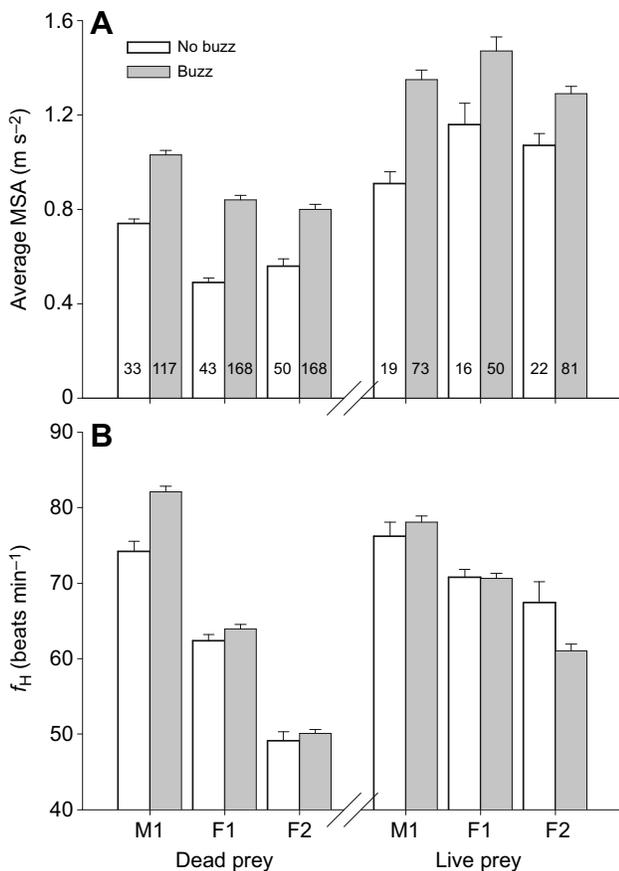


Fig. 6. Activity (MSA) and f_H during a 10 s window early in the dive (10–20 s) before feeding events, and during feeding (centred on the start of buzz). All porpoises increased activity during feeding events (both dead and live prey) (A), but f_H did not increase to match this increased activity, except for Eigil (M1) during dead prey capture trials (B). In contrast, Freja (F2) showed a decrease in f_H during live prey capture events despite an increase in activity. Data are means \pm s.e.m., sample size is indicate in the bars.

bradycardia. For example, f_H was lower in dives in which porpoises performed prey captures with silicone cups over their eyes, ensuring they only used echolocation to locate prey. This suggests that in situations when they cannot use all their senses to evaluate their surroundings, they are more conservative with their O₂ use, indicating volatile control over f_H . This is further supported by the weak relationship between dive f_H and dive duration when they do not know how long the dive task will be, in contrast to the strong relationship during free dives. When porpoises are unsure whether they will be asked to perform a short or long dive, they appear to manage O₂ in a fashion that will allow them to successfully perform a long dive. Interestingly, in some long prey capture dives, f_H was variable at the beginning of the dive, but then stabilized at a lower value when fish continued to be provided (Fig. 2). The ability to modify f_H based on expectations has recently been documented in the same porpoises performing stationary dives (Elmegaard et al., 2016) and has been suggested for other species including sea lions and bottlenose dolphins (Elsner et al., 1966; Kooyman and Campbell, 1972; McDonald and Ponganis, 2014; Noren et al., 2012; Ridgway et al., 1975).

Although dive duration and activity influenced f_H for all three porpoises, the porpoises had different surface and dive f_H . Eigil (M1), the small male (151 cm, ~45 kg), had both the highest surface f_H and highest dive f_H (Fig. 4; Table S1). Previous studies

Table 3. Effect tests results from comparison of f_H and activity (MSA) during 10 s windows centred on a feeding buzz and 10 s window with no buzzes

	f_H		MSA	
	Dead prey capture	Live prey capture	Dead prey capture	Live prey capture
Feeding state (increase)	$F_{1,573}=20.1$ $P<0.0001$	$F_{1,255}=1.7$ $P=0.187$	$F_{1,575}=187.1$ $P<0.0001$	$F_{1,257}=51.1$ $P<0.0001$
	(5%)	(30%)	(50%)	(30%)
Porpoise ID	$F_{2,573}=438.5$ $P<0.0001$	$F_{2,255}=44.8$ $P<0.0001$	$F_{2,575}=55.8$ $P<0.0001$	$F_{2,257}=6.6$ $P=0.002$
Feeding state \times ID	$F_{2,573}=7.4$ $P=0.001$	$F_{2,255}=4.9$ $P=0.008$		

Two-way ANOVA with feeding state and porpoise ID. If the interaction term was not significant, it was eliminated. MSA increased by 30–50% between non-feeding and feeding 10 s windows, while f_H did not increase or increased by 5% during feeding windows. A significant interaction term indicates that the f_H response to feeding state differed between individuals.

have also found smaller individuals tend to have higher f_H (McDonald and Ponganis, 2014; Williams et al., 2015). Sif (F1) and Freja (F2) were similar in size (160 and 158 cm, ~60–65 kg) and exhibited similar stationary dive f_H . However, Sif, the pregnant porpoise, exhibited surface f_H that was much higher, approximating values exhibited by Eigil at the surface. Also, as her activity level increased, her f_H increased during dives at a faster rate than that of Freja, resulting in dive f_H equivalent to that of Eigil in live prey capture dives (Fig. 4, Table 1). The higher f_H exhibited by Sif is probably due to pregnancy. In humans, cardiac output increases by 30–50% by late pregnancy, partly due to a ~15–25% increase in f_H (Hunter and Robson, 1992). Despite the differences in f_H , all porpoises exhibited lower f_H associated with longer dives and low activity.

Conclusions

In conclusion, we successfully obtained diving f_H profiles from porpoises performing both controlled experimental and free dives. We found that f_H decreases during all dives and that the f_H profile shape resembled that of aerobic dives of other breath-hold divers. In many dives, dive f_H did not differ appreciably from the f_H exhibited during short breath-holds within surface intervals. Both dive duration and activity influenced f_H , but the effect depended on whether porpoises were performing experimental or opportunistic dives. When the porpoises self-selected dive duration and activity during free dives, there was a strong relationship between dive duration and dive f_H . This was much weaker during experimental dives, where we found a stronger relationship between f_H and activity. This supports the hypothesis that during aerobic dives the dive response may be exercise modulated. As f_H was rarely below 40 beats min⁻¹, there was probably some muscle perfusion in most dives, and it increased slightly as the activity level increased. Although we found some support for the exercise-modulated dive response hypothesis, we did not find evidence of increases in f_H associated with feeding sprints. Additionally, the high variability in f_H and the differences observed between experimental and opportunistic dives indicate that other factors, such as expectation, influence dive f_H .

Acknowledgements

We thank the trainers at Fjord & Belt Aquarium, particularly Jakob Kristensen and Josefin Larson, for exceptional support and Danuta Wisniewska for assistance with use of the Dtag3 and data analysis. Rene Swift is thanked for producing and testing the suction cup electrodes.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: B.I.M., P.T.M.; Methodology: B.I.M., M.J., P.T.M.; Formal analysis: B.I.M.; Investigation: B.I.M.; Resources: M.J., P.T.M.; Tag development: M.J.; Writing - original draft: B.I.M.; Writing - review & editing: B.I.M., M.J., P.T.M.; Supervision: P.T.M.; Project administration: B.I.M.; Funding acquisition: B.I.M., M.J., P.T.M.

Funding

This research was supported by a National Science Foundation International Research Postdoctoral Fellowship (OISE – 1159123 awarded to B.I.M.); and grants from the Office of Naval Research (N000141410416) and the Carlsbergfondet (awarded to P.T.M. and B.I.M.). M.J. was funded by a Marie Curie Skłodowska Career Integration Grant (FP7-PEOPLE-2011_CIG) and by the Marine Alliance for Science and Technology Scotland.

Data availability

Data are available from figshare digital repository: 10.6084/m9.figshare.5549329.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.168740.supplemental>

References

- Aguilar Soto, N., Johnson, M. P., Madsen, P. T., Díaz, F., Domínguez, I., Brito, A. and Tyack, P.** (2008). Cheetahs of the deep sea: deep foraging sprints in short-finned pilot whales off Tenerife (Canary Islands). *J. Anim. Ecol.* **77**, 936-947.
- Andrews, R. D., Jones, D. R., Williams, J. D., Thorson, P. H., Oliver, G. W., Costa, D. P. and LeBoeuf, B. J.** (1997). Heart rates of northern elephant seals diving at sea and resting on the beach. *J. Exp. Biol.* **200**, 2083-2095.
- Boyd, I., Bevan, R., Woakes, A. and Butler, P.** (1999). Heart rate and behavior of fur seals: implications for measurement of field energetics. *Am. J. Physiol.* **276**, H844-H857.
- Davis, R. W. and Kanatous, S. B.** (1999). Convective oxygen transport and tissue oxygen consumption in Weddell seals during aerobic dives. *J. Exp. Biol.* **202**, 1091-1113.
- Davis, R. W. and Williams, T. M.** (2012). The marine mammal dive response is exercise modulated to maximize aerobic dive duration. *J. Comp. Physiol. A* **198**, 583-591.
- Deruiter, S. L., Bahr, A., Blanchet, M.-A., Hansen, S. F., Kristensen, J. H., Madsen, P. T., Tyack, P. L. and Wahlberg, M.** (2009). Acoustic behaviour of echolocating porpoises during prey capture. *J. Exp. Biol.* **212**, 3100-3107.
- Elmegegaard, S. L., Johnson, M., Madsen, P. T. and McDonald, B. I.** (2016). Cognitive control of heart rate in diving harbor porpoises. *Curr. Biol.* **26**, R1175-R1176.
- Elsner, R., Kenney, D. W. and Burgess, K.** (1966). Diving bradycardia in the trained dolphin. *Nature* **212**, 407-408.
- Fedak, M. A., Pullen, M. R. and Kanwisher, J.** (1988). Circulatory responses of seals to periodic breathing: heart rate and breathing during exercise and diving in the laboratory and open sea. *Can. J. Zool.* **66**, 53-60.
- Hill, R. D., Schneider, R. C., Liggins, G. C., Schuette, A. H., Elliott, R. L., Guppy, M., Hochachka, P. W., Qvist, J., Falke, K. J. and Zapol, W. M.** (1987). Heart rate and body temperature during free diving of Weddell seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **253**, R344-R351.
- Hindle, A. G., Young, B. L., Rosen, D. A. S., Haulena, M. and Trites, A. W.** (2010). Dive response differs between shallow- and deep-diving Steller sea lions (*Eumetopias jubatus*). *J. Exp. Mar. Biol. Ecol.* **394**, 141-148.
- Houser, D. S., Dankiewicz-Talmadge, L. A., Stockard, T. K. and Ponganis, P. J.** (2010). Investigation of the potential for vascular bubble formation in a repetitively diving dolphin. *J. Exp. Biol.* **213**, 52-62.
- Hunter, S. and Robson, S. C.** (1992). Adaptation of the maternal heart in pregnancy. *Br. Heart J.* **68**, 540.
- Johnson, M. P. and Tyack, P. L.** (2003). A digital acoustic recording tag for measuring the response of wild marine mammals to sound. *IEEE. J. Oceanic. Eng.* **28**, 3-12.
- Kooyman, G. L. and Campbell, W. B.** (1972). Heart rates in freely diving Weddell seals, *Leptonychotes weddelli*. *Comp. Biochem. Physiol. A Physiol.* **43**, 31-36.
- McDonald, B. I. and Ponganis, P. J.** (2013). Insights from venous oxygen profiles: oxygen utilization and management in diving California sea lions. *J. Exp. Biol.* **216**, 3332-3341.
- McDonald, B. I. and Ponganis, P. J.** (2014). Deep-diving sea lions exhibit extreme bradycardia in long-duration dives. *J. Exp. Biol.* **217**, 1525-1534.
- Meir, J. U., Champagne, C. D., Costa, D. P., Williams, C. L. and Ponganis, P. J.** (2009). Extreme hypoxic tolerance and blood oxygen depletion in diving elephant seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R927-R939.
- Noren, S. R., Cuccurullo, V. and Williams, T. M.** (2004). The development of diving bradycardia in bottlenose dolphins (*Tursiops truncatus*). *J. Comp. Physiol. B* **174**, 139-147.
- Noren, S. R., Kendall, T., Cuccurullo, V. and Williams, T. M.** (2012). The dive response redefined: underwater behavior influences cardiac variability in freely diving dolphins. *J. Exp. Biol.* **215**, 2735-2741.
- Noren, S. R., Noren, D. P. and Gaydos, J. K.** (2014). Living in the fast lane: rapid development of the locomotor muscle in immature harbor porpoises (*Phocoena phocoena*). *J. Comp. Physiol. B* **184**, 1065-1076.
- Otani, S., Naito, Y., Kawamura, A., Kawasaki, M., Nishiwaki, S. and Kato, A.** (1998). Diving behavior and performance of harbor porpoises, *Phocoena phocoena*, in Funka Bay, Hokkaido, Japan. *Mar. Mamm. Sci.* **14**, 209-220.
- Ponganis, P. J.** (2015). *Diving Physiology of Marine Mammals and Seabirds*. Cambridge: Cambridge University Press.
- Ponganis, P. J., Kooyman, G. L., Winter, L. M. and Starke, L. N.** (1997). Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions, *Zalophus californianus*. *J. Comp. Physiol. B* **167**, 9-16.
- Reed, J. Z., Chambers, C., Hunter, C. J., Lockyer, C., Kastelein, R., Fedak, M. A. and Boutillier, R. G.** (2000). Gas exchange and heart rate in the harbour porpoise, *Phocoena phocoena*. *J. Comp. Physiol. B* **170**, 1-10.
- Ridgway, S. H., Carder, D. A. and Clark, W.** (1975). Conditioned bradycardia in the sea lion *Zalophus californianus*. *Nature* **256**, 37-38.
- Scholander, P. F.** (1940). *Experimental Investigations on the Respiratory Function in Diving Mammals and Birds*. *Hvalradets Skrifter*. **22**, 1-131.
- Scholander, P., Irving, L. and Grinnell, S.** (1942). Aerobic and anaerobic changes in seal muscles during diving. *J. Biol. Chem.* **142**, 431-440.
- Simon, M., Johnson, M. and Madsen, P. T.** (2012). Keeping momentum with a mouthful of water: behavior and kinematics of humpback whale lunge feeding. *J. Exp. Biol.* **215**, 3786-3798.
- Teilmann, J., Tougaard, J., Miller, L. A., Kirketerp, T., Hansen, K. and Brando, S.** (2006). Reactions of captive harbor porpoises (*Phocoena phocoena*) to pinger-like sounds. *Mar. Mamm. Sci.* **22**, 240-260.
- Thompson, D. and Fedak, M. A.** (1993). Cardiac responses of grey seals during diving at sea. *J. Exp. Biol.* **174**, 139-154.
- Westgate, A. J., Head, A. J., Berggren, P., Koopman, H. N. and Gaskin, D. E.** (1995). Diving behaviour of harbour porpoises, *Phocoena phocoena*. *Can. J. Fish. Aquat. Sci.* **52**, 1064-1073.
- White, C. R. and Kearney, M. R.** (2014). Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Compr. Physiol.* **4**, 231-256.
- Williams, T. M. and Maresh, J. L.** (2015). Exercise energetics. In *Marine Mammal Physiology. Requisites for Ocean Living* (ed. M. A. Castellini and J.-A. Mellish), pp. 47-68. Boca Raton: CRC Press.
- Williams, T. M., Friedl, W. A. and Haun, J. E.** (1993). The physiology of bottlenose dolphins (*Tursiops truncatus*) - Heart-rate, metabolic-rate and plasma lactate concentration during exercise. *J. Exp. Biol.* **179**, 31-46.
- Williams, T. M., Haun, J. E. and Friedl, W. A.** (1999). The diving physiology of bottlenose dolphins (*Tursiops truncatus*). I. Balancing the demands of exercise for energy conservation at depth. *J. Exp. Biol.* **202**, 2739-2748.
- Williams, T. M., Fuiman, L. A., Kendall, T., Berry, P., Richter, B., Noren, S. R., Thometz, N., Shattock, M. J., Farrell, E., Stamper, A. M. et al.** (2015). Exercise at depth alters bradycardia and incidence of cardiac anomalies in deep-diving marine mammals. *Nat. Commun.* **6**, 6055.
- Wilson, R. P., White, C. R., Quintana, F., Halsey, L. G., Liebsch, N., Martin, G. R. and Butler, P. J.** (2006). Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. *J. Anim. Ecol.* **75**, 1081-1090.
- Wisniewska, D. M., Johnson, M., Teilmann, J., Rojano-Doñate, L., Shearer, J., Sveegaard, S., Miller, L. A., Siebert, U. and Madsen, P. T.** (2016). Ultra-high foraging rates of harbor porpoises make them vulnerable to anthropogenic disturbance. *Curr. Biol.* **26**, 1441-1446.
- Wright, A. K., Ponganis, K. V., McDonald, B. I. and Ponganis, P. J.** (2014). Heart rates of emperor penguins diving at sea: implications for oxygen store management. *Mar. Ecol. Prog. Ser.* **496**, 85-98.
- Zapol, W. M., Liggins, G. C., Schneider, R. C., Qvist, J., Snider, M. T., Creasy, R. K. and Hochachka, P. W.** (1979). Regional blood flow during simulated diving in the conscious Weddell seal. *J. Appl. Physiol.* **47**, 968-973.