

RESEARCH ARTICLE

Activity, not submergence, explains diving heart rates of captive loggerhead sea turtles

Cassandra L. Williams^{1,*}, Katsufumi Sato² and Paul J. Ponganis¹

ABSTRACT

Marine turtles spend their life at sea and can rest on the seafloor for hours. As air-breathers, the breath-hold capacity of marine turtles is a function of oxygen (O_2) stores, O_2 consumption during dives and hypoxia tolerance. However, some physiological adaptations to diving observed in mammals are absent in marine turtles. This study examined cardiovascular responses in loggerhead sea turtles, which have even fewer adaptations to diving than other marine turtles, but can dive for extended durations. Heart rates (f_H) of eight undisturbed loggerhead turtles in shallow tanks were measured using self-contained ECG data loggers under five conditions: spontaneous dives, resting motionless on the tank bottom, resting in shallow water with their head out of water, feeding on squid, and swimming at the surface between dives. There was no significant difference between resting f_H while resting on the bottom of the tank, diving or resting in shallow water with their head out of water. f_H rose as soon as turtles began to move and was highest between dives when turtles were swimming at the surface. These results suggest cardiovascular responses in captive loggerhead turtles are driven by activity and apneic f_H is not reduced by submergence under these conditions.

KEY WORDS: ECG, *Caretta caretta*, Cardiovascular, Diving

INTRODUCTION

Loggerhead sea turtles can remain submerged at sea for as long as 7 to 8 h (Hawkes et al., 2007; Hochscheid et al., 2007). Although not as extraordinary as the initial reports in the 1970s of marine turtles hibernating on the ocean floor for months (Carr et al., 1980; Felger et al., 1976), these are notable durations considering that, unlike freshwater turtles, marine turtles do not have significant extrapulmonary respiration capacity (Lutz and Bentley, 1985). Thus, as in marine mammals, the breath-hold capacity of marine turtles is a function of the amount of stored oxygen (O_2), how fast they consume O_2 during dives, and how tolerant they are to low O_2 levels. However, some of the key physiological adaptations observed in diving mammals are not present or are unknown in marine turtles. Loggerhead turtles in particular represent a unique model to study cardiovascular responses in a diving reptile because,

despite their long dive durations, they have even fewer adaptations to diving than other marine turtles.

First, unlike marine mammals, which cut off muscle blood flow during forced submersions (Scholander, 1940), muscle blood flow appears to occur in forcibly submerged loggerhead turtles (Lutz and Bentley, 1985). An intense peripheral vasoconstriction during forced submersion was initially described in seals (Scholander, 1940), the archetypal diving mammal, and considered a mechanism to limit O_2 uptake by tissues and to defend central arterial pressure in the face of a severe reduction in heart rate (f_H). Peripheral vasoconstriction was sufficient to result in the cessation of muscle perfusion, which was demonstrated by the simultaneous build-up of muscle lactate content and the lack of blood lactate accumulation during the forced submersion, and, then, the subsequent large increase in blood lactate after the seal was allowed to breathe again (Scholander, 1940). In green turtles, blood lactate remained low during the initial 30 to 60 min of a forced submersion, but then increased in the late stages of the submersion, suggesting peripheral vasoconstriction occurred initially, but could not be maintained for the entire submersion. In contrast, blood lactate accumulated throughout forced submersions of loggerhead turtles, implying continuous muscle perfusion in this species (Lutz and Bentley, 1985).

Second, although O_2 stores are elevated in marine mammals and, to a lesser degree, leatherback turtles, other marine turtles do not have increased O_2 stores (Lutcavage et al., 1990, 1992; Ponganis, 2015). In many homeothermic divers, myoglobin (Mb) concentrations are up to 15 times higher than in terrestrial animals, providing a significant O_2 store in muscle (Butler and Jones, 1997). The Mb- O_2 store can support maintenance of aerobic metabolism if muscle blood flow is limited during diving (Scholander, 1940; Williams et al., 2011). However, Mb concentrations in the muscle of loggerhead turtles are not elevated above those of terrestrial animals (Lutz and Bentley, 1985).

Although reduced f_H is the hallmark of the dive response, few studies have examined cardiac responses to diving in marine turtles. The classic dive response includes a rapid drop in f_H as the dive begins and low f_H during the dive until the ascent, when f_H increases to near surface levels (Butler and Jones, 1997; Ponganis, 2015). The reduction in f_H in diving mammals is variable: ranging from minimal in manatees and moderate in the majority of dives of many pinnipeds and dolphins to severe (equivalent to forced submersion) in long dives of some animals (Andrews et al., 1997; Gallivan et al., 1986; McDonald and Ponganis, 2014; Thompson and Fedak, 1993; Williams et al., 2017; Wright et al., 2014). The one study on marine turtles diving at sea described only mild f_H reduction in diving leatherback turtles relative to surface f_H (Southwood et al., 1999).

The high cost of endothermy at least partially necessitates the extreme mechanisms required for marine mammals to remain submerged for extended durations. However, with marine turtles' low metabolic rate and hypoxic tolerance (including of the brain) (Berkson, 1966; Lutz et al., 1980; Lutz and Bentley, 1985), cardiac

¹Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, 8655 Kennel Way, La Jolla, CA 92037, USA. ²Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan.

*Present address: National Marine Mammal Foundation, 2240 Shelter Island Drive, Suite 200, San Diego, CA 92106, USA.

†Author for correspondence (cassandra.williams@nmmpfoundation.org)

© C.L.W., 0000-0002-3977-2900; K.S., 0000-0002-7557-4784; P.J.P., 0000-0002-1556-770X

List of symbols and abbreviations

A	activity
ADL	aerobic dive limit
cADL	calculated aerobic dive limit
ECG	electrocardiogram
f_H	heart rate
Mb	myoglobin
PSI	post-dive surface interval
TDR	time–depth recorder
T_w	water temperature
\dot{V}_{O_2}	oxygen consumption rate

adjustments to reduce O_2 consumption during dives may not be as critical to their aquatic lifestyle. Studies on freshwater turtles suggest f_H changes are related to activity level and not whether turtles are submerged or on land (e.g. Krošniunas and Hicks, 2003). With few studies on f_H in marine turtles, the effect of activity level and submergence on f_H is unknown.

During summer, sub-adult and adult-sized loggerhead turtles forage in the nearshore areas of the Sanriku Coast of Japan (Narazaki et al., 2015). On occasion, they are incidentally caught in set nets by local fishermen. We took advantage of this seasonal incidental capture of loggerhead turtles to investigate their cardiac responses to voluntary dives. Our goals were to describe f_H patterns and investigate three questions about f_H of loggerhead turtles. First, is f_H during dives reduced relative to (1) surface f_H and (2) f_H when resting in shallow water with their head out of water? We predicted that f_H at the surface would be higher owing to the costs of ventilation and increased activity during surface intervals. However, with the apparent persistence of muscle blood flow during forced submersions, low Mb concentrations and the shallow depth of the experimental tanks, we predicted that turtles would not exhibit a significant decrease in f_H during dives versus resting out of water. Second, is f_H influenced by underwater activity of turtles? Again, because of the apparent persistence of muscle blood flow during forced submersions, we predicted that the more turtles moved, the higher f_H would be in order to meet O_2 demands during activity. And third, does f_H of turtles decrease as dive duration increases? We predicted that, because of their low metabolic rate and tolerance to hypoxia, turtles would not need to further reduce f_H in longer dives and that total heart beats would be closely correlated with dive duration. We attached time–depth recorders (TDRs) and self-contained physiological data loggers to record f_H and behavior. Turtles swam, rested and foraged in large outdoor tanks during the experimental period and then were released back to the ocean after experiments were completed.

MATERIALS AND METHODS**Animals and study site**

Eight loggerhead turtles, *Caretta caretta* (Linnaeus 1758), were incidentally caught in or around Otsuchi Bay, Japan, and transported to the University of Tokyo marine station (International Coastal Research Center, 39°21'05N, 141°56'04E) in the summers of 2013 and 2015. The sex of the eight turtles (58.5±18.9 kg) was not determined. During the experiments, turtles were kept in outdoor, individual seawater tanks (6×3×1 m) with continuous flow.

Instrument configurations

Each turtle was outfitted with a physiological data logger (model UUB/3-EPTb, UFI, Morro Bay, CA, USA) enclosed in a custom-made plastic waterproof housing (6.3×3×2 cm, 99 g in air) as

previously described (Williams and Hicks, 2016). The data loggers continuously recorded electrocardiogram (ECG) data at 50 Hz. A TDR (2013: DT or D2GT, Little Leonardo, Tokyo, Japan, 16 g in air, 1 s sampling interval; 2015: DST-Pitch and Roll, Star-Oddi, Gardabaer, Iceland; 4×1.3 cm, 9 g in air; 1 s sampling interval, resolution: ±0.6 cm; <0.1°C) was attached to the turtle's head with 5-min epoxy (Loctite Quick Set Epoxy, Henkel Corporation, Düsseldorf, Germany).

ECG implant

After mask induction of the turtle with 2–5% isoflurane in 100% O_2 , anesthesia was maintained at 1–2.5% isoflurane with spontaneous ventilation. Respiration was continuously monitored. In 2013, ECG wires, sterilized with Cidex OPA (Advanced Sterilization Products, Johnson & Johnson, Irvine, CA, USA), were implanted percutaneously in the first two turtles, with one electrode implanted in the neck near the left edge of the carapace and the other in the skin below the carapace and above the right rear flipper. To improve the ECG signal for the last two turtles in 2013, one electrode was implanted in the skin of the neck as above and the other electrode was guided through a 2 mm diameter hole drilled through the carapace near the right rear flipper. The carapace was cleaned with a topical povidone iodine solution before and after drilling and the hole was treated with 2–3 ml of 2% lidocaine as soon as it was drilled. All percutaneous implants were secured with sutures and tissue glue (3M; Vet-Bond, St Paul, MN, USA) and electrodes placed through the carapace were secured with tissue glue and holes were sealed with epoxy. Owing to the high level of noise in all ECG signals in 2013, an intravascular bipolar pacing catheter (model D97120F5, Edwards Life Sciences, Irvine, CA, USA), modified to record ECG signals, was used in all four loggerhead turtles in 2015. ECG catheters were inserted 24 to 32 cm percutaneously in the jugular vein through a peel-away catheter (5.5Fr or 6Fr, Cook Medical, Bloomington, IN, USA) (Meir et al., 2009; Ponganis et al., 2007) using ultrasound (MicroMaxx Ultrasound System, SonoSite Inc., Bothell, WA, USA) visualization as a guide to placement (Di Bello et al., 2010).

Instrument attachment

A piece of 0.25 inch square polyester mesh (1006, Delta knotless netting, Memphis Net and Twine, Memphis, TN, USA) approximately 8×5 cm in dimension with a 1 cm² Velcro™ patch in the center was secured to the upper midline of the carapace with 5-min epoxy. Data loggers were attached to the netting with a Velcro™ patch on the bottom of the housing and with plastic cable ties. With this attachment method, loggers could be removed and replaced easily. ECG catheters were connected to the data logger through underwater connectors (HUMG2-BCR and CCP, Sea-Con Brantner & Associates, Inc., El Cajon, CA, USA) and all cables or wires were secured to the carapace using tesa tape (tesa SE, Beiersdorf AG, Hamburg, Germany).

Diving protocol

After recovery overnight, turtles were left alone in the tanks, free to dive, swim or rest. Turtle behavior was monitored with a TDR and visually from a third-story stairwell in a nearby building. In some cases, turtle behavior was also video recorded. In 2015, turtles were fed dead squid several times a week.

Removal

Loggers and TDRs were taken off at the end of each experiment and data were downloaded. All netting, epoxy and tape were removed

from the carapace. Implanted sensors were taken out and skin incisions were closed with tissue glue. The holes through the carapace were treated with topical povidone iodine solution and then resealed with epoxy. After several days, skin incisions were no longer visible and holes through the carapace had begun to heal. These holes heal completely within 4 weeks (Southwood et al., 2003). At the end of all experiments, turtles were released into Otsuchi Bay. All procedures were performed in accordance with the guidelines of the Animal Ethics Committee of the University of Tokyo, and the protocol of the study was approved by this committee (permit nos. P13-17 and P15-16).

Data analysis

ECG data were plotted in a graphics program (Origin 2015-18, OriginLab Corporation, Northampton, MA, USA) and a custom peak detection script (K. Ponganis, unpublished) was used to detect and mark QRS complexes in the ECG profiles. The intervals between marked R waves were used to calculate instantaneous f_H (Meir et al., 2008). To ensure accuracy, all marked R waves were visually confirmed.

An aerobic dive limit (ADL) is the duration beyond which lactate begins to accumulate above resting values; however, as ADLs are difficult to measure because they require a post-dive blood sample, a calculated aerobic dive limit (cADL) can be used to assess dive durations (Kooyman et al., 1983, 1980). In this study, a range of cADLs (O_2 stores/ O_2 consumption rates) was determined for each turtle based on previously estimated mass-specific O_2 stores ($22.2 \text{ ml } O_2 \text{ kg}^{-1}$), and a previously measured O_2 consumption rate for North Pacific loggerhead turtles corrected for water temperature and for body mass with a metabolic scaling factor (Kinoshita et al., 2018; Lutz and Bentley, 1985; Prange and Jackson, 1976). In this equation:

$$\dot{V}O_2 = 0.1098 \exp(0.0581T_w + 0.0075A), \quad (1)$$

T_w is the water temperature in $^{\circ}\text{C}$, A is the percentage of time turtle was active, and units are expressed as $\text{ml } O_2 \text{ min}^{-1} \text{ kg}^{-0.83}$. The cADL range was calculated for activity levels of the turtles from 100% to 0% (Kinoshita et al., 2018).

Conditions measured

f_H was determined during five conditions, which were associated with different turtle behaviors and activity levels. The dive condition encompassed the time between a turtle's last breath prior to putting its head underwater and re-surfacing. Dives were identified based on DST pitch and roll logger profiles and visual observations. The pitch and roll profiles were used to identify when turtles submerged or surfaced and when they were motionless at the bottom of tanks, but were not used to quantify activity levels within conditions. A dive was defined as having a minimum duration of 2 min, and all shorter submergences were excluded from analyses. Post-dive surface intervals (PSIs) were defined as the time periods between dives when turtles were at the surface and did not include any short (<2 min) submergences. Because 92% of PSIs were 15 min or less, PSIs longer than 30 min were also excluded from analyses. In 2015, feeding events were defined as the time turtles spent underwater either moving toward the squid or consuming squid, and were identified from behavioral observations, TDR profiles and video recordings. Dives with feeding events were not included in any analysis of dives. One feeding event was analyzed per turtle. Two types of 10-min resting periods were examined for each 2015 turtle: lying in a tank filled with water that reached the

bottom of their carapace, but shallow enough so their head remained out of water ('shallow-water rest'), and submerged and motionless on the bottom of the tank ('bottom rest').

For shallow-water rest, we confirmed that each turtle's nares did not reach the water. Further, to minimize potential disturbance during shallow-water rest, turtles were not visually observed and DST pitch and roll profiles were examined to select these periods. As a result, breathing was not recorded and turtles could be apneic or eupneic during shallow-water rest. Bottom rest periods occurred during dives and these periods were included as part of dives within the dive condition.

Analysis

ECG data were successfully obtained in four turtles in 2013 and four turtles in 2015. To obtain f_H , duration and water temperature of different conditions or events, instantaneous f_H and TDR profiles (time, depth and water temperature) were plotted over a single 24-h period in Origin for each turtle. The number and duration of dives, maximum duration per turtle, PSI duration and mean dive water temperature were determined during the 24-h period for all eight turtles. Because R waves were not clearly discernible during movement in 2013 but were easily identified in 2015, only 2015 turtles were used for most f_H analyses. However, minimum instantaneous diving f_H was determined for the eight turtles as ECG signals were evident even in 2013 turtles when turtles rested on the bottom of the tank (Fig. S1A). For the 2015 turtles, feeding duration, total heart beats per dive and diving f_H (total heart beats divided by duration) were calculated. In addition, in 2015 turtles, the mean f_H and range of f_H were determined for all (1) dives, (2) PSIs, (3) feeding events, (4) bottom rest periods and (5) shallow-water rest periods.

Statistics

We used linear mixed-effects models [packages lme4, lmerTest and psycho (Bates et al., 2015; Kuznetsova et al., 2017; Makowski, 2018; <https://cran.r-project.org/web/packages/nlme/index.html>) implemented in R (version 3.2.3; <https://www.r-project.org/>)] to analyze the data. In all models, individual turtles were included as a random effect to account for repeated sampling. Water temperature was also included in all initial models as a fixed effect because it has a known effect on f_H and duration (Bentivegna et al., 2003; Southwood et al., 2003). All models were fit by maximum likelihood using Akaike's information criterion (AIC). Models were compared using Chi-squared distributed likelihood ratios. P -values were calculated using Satterthwaite's method to estimate denominator degrees of freedom for t -statistics (Kuznetsova et al., 2017). Marginal and conditional R^2 (R^2_m and R^2_c , respectively) values were obtained to determine goodness of fit (Nakagawa and Schielzeth, 2013). Residuals were checked to confirm model requirements, including normality and homoscedasticity, for all models. Heart rates were compared under the five activity conditions (dive, bottom rest, shallow-water rest, feeding and PSI). Differences between conditions were compared with Tukey's *post hoc* pairwise comparison. To assess changes in f_H in longer dives and because the calculation of diving f_H includes duration (total heart beats divided by dive duration), we evaluated the total number of heart beats as a function of dive duration and water temperature in 2015 turtles. We also analyzed the minimum instantaneous f_H in relation to dive duration in all turtles. To investigate recovery after dives, we compared PSI mean f_H and diving f_H in 2015 turtles. Finally, we plotted PSI duration with dive duration in all turtles. Means are expressed \pm s.d.

Table 1. Summary of data for each loggerhead sea turtle from 2013 (turtles 1–4) and 2015 (turtles 5–8)

Turtle	Mass (kg)	Number of dives (PSIs)	Mean dive duration (min)	Maximum dive duration (min)	PSI duration (min)	cADL range (min)	Minimum dive instantaneous f_H (beats min^{-1})	Mean dive water temperature ($^{\circ}\text{C}$)
1	88	36 (35)	25.7 \pm 10.2	46.1	3.3 \pm 1.9	54.4–115.1	4.5 \pm 1.3	22.8 \pm 0.8
2	61	41 (39)	24.8 \pm 13.7	42.4	8.9 \pm 5.0	51.7–109.4	2.5 \pm 0.5	22.6 \pm 0.3
3	79	41 (41)	31.0 \pm 8.7	44.7	4.2 \pm 2.1	51.5–109.1	3.6 \pm 0.8	23.4 \pm 0.8
4	66	46 (45)	29.0 \pm 11.3	45.1	4.9 \pm 3.3	52.7–111.5	3.4 \pm 1.0	22.5 \pm 0.8
5	41	32 (32)	35.4 \pm 15.3	52.5	5.7 \pm 5.1	56.5–119.6	3.6 \pm 1.2	19.9 \pm 0.2
6	57	45 (44)	15.3 \pm 8.0	32.4	5.1 \pm 4.0	57.7–122.2	3.1 \pm 1.0	20.5 \pm 0.5
7	38	29 (27)	26.7 \pm 13.5	45.9	8.5 \pm 7.2	55.8–118.1	3.6 \pm 1.3	19.9 \pm 0.7
8	38	58 (57)	13.0 \pm 9.8	34.1	8.7 \pm 6.5	56.1–118.8	5.6 \pm 1.6	19.8 \pm 0.9
Grand mean	58.5 \pm 18.9	41.0 \pm 9.1	24.1 \pm 13.4	42.9 \pm 6.7	6.2 \pm 5.1	54.5–115.4	3.8 \pm 1.5	21.4 \pm 1.5

Calculated aerobic dive limit (cADL) range for turtles is from 100% to 0% active (Kinoshita et al., 2018). Means are reported \pm s.d. Mean dive water temperature is water temperature during dives. PSI, post-dive surface interval; f_H , heart rate. $N=8$ turtles.

RESULTS

Behavior

Deployments ranged from 2 to 8 days. During the 24-h observation period, turtles typically submerged and rested on the bottom of the tank for up to 53 min (Table 1). Dives per turtle during the 24-h periods ranged from 29 to 58 for a total 328 dives (Table 1). Over 40% of dives were longer than 30 min and almost 60% were longer than 20 min (Fig. 1). cADLs ranged between 52 and 58 min for turtles at rest (Table 1). Mean and maximum dive durations for each turtle are reported in Table 1. Maximum dive durations were not followed by consistently high PSI durations; however, there was an evident increase in the minimum PSI duration in longer dives (Fig. 2).

During shallow-water rest, turtles were left alone in a tank with water that reached the bottom of their carapace, but shallow enough so their head remained out of water. Although turtles were primarily motionless during both dive and shallow-water rest, there was some intermittent activity. Dives included movement to and from the surface and shallow-water rest included some turtles moving, including head movements potentially during breathing, although breathing was not quantified. The lack of movement during bottom rest periods was confirmed from the pitch and roll profiles of turtles lying on the bottom of tanks. When turtles were fed squid, they slowly swam underwater toward the squid and paused briefly as they consumed the squid. Feeding was the most active underwater condition as turtles moved almost continuously. During PSIs, turtles

swam continuously at the surface back and forth across the length of tanks, intermittently lifting their head to breathe. Turtles did not float at the surface or have periods of inactivity during PSIs.

Diving f_H was lower than surface f_H , but not different from shallow-water rest f_H

Heart rates during diving, bottom rest, shallow-water rest, feeding and PSIs are reported in Table 2. In the model comparing f_H under five conditions, water temperature did not have a significant effect and was removed in the final, best-fit model. Condition had a significant effect on f_H ($F_{4,12}=18.3$, $P<0.001$; Fig. 3) and explained 77.4% of variation under the model, whereas the effect of individual contributed 2.5% of explanatory power ($R^2_m=77.4$, $R^2_c=79.9$). A *post hoc* pairwise comparison showed that PSI f_H was significantly higher than diving f_H ($P<0.001$, $t=7.2$, d.f.=12). However, there were no significant differences in f_H between shallow-water rest and dive ($P=0.18$, $t=2.4$, d.f.=12) or shallow-water rest and bottom rest ($t=2.7$, d.f.=14, $P=0.12$; Fig. 3).

Heart rate increased as underwater activity levels increased

When turtles moved underwater, f_H increased by 2- to 3-fold and occasionally reached PSI f_H (Fig. 4, Table 2). Feeding f_H was significantly higher than bottom rest f_H ($P<0.05$, $t=3.5$, d.f.=12) and

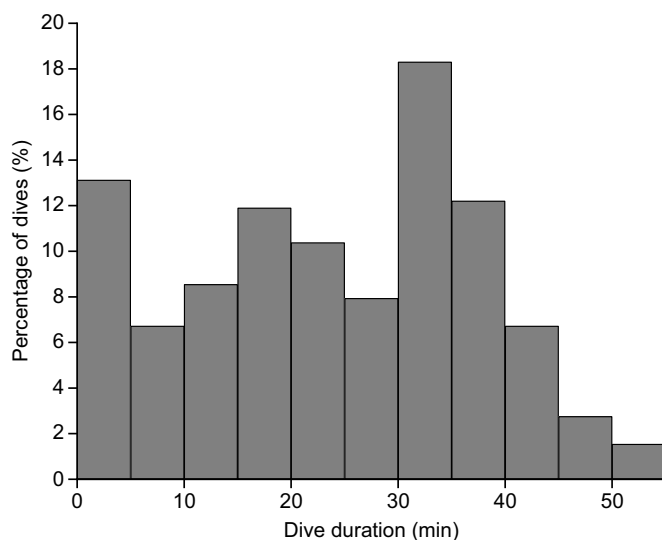


Fig. 1. Frequency distribution of dive durations. $n=8$ turtles, 328 dives.

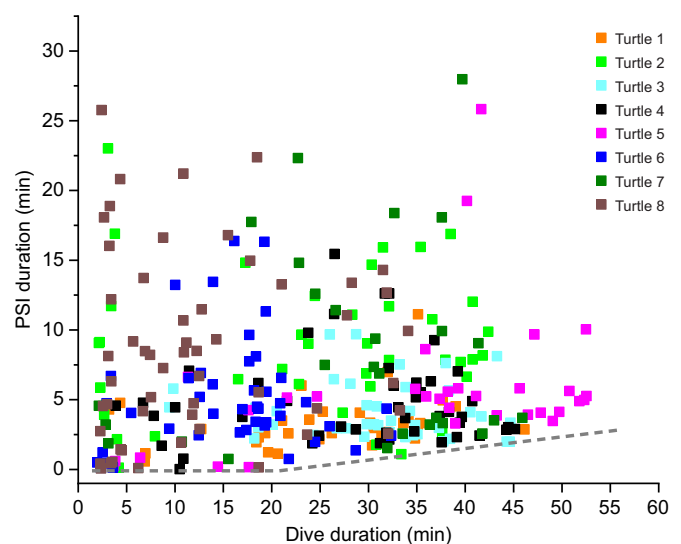


Fig. 2. Post-dive surface interval (PSI) duration versus dive duration. Dives with PSIs in excess of 30 min were excluded. Dashed line represents minimum PSI duration. Individual turtles are represented by different colors (see key). $n=8$ turtles, 317 dives.

Table 2. Heart rate (f_H) data for five conditions and feeding duration for the four 2015 turtles

Turtle	Diving f_H (beats min ⁻¹)	Bottom rest f_H (beats min ⁻¹)	Shallow-water rest f_H (beats min ⁻¹)	Feeding f_H (beats min ⁻¹)	Feeding duration (min)	PSI f_H (beats min ⁻¹)
5	7.3±1.7 (5.4–12.4)	6.1±0.8 (3.7–7.4)	9.5±0.9 (7.3–11.7)	8.9±2.5 (4.9–13.5)	4.1	18.9±1.9 (14.1–22.0)
6	5.2±1.4 (3.3–10.9)	5.5±0.6 (4.1–7.7)	9.9±2.9 (4.5–16.2)	9.8±3.1 (4.6–14.6)	5.7	13.9±4.0 (7.6–20.7)
7	6.8±1.6 (4.9–11.6)	5.8±0.8 (3.9–7.2)	11.8±2.6 (6.0–16.8)	13.9±3.2 (5.7–19.7)	2.5	18.7±3.0 (13.6–24.6)
8	7.6±1.7 (4.9–11.5)	7.8±3.4 (5.0–15.1)	8.2±0.7 (6.6–10.2)	11.0±1.5 (7.9–13.2)	1.9	12.9±2.0 (9.3–17.2)
Grand mean	6.7±1.1	6.4±1.0	9.9±1.5	10.9±2.2	3.6±1.7	16.1±3.2

There was one bottom rest, shallow-water rest and feeding event per turtle. The numbers of dives and PSIs are different for each turtle and specified in Table 1. For f_H columns, the first line is the mean±s.d. and the second line is the range (in parentheses). PSI, post-dive surface interval; $N=4$ turtles.

diving f_H ($P<0.05$, $t=3.2$, d.f.=12), but not shallow-water rest f_H ($P=0.9$, $t=0.81$, d.f.=12).

Relationship between f_H and dive duration

The total number of heart beats was driven by dive duration and water temperature ($P<0.001$, $\chi^2=476.3$, d.f.=4, $R^2_m=0.87$, $R^2_c=0.97$; Fig. 5A) as total beats increased by 5.4 and 15.0 beats for each additional minute submerged and 1°C increase in water temperature, respectively (duration: $P<0.001$, $F_{1,2.8}=373.2$; water temperature: $P<0.001$, $F_{1,158.4}=62.5$). Mean diving f_H versus duration is plotted in Fig. 5B for illustration. Mean minimum instantaneous f_H was significantly related to dive duration and water temperature ($P<0.001$, $\chi^2=187.3$, d.f.=5, $R^2_m=0.30$, $R^2_c=0.77$; Fig. 6). The best predictive model for mean minimum instantaneous diving f_H included dive duration ($P<0.05$, $F_{1,20.5}=7.1$), water temperature ($P<0.05$, $F_{1,95.4}=38.9$) and their interaction ($P<0.01$, $F_{1,20.7}=12.1$).

Heart rate patterns

When turtles began to descend during dives, f_H dropped quickly from a range of 12 to 20 beats min⁻¹ down to less than 10 beats min⁻¹ (Fig. 4). While resting on the bottom, mean minimum instantaneous f_H was less than 6 beats min⁻¹ for all turtles (Table 1). There was no significant difference between bottom rest f_H and diving f_H ($P=1.0$, $t=0.29$, d.f.=12; Fig. 3). After turtles returned to the surface, f_H was always the highest f_H as turtles swam continuously during the PSI (Table 2, Fig. 3). In addition to being higher than diving f_H , PSI f_H was significantly higher than

bottom rest f_H ($P<0.001$, $t=7.5$, d.f.=12), shallow-water rest f_H ($P<0.01$, $t=4.8$, d.f.=12) and feeding f_H ($P<0.05$, $t=4.0$, d.f.=12). Dive duration and water temperature were significantly related to instantaneous mean PSI f_H ($\chi^2=91.3$, $P<0.001$, d.f.=4, $R^2_m=0.38$, $R^2_c=0.68$, $n=4$; Fig. 7), such that for each additional minute submerged, post-dive f_H increased by 0.13 beats min⁻¹ ($P<0.05$, $F_{1,3.6}=13.3$). An increase in water temperature also had a significant effect on PSI f_H , raising f_H by 1.7 beats min⁻¹ °C⁻¹ ($P<0.001$, $F_{1,156.6}=37.9$).

Effect of temperature

Mean water temperature was slightly higher in 2013 ($23.0\pm0.4^\circ\text{C}$) than in 2015 ($20.0\pm0.3^\circ\text{C}$) and varied among individual turtles (Table 1). Water temperature was not significant in all models, likely because it did not vary widely, especially among 2015 turtles (Table 1).

DISCUSSION

The most important findings in this study are that (a) diving f_H and bottom rest f_H were not significantly different from f_H when turtles were resting with their head out of water, (b) changes in f_H were largely driven by activity, and (c) f_H was not further reduced in longer dives.

Diving f_H was lower than PSI f_H , but not different from shallow-water rest f_H

Although it is often assumed that all air-breathing vertebrates have a diving bradycardia (e.g. Andersen, 1966), the question of whether

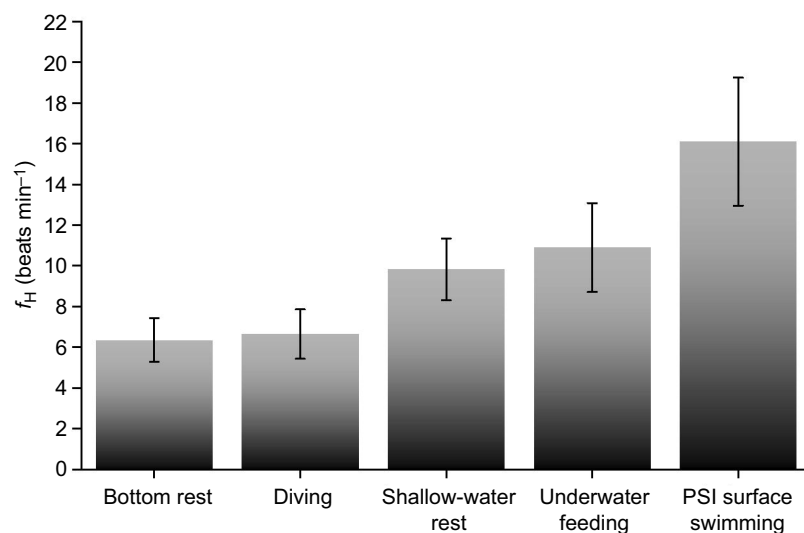


Fig. 3. Mean±s.d. heart rate (f_H) for five routine activities of loggerhead sea turtles from 2015. Bottom rest indicates turtles resting motionless on the bottom of tanks, whereas shallow-water rest occurred when turtles were in shallow water with their head out of water. During underwater feeding, turtles swam (without breathing) after squid were placed in the tank. Dives began when the turtle's head was underwater and ended when the turtle raised its head to breathe. Surface swimming includes breathing. $n=4$ turtles.

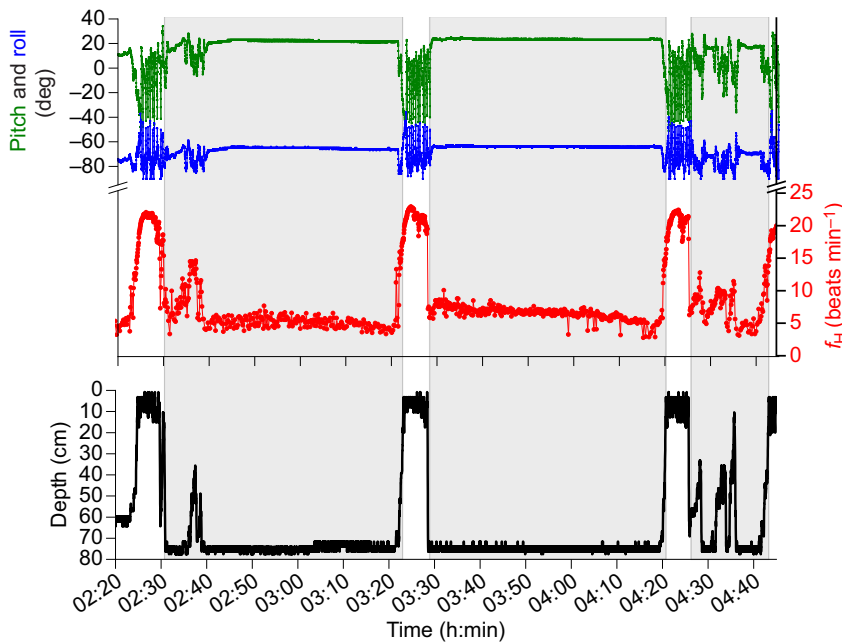


Fig. 4. f_H (red), pitch (green), roll (blue) and depth (black) profiles during three dives and three surface intervals of turtle 5 on 8 September 2015. Shaded areas indicate turtle is submerged. Heart rate during dives is low unless the turtle is moving, as indicated by the pitch and roll profiles.

turtles experience a diving bradycardia is complicated by the control f_H with which the diving f_H is compared. If a diving bradycardia is defined as a lower f_H during diving compared with at the surface, then loggerhead turtles experienced a diving bradycardia under the conditions of the present study. Diving f_H was less than half the PSI f_H . In marine turtles freely diving at sea, leatherback turtles' diving f_H (17 beats min^{-1}) was only moderately lower than post-dive surface f_H (25 beats min^{-1}) (Southwood et al., 1999). However, it is difficult to compare these results because, in the present study, turtles were primarily resting on the bottom during dives, whereas leatherbacks were diving at sea and, thus, were potentially more active (Southwood et al., 1999). While freely diving at sea, loggerhead turtles may also have higher f_H ; however, to date there have been no studies examining diving f_H in loggerhead turtles at sea.

Conversely, if a diving bradycardia is defined as a significantly lower f_H during dives compared with f_H at the same activity level either at the surface or on land (Stephenson et al., 1986), then loggerhead turtles did not experience a diving bradycardia. There was no significant difference in f_H of these turtles either during the entire dive or during rest in the bottom phase of the dive compared with f_H at rest in shallow water with the turtle's head out of the water. Although this study will not resolve how to define a diving bradycardia in marine turtles, at a minimum, these results suggest that f_H is not further reduced by being submerged. These results are consistent with the lack of difference in resting f_H found in red-eared sliders, whether on land or underwater (Krosniunas and Hicks, 2003). The same study also found no difference in f_H between walking on land, underwater swimming or diving in red-eared sliders (Krosniunas and Hicks, 2003).

The comparison between shallow-water rest and bottom rest or diving f_H values is also complicated by the cost of ventilation during shallow-water rest. Although breathing was not monitored in the present study during shallow-water rest, the pitch and roll profiles indicated that, in some instances, breathing may have occurred. Ventilation in marine turtles requires the use of respiratory muscles to expand the lungs and neck muscles to raise the head, both of which should come at an energetic cost. However, O_2 consumption in resting loggerhead turtles did not increase during ventilation (Lutz et al., 1989). Similarly, estimates of the oxidative costs of

breathing in freshwater turtles suggest it is low (from 1% of resting metabolic rate) (Jackson et al., 1991). The lack of a significant difference between shallow-water rest f_H and diving f_H or bottom rest f_H supports the hypothesis that marine turtles exhibit low metabolic costs of ventilation or that the turtles in the present study were primarily apneic during the shallow-water rest periods.

Heart rate increased as underwater activity levels increased

Changes in the f_H of submerged loggerhead turtles were driven by activity, similar to terrestrial animals as part of the exercise response. When turtles were motionless on the bottom of tanks, f_H was lowest at 6.3 beats min^{-1} . However, with any discernible movement, f_H increased (Fig. 4), at times nearing PSI f_H levels. The highest underwater f_H occurred as turtles swam underwater during feeding; it was significantly higher than bottom rest f_H and diving f_H . While diving at sea, the most vigorous underwater swimming occurs as turtles begin to descend, when stroke frequency and amplitude are highest (Hays et al., 2007). Our findings suggest that f_H may be highest during the descent phase of dives. This activity-related increase in f_H also occurs in some diving endotherms. A number of marine mammals and birds have a graded dive response in which exercise appears to decrease the level of bradycardia (i.e. increase f_H) in some, but not necessarily all, dives (Butler and Jones, 1997; Davis and Williams, 2012; Hindle et al., 2010; Noren et al., 2012; Signore and Jones, 1996; Williams et al., 2015). For example, in Steller sea lions, f_H was related to activity levels during shallow dives, but not deep dives (Hindle et al., 2010). Nonetheless, despite these varied responses, f_H typically remained below surface or resting levels in most dives of these other species.

However, this effect of underwater activity on turtle f_H is in stark contrast to effects in other marine mammals and birds, including emperor penguins diving at sea. During the bottom phases of the emperor penguin's deepest dives at sea, stroke rate was the highest (Williams et al., 2012). Yet, Wright et al. (2014) found that during these deepest segments, emperor penguin f_H was at its lowest. Similarly, during the initial post-release dives of narwhals, f_H was maintained near 10 beats min^{-1} , independent of stroke activity (Williams et al., 2017). By uncoupling workload from f_H , these divers conserve O_2 for hypoxia-sensitive organs (the heart and brain). This

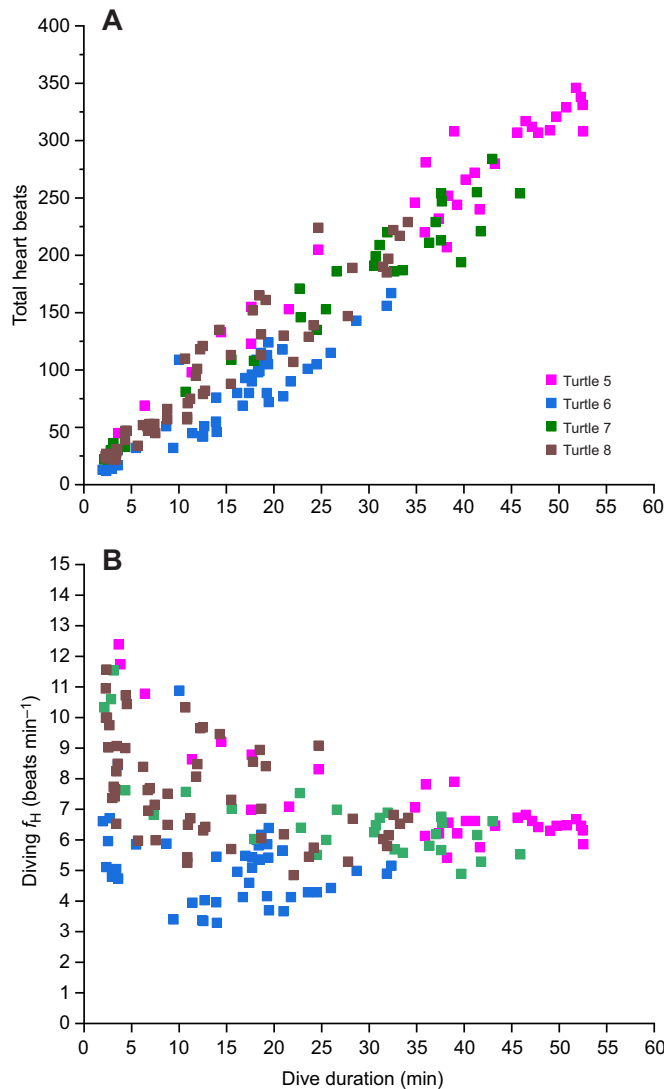


Fig. 5. f_H during dives of 2015 loggerhead turtles. (A) Total heart beats versus dive duration. (B) Diving f_H versus dive duration. Individual turtles are indicated by different colors (see key). $n=4$ turtles, 164 dives.

contrast between turtles and diving endotherms on the effect of activity was also observed in forced submersions of marine turtles and seals, where struggling elicited a significant increase in f_H in green turtles, but not in seals (Berkson, 1966; Scholander, 1940).

Like marine turtles, red-eared sliders also demonstrate differences in f_H related to their activity level (Krosniunas and Hicks, 2003). f_H increased when red-eared sliders were active; however, in these turtles, the increases in f_H appeared to be constrained to a narrow range set by body temperature (Krosniunas and Hicks, 2003). In the present study, water temperature did not have a significant effect on f_H during different activities, although there was only a minimal temperature difference among the turtles in 2015 (Table 1). Further research is needed to determine whether there is a similar effect of activity level on f_H in marine turtles freely diving at sea and whether cardiovascular responses to activity at different water temperatures are similarly constrained by narrow f_H ranges.

Relationship between f_H and dive duration

Marine mammals and emperor penguins frequently extend aerobic dive durations by reducing f_H , which reduces O_2 consumption in

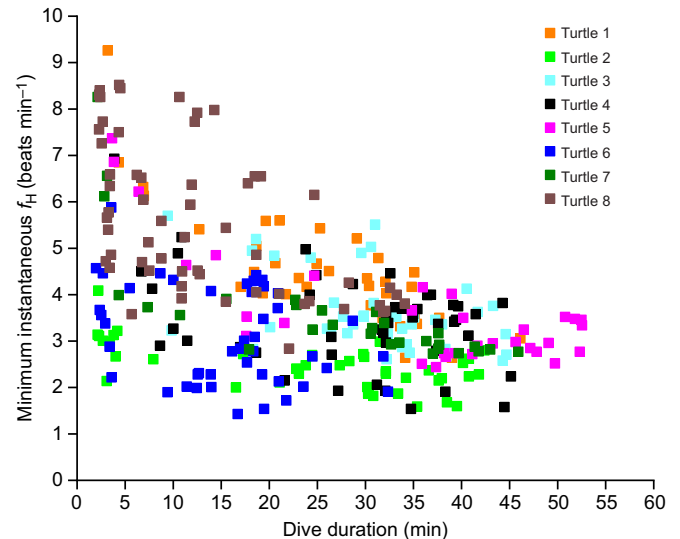


Fig. 6. Minimum instantaneous f_H during dives versus dive duration. Individual turtles are represented by different colors (see key). $n=8$ turtles, 328 dives.

perfusion dependent organs. In emperor penguins and grey seals diving at sea, the slopes of the relationship between total heart beats and dive duration begin with a linear relationship similar to that in loggerhead turtles (Fig. 5A), but then the number of heart beats flattens out in dives beyond 6 and 7 min, respectively (Thompson and Fedak, 1993; Wright et al., 2014). This plateau, which is consistent with greater reductions in f_H during longer dives of seals and penguins, was not observed in the loggerhead turtles (Fig. 5). The lack of a plateau suggests these turtles did not need to further reduce f_H and O_2 consumption to maintain aerobic metabolism during the long dives in this study. Both this linear increase in total number of heart beats with dive duration and the fact that all dive durations were less than cADLs (Table 1) support the hypothesis that metabolism was primarily aerobic in these turtles.

It is possible that, in loggerhead turtles at sea, f_H decreases further in longer dives. The longest dives in the present study (30–50 min)

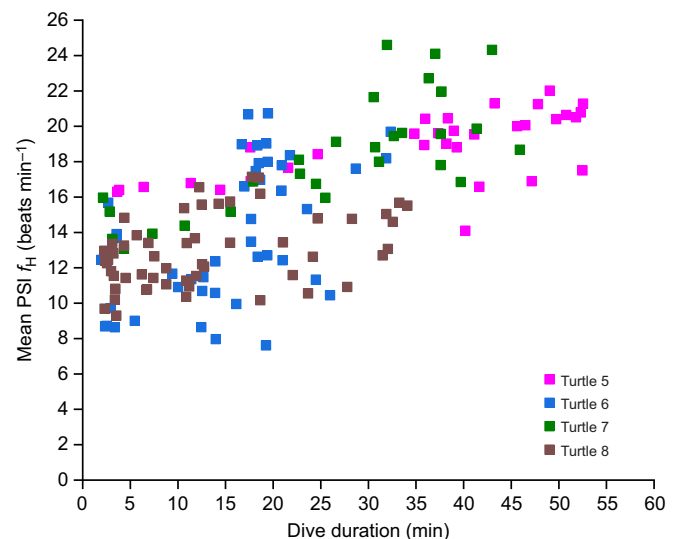


Fig. 7. Mean f_H during surface intervals after dives compared with the duration of the previous dive. Individual turtles are represented by different colors (see key). $n=4$ turtles, 160 dives.

were considerably shorter than the longest dives of similarly sized loggerhead turtles diving at sea (80–150 min) in comparable temperatures (Hochscheid et al., 2007, 2005). Further, minimum instantaneous f_H during dives in the present study declined slightly, but significantly with dive duration (Fig. 6), raising the possibility that diving f_H may significantly decrease in dives over 50 min.

However, the shorter dive durations in the present study have several possible explanations with different implications for changes in diving f_H . First, loggerhead turtles in the North Pacific may dive for shorter durations with higher f_H because they have a higher metabolic rate than other populations. North Pacific juvenile loggerhead turtles maintain higher resting metabolic rates than Mediterranean loggerhead turtles, which results in lower cADLs of North Pacific turtles (Hochscheid et al., 2004; Kinoshita et al., 2018) (see Table S1 for differences in \dot{V}_{O_2} and cADL calculated using equations derived from data of the two populations). Future heart rate studies comparing Mediterranean and North Pacific loggerhead turtles might reveal lower resting f_H in Mediterranean turtles considering their lower metabolic rate. Second, anaerobic metabolism during dives at sea may contribute to longer dive durations, which would not necessitate lower f_H in longer dives. Marine turtles have a high capacity for anaerobic metabolism; however, anaerobic metabolism is believed to be used during intense activities rather than to extend dive durations (Southwood et al., 2003, 2006). Third, dives in the present study could be shorter because turtles are diving with a reduced lung volume. To remain at their preferred depth, loggerhead turtles alter their depth to maintain neutral buoyancy or slightly negative buoyancy, which conserves O_2 by reducing the work effort required to move underwater or rest on the ocean floor (Hays et al., 2004; Hochscheid et al., 2003; Minamikawa et al., 2000). Because turtles could not alter their depth in the shallow tanks, to become slightly negatively buoyant they would have to decrease their inhaled lung volume, reducing the lung O_2 store. As a result, loggerhead turtles diving at sea may have similar f_H , but longer durations as a result of diving with a full lung volume, although extended dive durations were not shown in weighted loggerhead turtles diving in shallow tanks (Hochscheid et al., 2003). Thus, regardless of whether dive durations are shorter or longer at sea, f_H may not be lower than in the present study because of differences in (1) metabolic rates between populations of loggerhead turtles, (2) preferred metabolic pathways at sea, or (3) diving lung volume. Additional research is needed to investigate f_H in freely diving loggerhead turtles and determine if there is a relationship between at sea dive durations and f_H .

PSI f_H and minimum PSI duration increased after longer dive durations

Although loggerhead turtles did not extend dive durations by reducing f_H , they did increase PSI f_H after longer dives (Fig. 7). A faster f_H will reload O_2 stores and unload CO_2 more quickly, minimize time at the surface and allow turtles to maintain high dive-duration-to-surface-interval ratios. Although the longest dives did not have the longest PSI durations, there was a clear increase in the minimum PSI duration as dive durations increased (Fig. 2), suggesting additional minimum recovery times were necessary for longer dives. However, the increase was minor as the longest dives only increased the minimum PSI by a few minutes.

Heart rate patterns

The profiles of loggerhead turtle f_H are similar to those of other turtle studies in that underwater resting f_H was lower compared with swimming f_H or eupneic f_H (Table 3). However, it is difficult to draw any other conclusions from comparisons with prior studies because of differences in measurement conditions, turtle size, species and water temperature (Table 3). Despite these differences, resting f_H and swimming f_H in the present study were much lower than in turtles from past studies, regardless of whether other turtles were bigger or smaller, in colder or warmer temperatures, or swimming in tanks or at sea. These exceptionally low f_H values were apparently adequate for maintenance of homeostasis out of water or underwater in these captive turtles.

The highest overall f_H in the present study occurred when turtles were at the surface. The high PSI f_H was likely driven by ventilation, as well as activity. Activity at the surface included swimming and movements required for breathing. Marine turtles swim with their head underwater, but use their flippers to help lift their head to breathe (Prange, 1976). We could not separate the activity costs from ventilatory costs because turtles did not stop moving during PSIs, preventing a comparison between f_H while floating at the surface and f_H while surface swimming. However, a transient increase in f_H was associated with breathing in green turtles (Butler et al., 1984; Davenport et al., 1982; West et al., 1992). Although ventilatory costs may be very low in marine turtles, increased ventilation likely contributes to a higher PSI f_H because PSI f_H increased with longer dives.

Conservation implications

Mortality rates from turtle interactions with fisheries are high, with loggerhead turtles having interactions with fisheries more than other marine turtle species in the United States (Finkbeiner et al., 2011).

Table 3. f_H of different marine turtle species during underwater rest and swimming or breathing

Marine turtle species	Mass (kg)	Water temperature (°C)	Study conditions	Sample size	Underwater rest f_H (beats min ⁻¹)	Swimming/eupneic f_H (beats min ⁻¹)	Reference
Loggerhead	38–88	20–23	Data logger in tanks	8	6.4	10.9	Present study
Loggerhead	11.8	19.3–22.5	Flipper blood flow study	1	18	19	Hochscheid et al., 2002
Loggerhead	25	15.5	Attached to cord in tank	1	7.5	15	Lanteri et al., 1980
Leatherback	172–377	25–27.5	Freely diving at sea	5	N/A	17.4	Southwood et al., 1999; Southwood et al., 2005
Green (winter)	19–43	17	Data logger in tanks	3–5	10.2	12.9	Southwood et al., 2003
Green (summer)	19–43	26	Data logger in tanks	3–5	19.6	24.0	Southwood et al., 2003

f_H from present study is lower than marine turtles in other studies. Note differences in turtle size, water temperature and study conditions.

Recent discovery of gas emboli in sea turtles suggest that turtles may suffer from decompression sickness after being entangled in nets at depth (García-Párraga et al., 2014). Although turtles typically lower heart rate during forced submergences (Berkson, 1966), the physiological response to net entanglement is likely different. Our results suggest that if turtles are struggling in nets, f_H will increase from vigorous activity, which will result in rapid oxygen depletion. A faster oxygen depletion can contribute to lactate accumulation, and turtles may suffer from lactic acidosis. Further, a higher f_H will increase pulmonary blood flow, which may contribute to increased nitrogen absorption, potentially resulting in the development of gas emboli, recently observed in marine turtles after being entangled in nets (García-Párraga et al., 2018). Future studies designed to increase our understanding of physiological responses to both baseline activities and net entanglement is vital for understanding how to protect and manage marine turtle populations.

Intravascular ECG electrodes resulted in clear ECG records even during activity

ECG signals in 2013 were clear when the turtles were resting on the bottom of the tanks, but became increasingly noisy as the turtle began to move, similar to what was observed in green turtles during movement (Southwood et al., 2003). The noise is likely due to interference from electromyography signals from muscle contractions during movements as it was highest when turtles were the most active – swimming at the surface. The use of a bipolar pacing catheter in 2015 resolved this problem, providing clear ECG signals during all activities, with only rare instances of unreadable signals (Fig. S1).

Conclusions

Our results that f_H while resting out of water was no different from f_H during dives or bottom rest indicate that, under the conditions of this study, f_H is not further reduced by submergence. Further, our finding that f_H also remained relatively constant in short and long dives suggests that loggerhead turtles do not rely on further f_H reductions to reduce O_2 consumption and maintain aerobic metabolism during the longer dive durations of this study. Rather, f_H appears driven by underwater activity levels as adjustments in f_H during dives were in line with an exercise response. With their lack of elevated muscle O_2 stores and the apparent absence of reduced muscle blood flow during forced submersions (Lutz and Bentley, 1985), loggerhead turtles must maintain high enough f_H to support routine O_2 consumption, as well as the increased O_2 requirements in muscle during activity. Thus, the increase in f_H during feeding may be at least partially in response to the additional muscle O_2 demands from underwater swimming.

The f_H values we report here highlight the value of recording ECG using self-contained data loggers. Our findings of lower overall f_H compared with values in previously measured turtles suggest that, in some cases, f_H in other studies may have been affected by the presence of investigators or the turtles being attached to external equipment. The f_H of undisturbed loggerhead turtles in our study provide a baseline for understanding cardiac responses to resting, surface swimming and underwater feeding. These are key activities for the loggerhead turtle, which is known to rest on the ocean bottom for over 7 h in cold temperatures (Hawkes et al., 2007; Hochscheid et al., 2007), spend significant time at the surface (traveling or foraging on surface prey) (Hochscheid et al., 2010; Polovina et al., 2004), and forage within the water column (Casale et al., 2008; Narazaki et al., 2013). f_H at the surface was the highest likely because of increased O_2 demands from continuous swimming

and ventilation. $PSI f_H$ increased as dive duration increased. This strategy allows loggerhead turtles to maximize underwater foraging time by minimizing recovery time at the surface.

These results are the first f_H records of undisturbed loggerhead turtles and provide valuable information to assess captive loggerhead turtles as well as to make comparisons with future studies on marine turtles at sea. Whether cardiac responses in loggerhead turtles freely diving at sea are different from the present study awaits further investigation.

Acknowledgements

We thank Takuya Fukuoka, Misaki Yamane and Chihiro Kinoshita for their assistance in the field. We also thank Tomoko Narazaki for valuable input on the initial design of the experiment, and James Hicks, Allyson Hindle, Graeme Hays and an anonymous reviewer for helpful comments on earlier versions of the manuscript. We are grateful to the volunteers from the Fisheries Cooperative Association of Funakoshi Bay, Hirota Bay, Kamaishi Bay, Kamaish-Tobu, Miyako, Ofunato, Okitai, Omoe, Shin-Otsuchi, Ryori, Sanriku-Yamada, Sasaki, Toni, Yamaichi and Yoshiama for providing the incidentally caught, wild loggerhead sea turtles used in this study.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.L.W., P.J.P.; Methodology: C.L.W., K.S., P.J.P.; Software: C.L.W.; Validation: C.L.W., P.J.P.; Formal analysis: C.L.W.; Investigation: C.L.W., K.S., P.J.P.; Resources: K.S., P.J.P.; Writing - original draft: C.L.W.; Writing - review & editing: C.L.W., K.S., P.J.P.; Visualization: C.L.W.; Supervision: P.J.P.; Project administration: K.S., P.J.P.; Funding acquisition: C.L.W., P.J.P.

Funding

This study was funded by the National Science Foundation (IOS-1121324 to P.J.P.) and Tohoku Ecosystem-Associated Marine Science (TEAMS). C.L.W. was supported by a National Institutes of Health training grant (2T32AR047752 to V. J. Caiozzo). The present study was performed under the Cooperative Program of Atmosphere and Ocean Research Institute, the University of Tokyo. Deposited in PMC for release after 12 months.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.200824.supplemental>

References

- Andersen, H. T. (1966). Physiological adaptations in diving vertebrates. *Physiol. Rev.* **46**, 212–243. doi:10.1152/physrev.1966.46.2.212
- Andrews, R. D., Jones, D. R., Williams, J. D., Thorson, P. H., Oliver, G. W., Costa, D. P. and Le Boeuf, B. J. (1997). Heart rates of northern elephant seals diving at sea and resting on the beach. *J. Exp. Biol.* **200**, 2083–2095.
- Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. doi:10.18637/jss.v067.i01
- Bentivegna, F., Hochscheid, S. and Minucci, C. (2003). Seasonal variability in voluntary dive duration of the Mediterranean loggerhead turtle, *Caretta caretta*. *Sci. Mar.* **67**, 371–375. doi:10.3989/scimar.2003.67n3371
- Berkson, H. (1966). Physiological adjustments to prolonged diving in the Pacific green turtle (*Chelonia mydas agassizii*). *Comp. Biochem. Physiol.* **18**, 101–119. doi:10.1016/0010-406X(66)90335-5
- Butler, P. J. and Jones, D. R. (1997). Physiology of diving of birds and mammals. *Physiol. Rev.* **77**, 837–899. doi:10.1152/physrev.1997.77.3.837
- Butler, P. J., Milsom, W. K. and Woakes, A. J. (1984). Respiratory, cardiovascular and metabolic adjustments during steady state swimming in the green turtle, *Chelonia mydas*. *J. Comp. Physiol. B* **154**, 167–174. doi:10.1007/BF00684141
- Carr, A., Ogren, L. and McVea, C. (1980). Apparent hibernation by the Atlantic loggerhead turtle *Caretta caretta* off cape canaveral, Florida. *Biol. Conserv.* **19**, 7–14. doi:10.1016/0006-3207(80)90011-7
- Casale, P., Abbate, G., Freggi, D., Conte, N., Oliverio, M. and Argano, R. (2008). Foraging ecology of loggerhead sea turtles *Caretta caretta* in the central Mediterranean Sea: evidence for a relaxed life history model. *Mar. Ecol. Prog. Ser.* **372**, 265–276. doi:10.3354/meps07702
- Davenport, J., Inagle, G. and Hughes, A. K. (1982). Oxygen uptake and heart rate in young green turtles (*Chelonia mydas*). *J. Zool.* **198**, 399–412. doi:10.1111/j.1469-7998.1982.tb02084.x
- 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*

- Neuroethol. Sens. Neural Behav. Physiol.* **198**, 583–591. doi:10.1007/s00359-012-0731-4
- Di Bello, A., Valastro, C., Freggi, D., Saponaro, V. and Grimaldi, D. (2010). Ultrasound-guided vascular catheterization in loggerhead sea turtles (*Caretta caretta*). *J. Zoo Wildl. Med.* **41**, 516–518. doi:10.1638/2008-0195.1
- Felger, R. S., Clifton, K. and Regal, P. J. (1976). Winter dormancy in sea turtles: independent discovery and exploitation in the Gulf of California by two local cultures. *Science* **191**, 283–285. doi:10.1126/science.191.4224.283
- Finkbeiner, E. M., Wallace, B. P., Moore, J. E., Lewison, R. L., Crowder, L. B. and Read, A. J. (2011). Cumulative estimates of sea turtle bycatch and mortality in USA fisheries between 1990 and 2007. *Biol. Conserv.* **144**, 2719–2727. doi:10.1016/j.biocon.2011.07.003
- Gallivan, G. J., Kanwisher, J. W. and Best, R. C. (1986). Heart rates and gas exchange in the Amazonian manatee (*Trichechus inunguis*) in relation to diving. *J. Comp. Physiol. B* **156**, 415–423. doi:10.1007/BF01101104
- García-Párraga, D., Crespo-Picazo, J. L., de Quirós, Y. B., Cervera, V., Martí-Bonmati, L., Díaz-Delgado, J., Arbelo, M., Moore, M. J., Jepson, P. D. and Fernández, A. (2014). Decompression sickness ('the bends') in sea turtles. *Dis. Aquat. Org.* **111**, 191–205. doi:10.3354/dao02790
- García-Párraga, D., Lorenzo, T., Wang, T., Ortiz, J.-L., Ortega, J., Crespo-Picazo, J.-L., Cortijo, J. and Fahlman, A. (2018). Deciphering function of the pulmonary arterial sphincters in loggerhead sea turtles (*Caretta caretta*). *J. Exp. Biol.* **221**, jeb179820. doi:10.1242/jeb.179820
- Hawkes, L. A., Broderick, A. C., Coyne, M. S., Godfrey, M. H. and Godley, B. J. (2007). Only some like it hot — quantifying the environmental niche of the loggerhead sea turtle. *Divers. Distrib.* **13**, 447–457. doi:10.1111/j.1472-4642.2007.00354.x
- Hays, G. C., Metcalfe, J. D. and Walne, A. W. (2004). The implications of lung-regulated buoyancy control for dive depth and duration. *Ecology* **85**, 1137–1145. doi:10.1890/03-0251
- Hays, G. C., Marshall, G. J. and Seminoff, J. A. (2007). Flipper beat frequency and amplitude changes in diving green turtles, *Chelonia mydas*. *Mar. Biol.* **150**, 1003–1009. doi:10.1007/s00227-006-0412-3
- 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. doi:10.1016/j.jembe.2010.08.006
- Hochscheid, S., Bentivegna, F. and Speakman, J. R. (2002). Regional blood flow in sea turtles: Implications for heat exchange in an aquatic ectotherm. *Physiol. Biochem. Zool.* **75**, 66–76. doi:10.1086/339050
- Hochscheid, S., Bentivegna, F. and Speakman, J. R. (2003). The dual function of the lung in chelonian sea turtles: buoyancy control and oxygen storage. *J. Exp. Mar. Biol. Ecol.* **297**, 123–140. doi:10.1016/j.jembe.2003.07.004
- Hochscheid, S., Bentivegna, F. and Speakman, J. R. (2004). Long-term cold acclimation leads to high Q_{10} effects on oxygen consumption of loggerhead sea turtles *Caretta caretta*. *Physiol. Biochem. Zool.* **77**, 209–222. doi:10.1086/381472
- Hochscheid, S., Bentivegna, F. and Hays, G. C. (2005). First records of dive durations for a hibernating sea turtle. *Biol. Lett.* **1**, 82–86. doi:10.1098/rsbl.2004.0250
- Hochscheid, S., Bentivegna, F., Bradai, M. N. and Hays, G. C. (2007). Overwintering behaviour in sea turtles: dormancy is optional. *Mar. Ecol. Prog. Ser.* **340**, 287–298. doi:10.3354/meps340287
- Hochscheid, S., Bentivegna, F., Hamza, A. and Hays, G. C. (2010). When surfacers do not dive: multiple significance of extended surface times in marine turtles. *J. Exp. Biol.* **213**, 1328–1337. doi:10.1242/jeb.037184
- Jackson, D. C., Singer, J. H. and Downey, P. T. (1991). Oxidative cost of breathing in the turtle *Chrysemys picta bellii*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **261**, R1325–R1328. doi:10.1152/ajpregu.1991.261.5.R1325
- Kinoshita, C., Fukuoka, T., Niizuma, Y., Narazaki, T. and Sato, K. (2018). High resting metabolic rates with low thermal dependence induce active dives in overwintering Pacific juvenile loggerhead turtles. *J. Exp. Biol.* **221**, jeb.175836. doi:10.1242/jeb.175836
- Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnett, E. E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **138**, 335–346. doi:10.1007/BF00691568
- Kooyman, G. L., Castellini, M. A., Davis, R. W. and Maue, R. A. (1983). Aerobic diving limits of immature Weddell seals. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **151**, 171–174. doi:10.1007/BF00689915
- Krosniunas, E. H. and Hicks, J. W. (2003). Cardiac output and shunt during voluntary activity at different temperatures in the turtle, *Trachemys scripta*. *Physiol. Biochem. Zool.* **76**, 679–694. doi:10.1086/377745
- Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* **82**, 1–26. doi:10.18637/jss.v082.i13
- Lanteri, A., Lloze, R. and Roussel, H. (1980). Diving and heart beat compounds in the marine turtle *Caretta caretta* (LINNÉ) (Reptilia, Testudines). *Amphibia-Reptilia* **1**, 337–341. doi:10.1163/156853881x00429
- Lutcavage, M. E., Bushnell, P. G. and Jones, D. R. (1990). Oxygen transport in the leatherback sea turtle *Dermochelys coriacea*. *Physiol. Zool.* **63**, 1012–1024. doi:10.1086/physzool.63.5.30152626
- Lutcavage, M. E., Bushnell, P. G. and Jones, D. R. (1992). Oxygen stores and aerobic metabolism in the leatherback sea turtle. *Can. J. Zool.* **70**, 348–351. doi:10.1139/z92-051
- Lutz, P. L. and Bentley, T. B. (1985). Respiratory physiology of diving in the sea turtle. *Copeia* **1985**, 671–679. doi:10.2307/1444761
- Lutz, P. L., LaManna, J. C., Adams, M. R. and Rosenthal, M. (1980). Cerebral resistance to anoxia in the marine turtle. *Respir. Physiol.* **41**, 241–251. doi:10.1016/0034-5687(80)90074-2
- Lutz, P. L., Bergoy, A. and Bergoy, M. (1989). Effects of temperature on gas exchange and acid-base balance in the sea turtle *Caretta caretta* at rest and during routine activity. *J. Exp. Biol.* **144**, 155–169.
- Makowski, D. (2018). The psycho package: an efficient and publishing-oriented workflow for psychological science. *J. Open Source Software* **3**, 470. doi:10.21105/joss.00470
- McDonald, B. I. and Ponganis, P. J. (2014). Deep-diving sea lions exhibit extreme bradycardia in long-duration dives. *J. Exp. Biol.* **217**, 1525–1534. doi:10.1242/jeb.098558
- Meir, J. U., Stockard, T. K., Williams, C. L., Ponganis, K. V. and Ponganis, P. J. (2008). Heart rate regulation and extreme bradycardia in diving emperor penguins. *J. Exp. Biol.* **211**, 1169–1179. doi:10.1242/jeb.013235
- Meir, J. U., Champagne, C. D., Costa, D. P., Williams, C. L. and Ponganis, P. J. (2009). Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R927–R939. doi:10.1152/ajpregu.00247.2009
- Minamikawa, S., Naito, Y., Sato, K., Matsuzawa, Y., Bando, T. and Sakamoto, W. (2000). Maintenance of neutral buoyancy by depth selection in the loggerhead turtle *Caretta caretta*. *J. Exp. Biol.* **203**, 2967–2975.
- Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142. doi:10.1111/j.2041-210x.2012.00261.x
- Narazaki, T., Sato, K., Abernathy, K. J., Marshall, G. J. and Miyazaki, N. (2013). Loggerhead turtles (*Caretta caretta*) use vision to forage on gelatinous prey in mid-water. *PLoS ONE* **8**, e66043. doi:10.1371/journal.pone.0066043
- Narazaki, T., Sato, K. and Miyazaki, N. (2015). Summer migration to temperate foraging habitats and active winter diving of juvenile loggerhead turtles *Caretta caretta* in the western North Pacific. *Mar. Biol.* **162**, 1251–1263. doi:10.1007/s00227-015-2666-0
- Noren, S. R., Kendall, T., Cuccurullo, V. and Williams, T. M. (2012). The dive response redefined: underwater behavior influences cardiac variability in freely diving dolphins. *J. Exp. Biol.* **215**, 2735–2741. doi:10.1242/jeb.069583
- Polovina, J. J., Balazs, G. H., Howell, E. A., Parker, D. M., Seki, M. P. and Dutton, P. H. (2004). Forage and migration habitat of loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*) sea turtles in the central North Pacific Ocean. *Fish. Oceanogr.* **13**, 36–51. doi:10.1046/j.1365-2419.2003.00270.x
- Ponganis, P. J. (2015). *Diving Physiology of Marine Mammals and Seabirds*. Cambridge University Press.
- Ponganis, P. J., Stockard, T. K., Meir, J. U., Williams, C. L., Ponganis, K. V., van Dam, R. P. and Howard, R. (2007). Returning on empty: extreme blood O_2 depletion underlies dive capacity of emperor penguins. *J. Exp. Biol.* **210**, 4279–4285. doi:10.1242/jeb.011221
- Prange, H. D. (1976). Energetics of swimming of a sea turtle. *J. Exp. Biol.* **64**, 1–12.
- Prange, H. D. and Jackson, D. C. (1976). Ventilation, gas exchange and metabolic scaling of a sea turtle. *Respir. Physiol.* **27**, 369–377. doi:10.1016/0034-5687(76)90065-7
- Scholander, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalradets Skrifter* **22**, 1–131.
- Signore, P. E. and Jones, D. R. (1996). Autonomic nervous control of heart rate in muskrats during exercise in air and under water. *J. Exp. Biol.* **199**, 1563–1568.
- Southwood, A. L., Andrews, R. D., Lutcavage, M. E., Paladino, F. V., West, N. H., George, R. H. and Jones, D. R. (1999). Heart rates and diving behavior of leatherback sea turtles in the eastern Pacific Ocean. *J. Exp. Biol.* **202**, 1115–1125.
- Southwood, A. L., Darveau, C. A. and Jones, D. R. (2003). Metabolic and cardiovascular adjustments of juvenile green turtles to seasonal changes in temperature and photoperiod. *J. Exp. Biol.* **206**, 4521–4531. doi:10.1242/jeb.00689
- Southwood, A. L., Andrews, R. D., Paladino, F. V. and Jones, D. R. (2005). Effects of diving and swimming behavior on body temperatures of Pacific leatherback turtles in tropical seas. *Physiol. Biochem. Zool.* **78**, 285–297. doi:10.1086/427048
- Southwood, A. L., Reina, R. D., Jones, V. S., Speakman, J. R. and Jones, D. R. (2006). Seasonal metabolism of juvenile green turtles (*Chelonia mydas*) at Heron Island, Australia. *Can. J. Zool.* **84**, 125–135. doi:10.1139/z05-185
- Stephenson, R., Butler, P. and Woakes, A. (1986). Diving behaviour and heart rate in tufted ducks (*Aythya fuligula*). *J. Exp. Biol.* **126**, 341–359.

- Thompson, D. and Fedak, M. A.** (1993). Cardiac responses of grey seals during diving at sea. *J. Exp. Biol.* **174**, 139-164.
- West, N. H., Butler, P. J. and Bevan, R. M.** (1992). Pulmonary blood flow at rest and during swimming in the green turtle, *Chelonia mydas*. *Physiol. Zool.* **65**, 287-310. doi:10.1086/physzool.65.2.30158254
- Williams, C. L. and Hicks, J. W.** (2016). Continuous arterial P_{O_2} profiles in unrestrained, undisturbed aquatic turtles during routine behaviors. *J. Exp. Biol.* **219**, 3616-3625. doi:10.1242/jeb.141010
- Williams, C. L., Meir, J. U. and Ponganis, P. J.** (2011). What triggers the aerobic dive limit? Patterns of muscle oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **214**, 1802-1812. doi:10.1242/jeb.052233
- Williams, C. L., Sato, K., Shiomi, K. and Ponganis, P. J.** (2012). Muscle energy stores and stroke rates of emperor penguins: implications for muscle metabolism and dive performance. *Physiol. Biochem. Zool.* **85**, 120-133. doi:10.1086/664698
- 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. doi:10.1038/ncomms7055
- Williams, T. M., Blackwell, S. B., Richter, B., Sinding, M.-H. S. Heide-Jørgensen, M. P.** (2017). Paradoxical escape responses by narwhals (*Monodon monoceros*). *Science* **358**, 1328-1331. doi:10.1126/science.aao2740
- 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. doi:10.3354/meps10592

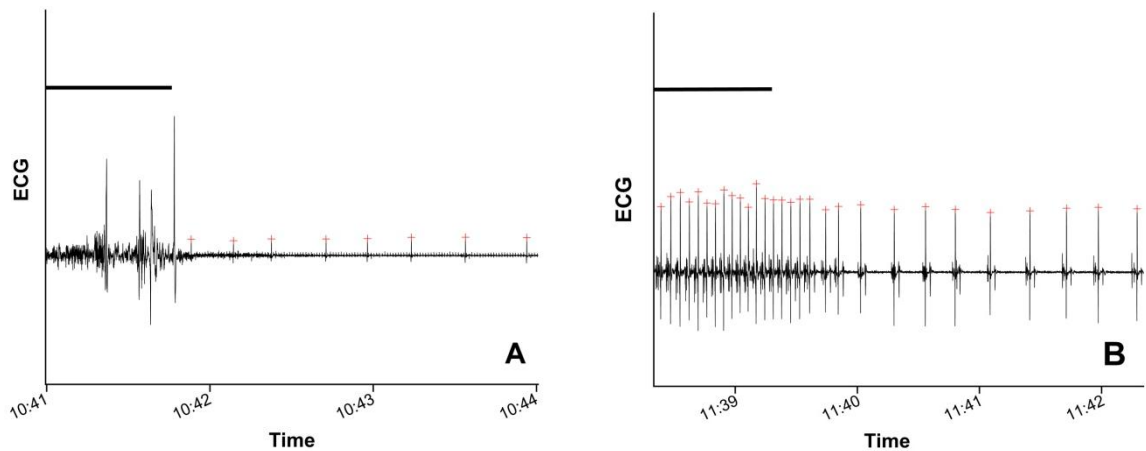


Figure S1. Example of ECG signals from A) Turtle 2 on Sept. 18, 2013 and B) Turtle 6 on Sept. 12, 2015. R waves are denoted by a red plus sign above the wave and the black bars above the ECG signals indicate when the turtle was at the surface. ECG signals were very difficult to read in 2013 (A) during surface periods, but legible during dives (~10:42 – 10:44). However, R waves were quite legible in 2015 (B), even during surface periods.

Table S1. Oxygen (O₂) stores, \dot{V}_{O_2} and calculated aerobic dive limits (cADLs) for eight turtles using different equations.

Turtle	Mass	O ₂ Stores	Water Temp.	\dot{V}_{O_2} 100% Active Kinoshita	cADL 100% Active Kinoshita	\dot{V}_{O_2} 0% Active Kinoshita	cADL 0% Active Kinoshita	\dot{V}_{O_2} Hochscheid	cADL Hochscheid
	Kg	mL O ₂	°C	mL O ₂ min ⁻¹	Mins	mL O ₂ min ⁻¹	Mins	mL O ₂ min ⁻¹	Mins
T1	88	1953.6	22.8	35.94	54.4	16.98	115.1	12.691	153.9
T2	61	1354.2	22.6	26.21	51.7	12.38	109.4	10.783	125.6
T3	79	1742.7	23.4	34.02	51.5	16.07	109.1	13.482	129.3
T4	66	1454.1	22.5	27.81	52.7	13.14	111.5	10.873	133.7
T5	41	910.2	19.9	16.11	56.5	7.61	119.6	5.954	152.9
T6	57	1265.4	20.5	21.93	57.7	10.36	122.2	7.398	171.0
T7	38	843.6	19.9	15.12	55.8	7.14	118.1	5.797	145.5
T8	38	843.6	19.8	15.04	56.1	7.10	118.8	5.700	148.0

Two calculations are based on the \dot{V}_{O_2} equation from Kinoshita et al.: $\dot{V}_{O_2} = 0.1098 \exp(0.0581T_w + 0.0075A)$, where T_w is the water temperature in Celsius, A is the percentage of time turtle was active and units are expressed as ml O₂ min⁻¹ kg^{-0.83}. The first \dot{V}_{O_2} and cADL are assuming the turtle are active 100% of the time and the second set assumes the turtles are active 0% of the time (Kinoshita et al., 2018). The second equation is from Hochscheid et al.: $\ln \dot{V}_{O_2} = -2.87 + 0.168 T_w + 0.353 \ln M_b$, where M_b is turtle mass (Hochscheid et al., 2004). The Kinoshita equation is based on data collected from North Pacific loggerhead turtles and the Hochscheid equation is based on data collected from Mediterranean loggerhead turtles (Hochscheid et al., 2004; Kinoshita et al., 2018).