

A novel technique for measuring heart rate in a free swimming marine vertebrate

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Abstract

A mouth opening sensor incorporating a magnet and Hall sensor attached to a data logging unit was used to monitor the breathing and foraging behavior of a free-swimming leatherback sea turtle (*Dermochelys coriacea*). Analysis of these data revealed a rhythmic low amplitude oscillation. Further investigation of the frequency of this signal lead us to believe that the movements (<0.1 mm) are caused by the movement of blood through the nearby blood vessels. Putative heart rate decreased during dive descent and increased considerably during dive ascent reflecting the bradycardia and anticipatory tachycardia recorded by other means in other air-breathing divers. Oscillation frequencies were also comparable to the heart rate recorded in leatherbacks by means of implanted electrodes. We therefore propose that this device which was already known to reliably record behaviour such as breathing, feeding and buccal oscillations in sea turtles also has potential for recording other signals which cause movement on the external surface of an animal.

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1. Introduction

Since the mid-1960s (Kooyman, 1965), sensors connected to data logging devices have routinely been used to record various aspects of the behaviour and environment of free-swimming animals. Throughout the evolution of data-logging technology the development of new sensors and smaller devices with increased memory has allowed for an ever increasing range of physical and biological parameters to be measured. For example,

devices are now routinely used to monitor depth, temperature, salinity, heart rate, and acceleration (e.g. Croxall et al., 1985; Charrassin et al., 2002; Hooker and Boyd, 2003; Greaves et al., 2005; Wilson et al., 2006). However, some measurements continue to require surgical implantation of equipment which reduces their utility. For example heart rate is conventionally recorded using subcutaneously implanted electrodes attached to a data logger (e.g. Southwood et al., 2003; Zimmer and Milsom, 2001; Andrews et al., 2000). Here we present a new technique for measuring the heart rate of free-swimming animals through movements of the skin, an approach that requires no surgery on the study animal.

There is now a long tradition of using the external motion of an animals' body to record detailed aspects of

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Fig. 1. *Dermochelys coriacea*. Photograph of a leatherback turtle head showing the positioning of the magnet (lower mandible) and hall sensor (upper mandible). The cable trailing from the sensor attaches to a data logging unit secured in the shoulder strap of a harness (photo: A Myers).

their behaviour, physiology and ecology. For example, over 15 years ago we developed low powered radar to non-invasively measure the tiny movements of the skin associated with breathing in bats (Hays et al., 1990). More recently such measurements have been combined with miniature data-loggers allowing measurements to be made on free-living animals for extended periods. Essentially, any behaviour or physiological component reflected in an external movement, no matter how small, can be measured and recorded. For example, the IMASEN (Immediate Mandibular Angle SENSOR) consists of a Hall Sensor that measures the proximity of a magnet positioned nearby. IMASENs were initially developed to monitor the

mouth opening behaviour of Adelie (*Pygoscelis adelia*) and gentoo (*P. papua*) penguins (Wilson et al., 2002). Since this time, the technology behind the motion sensor has been used to examine flipper beat frequency in seals, penguins and turtles (Wilson and Liebsch, 2003; Hays et al., 2004), defecation rates in penguins (Wilson et al., 2004) and the behaviour of bivalves (Wilson et al., 2005). We recently used this device to record the mouth opening behaviour of leatherback turtles during the breeding season, measuring breathing at the surface and sub-surface buccal oscillations (gular pumping) on dive descents, probably associated with attempts to use gustatory cues to locate prey (Myers and Hays, 2006). Serendipitously whilst examining these data we uncovered another regular, low amplitude signal. Here we detail how embedded within the mouth opening data may be the heart rate of the diving animal. Potentially this study opens up the way for several parameters to be measured by the same sensor on a single deployment: breathing, prey ingestion, buccal oscillations and heart rate.

2. Method

The mouth sensor consists of a Hall sensor connected to a one channel data-logger (Driesen-Kern, Kiel, Germany, $65 \times 35 \times 15$ mm, 25 g in air) with a resolution of 16 bits and an 8 Mb memory. The potential difference (Hall voltage) recorded by the data logger is dependent on the proximity of a neodymium boron magnet ($10 \times 10 \times 3$ mm) to the Hall sensor. For the purpose of this study the data logger was programmed to record the Hall voltage at a rate of 10 Hz which would permit continuous recording for

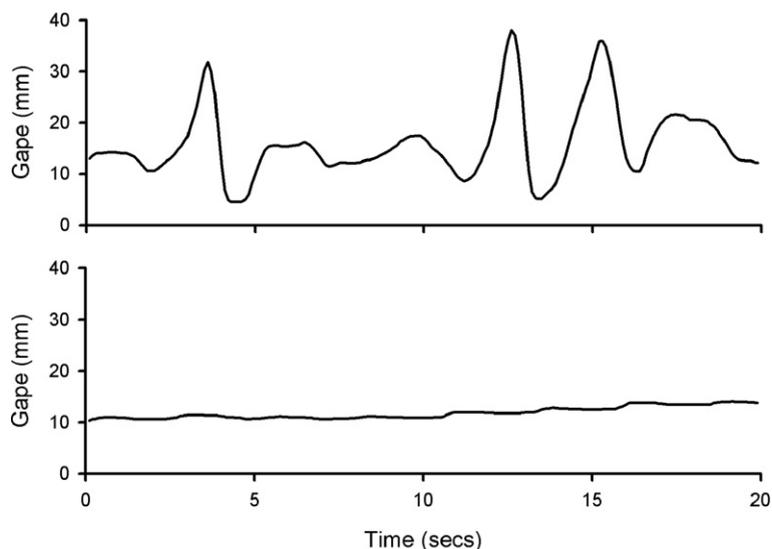


Fig. 2. Comparison of the magnitude of mouth opening for buccal oscillations (top) and the lower amplitude oscillation purported to be heart rate (bottom).

4.6 days. Both the magnet and the Hall sensor were embedded in a small amount of two-part epoxy to facilitate attachment ($15 \times 15 \times 8$ mm). The magnet and Hall sensor were attached to the lower and upper mandible, respectively, with cyanoacrylate glue (Fig. 1). Hall Sensor readings were converted to gape magnitude using the technique described in Myers and Hays (2006).

The data logger was housed inside the shoulder strap of a specially designed harness. The harness (described in Myers and Hays (2006)) incorporates a padded aluminium base plate to which a time-depth recorder (Star Oddi, Reykjavik) was attached with stainless steel wire and cable ties. The time-depth recorders (TDRs) were programmed to record temperature and depth at a sampling interval of 10 s, permitting continuous recording for ~ 2.5 days.

The two devices were recovered during a subsequent nesting event 10 days after the device was attached. No abnormal behaviour was observed during the attachment process or during the recovery of the devices, and nesting continued as normal for the duration of the nesting season. Although the long term effects of using a harness as a

means of attaching devices remains untested, the short term effects of such an attachment technique is likely to have nothing more than a negligible impact on the long term survival of the animal. This research was conducted on Levera Beach, Grenada (12°N 62°W) with permission from the Grenadian Ministry of Agriculture, Forestry, Land and Fisheries in conjunction with a local NGO (Ocean Spirits).

Data were manually coded to facilitate the isolation of individual dives. Sections longer than 10 s were extracted for further analysis if the gape at the sensor between any two points did not exceed 0.5 mm. As the signal could be detected even if the mouth was slightly open, the rate of change in gape was used to standardize the oscillation rate.

3. Results

We identified rhythmic oscillations of much smaller amplitude than the buccal oscillations we have described previously (Myers and Hays, 2006). The relative magnitude of these two signals is shown in Fig. 2. We termed the

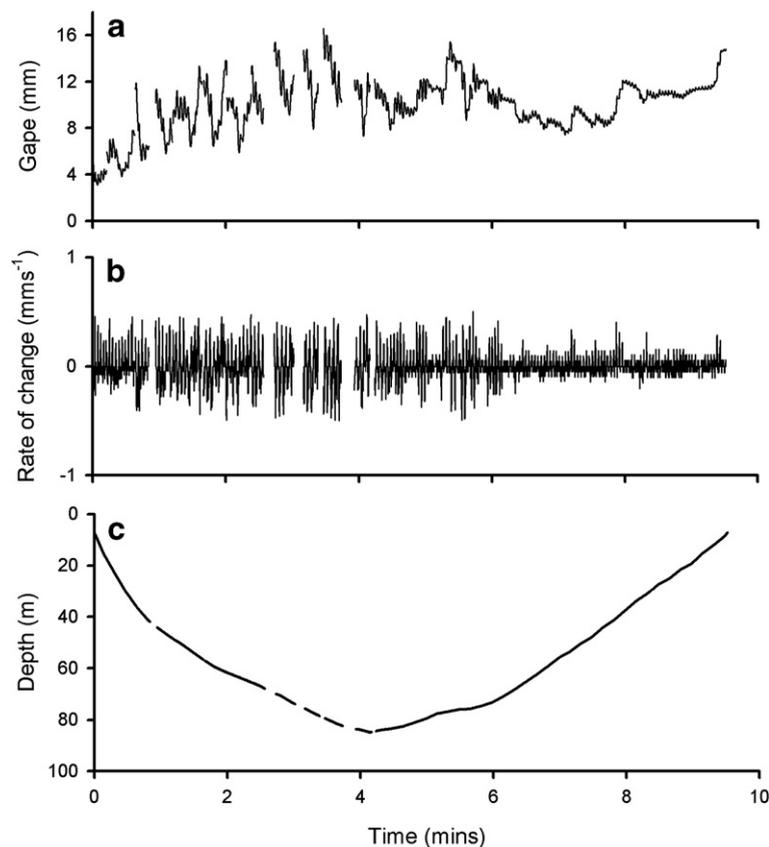


Fig. 3. Low amplitude oscillations were recorded by an IMASEN logging at 10 Hz (a). Transforming the data into a rate of change (mm s^{-1}) allows for individual oscillations to be easily identified (b). Gaps in the data represent periods when other mouth opening activity interrupted the record. This generally occurred during the dive descent (c).

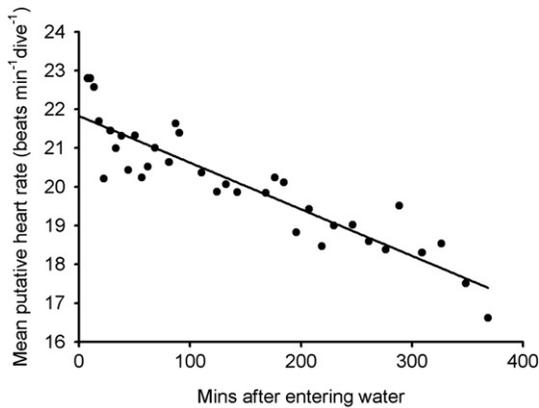


Fig. 4. The rate of oscillation decreased significantly ($r^2=0.83$, $p<0.0001$) with duration through the record with the relationship $y=21.826-0.012x$. Each point represents dives where a period of oscillations >10 s was detected during the descent, bottom phase and ascent. Mean dive putative heart rate was calculated by weighting the oscillation rate for the proportionate duration of each phase.

low amplitude signals the putative heart rate, for reasons outlined below.

A total of 299 sections of putative heart rate longer than 10 s were recorded during the six hour period that the mouth sensor functioned (e.g. Fig. 3). The total duration of these sections was approximately 190 min, with mean (\pm sd) duration of 38.4 ± 43 s.

The putative heart rate decreased significantly throughout the 6-h record (Fig. 4, $r^2=0.83$, $p<0.0001$) with the declining trend occurring during the descent (from the surface to 80% of the maximum depth), bottom (the deepest 20%) and ascent (from 80% of the maximum depth to the surface) phases of the dive. The mean (\pm sd) putative heart rates for the three phases were 20.2 ± 3 , 19.2 ± 2 and 21.8 ± 1 beats min^{-1} for the descent, bottom and ascent phase respectively. This difference between the putative heart rates during the three phases may not be completely representative of the oscillation rate since, due to the occurrence of other mouth opening activity, uninterrupted traces tended to be shorter during the descent (16.9 ± 7 s) and bottom (33.9 ± 32 s) phases than during the ascent (77.2 ± 61 s).

Although the majority of the detected sections of putative heart rate were interrupted by other mouth opening activities, one dive gave an almost uninterrupted trace for the entire dive (87% of the total duration, Fig. 5). By breaking down the longer traces in this record to 26 traces of approximate equal length (20 ± 5 s) a pattern can be observed regarding a change in oscillation rate as the dive proceeds. During the descent, the first rate (at 13 m depth) was 19.7 beats min^{-1} which decreased to a minimum of 13.6 beats min^{-1} at 72 m. Between this depth and the maximum depth of 84.9 m the rate of oscillation increased to 16.6 beats min^{-1} , during the ascent the rate

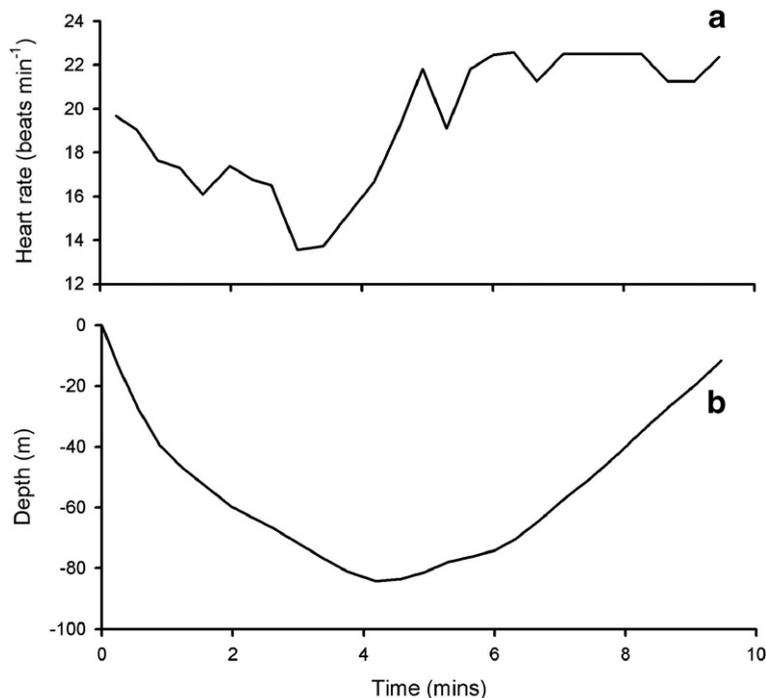


Fig. 5. Relationship between dive duration and heart rate (a) and depth (b) during a 580 s dive to 85 m.

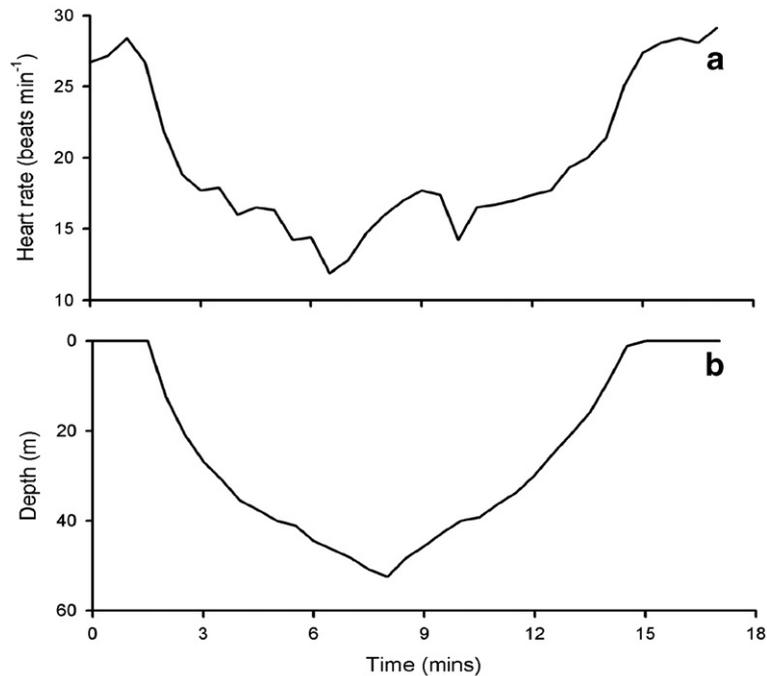


Fig. 6. Heart rate (a) during a dive to >50 m (b) digitized from Fig. 4 in Southwood et al. (1999).

increases rapidly to a rate of $21.8 \text{ beats min}^{-1}$ at 81.8 m, a rate which changes very little during the remainder of the ascent ($21.8 \pm 1 \text{ beats min}^{-1}$).

4. Discussion

It may be argued that the low amplitude movements detected in this study are attributable to influences unrelated to cardiac pumping. By drawing inferences from the literature we support the suggestion that this signal reflects the heart rate and is not caused by buccal oscillations or flipper beats.

During a comparative laboratory study Hochscheid et al. (2005) used IMASENS to record the mouth opening behaviour of loggerhead turtles (*Caretta caretta*) and validated individual movements by recording the behaviour of the animals with a video camera. Three types of behaviour were recorded during this study. Foraging, breathing and movements associated with buccal oscillations. These latter movements increased from a mean (\pm sd) of $0.52 \pm 0.15 \text{ mm}$ to $0.82 \pm 0.25 \text{ mm}$ during feeding episodes, although the frequency remained unchanged (24.3 to 34.6 min^{-1}). The oscillations were seen to coincide with buccal oscillations and stopped when the turtle ingested food, breathed or settled at the bottom of the tank to rest. In contrast the frequencies of the 299 records of oscillations recorded in this study fell in the range 10.9 to 29.46 min^{-1} . Of these 299 records 81

occurred during the descent, 138 during the bottom phase, 68 during the ascent and only 13 periods at the surface (these occurring during the initial period moving away from the beach when the turtle was likely to have been traveling at, or just under, the surface). All 13 of the surface periods produced the most rapid rate of oscillations ranging from 26.1 to $29.5 \text{ beats min}^{-1}$. It is unlikely that buccal oscillations (likely to be a mechanism with which to locate prey) would be important to turtles at this time, as any effort to detect food immediately on leaving the beach would be unlikely to be successful. Discounting these periods of oscillation leaves a range of 10.9 to $25.2 \text{ beats min}^{-1}$.

The amplitude of the oscillations recorded in this study (mean 0.14 mm) are also very different to the buccal oscillations recorded by Hochscheid et al. (2005) (0.52 mm). As part of a related study (Myers and Hays, 2006), higher amplitude oscillations were detected during dive descent. We suggested in this paper that these mouth opening events were related to the detection of gustatory cues (i.e. buccal oscillations). Fig. 2 shows that these oscillations had considerably greater amplitude than those put forward in this study. As buccal oscillations recorded by Hochscheid et al. (2005) and Myers and Hays (2006) were of much greater amplitude than those studied herein, the theory that these oscillations represent olfactory sensing can be rejected.

Using a device identical to the device used in this study Hays et al. (2004) recorded the flipper beat

frequencies of a free swimming green turtle. Intuitively, the flipper beat frequency was typically high during the initial phase of descent whereby the animal must overcome positive buoyancy. During this phase flipper beat frequencies were typically 30–40 beats min^{-1} although it is not specified at what depth this frequency drops as the animal achieves neutral buoyancy, it is noted that at the end of the descent the flipper beat frequency was around 12 beats min^{-1} . If the oscillations seen in this record occurred as a result of flipper beating a similar pattern would be expected for the leatherback turtle. Although the oscillations in this study do decrease as the turtle descends, the difference between the oscillation frequency in waters shallower than 10 m (20.64 min^{-1}) when the turtle is likely to be positively buoyant is not considerably different to the frequency between 20 and 30 m (19.2 min^{-1}) when it is likely that the animal would no longer be positively buoyant.

To our knowledge, there has only been one study examining the heart rate of leatherback turtles at sea. Southwood et al. (1999) monitored the heart rates of six interesting turtles at Playa Grande, Costa Rica. This was achieved by inserting electrodes into muscle at the front and rear of the turtle and recording the electrical signal emitted by the pulsing heart. The mean heart rate recorded during dives by this method was 17.4 ± 0.9 beats min^{-1} whereas the mean rate of oscillations recorded in this study was 19.53 beats min^{-1} . If, however, we consider that the recording period in the study by Southwood et al. (1999) was up to 3.5 days and that the heart rate is accelerated during activity on the nesting beach (43–48 beats min^{-1} when restrained (Lutcavage et al., 1992)) it is understandable that, if heart rate were being recorded in this study, that the rates would be higher than for a longer term study due to the increased stress and work rate involved in nesting. Towards the end of the period recorded in this study the mean rate of oscillation was approaching the leatherback heart rate previously recorded by Southwood et al. (1999). Indeed if all dives with the exception of the last five are excluded the mean rate of oscillation is 17.5 beats min^{-1} , considerably closer to the mean rate recorded by Southwood et al. (1999). This lends weight to the conclusion that the pulse being detected via the mouth sensor is that of heart rate.

The shift in heart rate during dives, as recorded by Southwood et al. (1999) saw heart rate begin to decrease upon submersion, and continue to fall throughout the descent, stabilize during mid-dive around the mean dive heart rate and then increase as the turtle began its ascent. Although the signal detected by the mouth sensor is interrupted by mouth opening events leading to a paucity of

data during dive descents, one exception to this is a dive which showed an oscillating record for 503.8 s (87% of total duration). This trace follows a pattern very similar to that described by Southwood et al. (1999), with oscillation frequency decreasing during dive descent and then increasing to a maximum (indicating anticipatory tachycardia) as the turtle ascends to the surface. The trace from this dive differs from the dive displayed in some detail by Southwood et al. (1999) (and reproduced here by digitizing the raw trace every 30 s, Fig. 6). There are similarities in that the lowest heart rate occurs just prior to maximum depth being reached in both cases, however, in our study the oscillation rate increases sharply at the start of the ascent whereas during the dive digitized from Southwood et al. (1999) the heart rate initially begins to increase rapidly and then drops slightly before continuing to increase at a slower rate, only reaching a stable rate when the turtle reaches the surface. This however, does not discount heart rate for being the reason for the oscillation rates noted above. The function of the two dives is likely to have been different and therefore elicited different responses regarding the heart rate. The dive represented below was over ten minutes and reached a maximum depth of just over 50 m whereas the dive in this study lasted for less than ten minutes and reached a maximum depth of over 80 m. If, for example the dive represented below was an exploratory or potentially foraging dive then anticipatory tachycardia at the start of the ascent then the turtle may not have been able to prolong the dive sufficiently to exploit the prey. We believe that all the evidence presented leads to the conclusion that the oscillations detected during the periods of apparent inactivity of the mouth are indicative of heart rate. Where the heart rate is actually detected is unknown, although based on proximity it is most likely to be due to blood moving through the common carotid or oesophageal arteries.

Reptiles generally have a less pronounced bradycardiac response than birds and mammals with, for example, freshwater crocodiles (*Crocodylus johnstoni*) showing a decrease in heart rate of 12% when submerged (Seebacher et al., 2005). Southwood et al. (1999) recorded a mean decrease in leatherback heart rate on submergence of 30% from 24.9 to 17.4 beats min^{-1} . Unfortunately the fact that other mouth opening activities interrupted the signal recorded in this study, means that very few surface heart rates were recorded, but the reduction in the putative heart rate during dive descents and subsequent increase during the ascent does support the work conducted by Southwood et al. (1999) and confirms that the low amplitude oscillations recorded in this study are related to the heart rate. The few records of non-diving heart rate occurred immediately after the turtle left the beach where, during

subsurface swimming, the heart rate was 27.3 beats min^{-1} , 29% greater than the overall mean diving rate of 19.5 beats min^{-1} .

In conclusion, our preliminary results suggest that heart rate can be reliably monitored in the leatherback turtle by means of a mouth sensor although follow up studies are clearly needed to test the ease with which these measurements can be made in other individuals. Importantly, a single IMASEN attached to the mouth can potentially record several useful parameters: breathing, prey ingestion, buccal oscillations (prey sensing) and heart rate. Multi-channel IMASENs now also record depth and water temperature so that a single device can be used to address a broad range of questions surrounding the physiological ecology of diving.

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