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RESEARCH ARTICLE

Energy expenditure of freely swimming adult green turtles (*Chelonia mydas*) and its link with body acceleration

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SUMMARY

Marine turtles are globally threatened. Crucial for the conservation of these large ectotherms is a detailed knowledge of their energy relationships, especially their at-sea metabolic rates, which will ultimately define population structure and size. Measuring metabolic rates in free-ranging aquatic animals, however, remains a challenge. Hence, it is not surprising that for most marine turtle species we know little about the energetic requirements of adults at sea. Recently, accelerometry has emerged as a promising tool for estimating activity-specific metabolic rates of animals in the field. Accelerometry allows quantification of the movement of animals (ODBA/PDBA, overall/partial dynamic body acceleration), which, after calibration, might serve as a proxy for metabolic rate. We measured oxygen consumption rates ($\dot{V}_{O_2}$) of adult green turtles (*Chelonia mydas*; 142.1±26.9 kg) at rest and when swimming within a 13 m-long swim channel, using flow-through respirometry. We investigated the effect of water temperature (T_w) on turtle $\dot{V}_{O_2}$ and tested the hypothesis that turtle body acceleration can be used as a proxy for $\dot{V}_{O_2}$. Mean mass-specific $\dot{V}_{O_2}$ ($s\dot{V}_{O_2}$) of six turtles when resting at a T_w of 25.8±1.0°C was 0.50±0.09 ml $min^{-1} kg^{-0.83}$. $s\dot{V}_{O_2}$ increased significantly with T_w and activity level. Changes in $s\dot{V}_{O_2}$ were paralleled by changes in respiratory frequency (f_R). Deploying bi-axial accelerometers in conjunction with respirometry, we found a significant positive relationship between $s\dot{V}_{O_2}$ and PDBA that was modified by T_w. The resulting predictive equation was highly significant (r^2=0.83, P<0.0001) and associated error estimates were small (mean algebraic error 3.3%), indicating that body acceleration is a good predictor of $\dot{V}_{O_2}$ in green turtles. Our results suggest that accelerometry is a suitable method to investigate marine turtle energetics at sea.

Key words: sea turtle energetics, accelerometry, diving, oxygen consumption rate, exercise, PDBA, respiratory frequency, body acceleration, resting metabolic rate.

INTRODUCTION

Marine turtles are large ectotherms with a general tropical to sub-tropical distribution. Six out of seven marine turtle species are currently listed in the International Union for Conservation of Nature (IUCN) red list of threatened species, with a status that ranges from 'vulnerable' to 'critically endangered'. Fundamental for conservation efforts is a clear understanding of marine turtle physiology and ecology. Especially important are the energy relationships of marine turtles; how they acquire and allocate energy. The rate at which organisms take up energy and materials from the environment, transform them, and allocate them to survival, growth and reproduction – in other words, their metabolic rate – controls ecological processes at all levels of organisation: from the fitness of the individual, to population dynamics and ecosystem processes (Brown et al., 2004). Most important for our understanding of the overall energy requirements of marine turtle populations, which will ultimately define population structure and size, is the knowledge of the at-sea metabolic rates of marine turtles (Jones et al., 2004). Measuring the metabolic rates of turtles at sea, however, is not an easy task, given the logistic and methodological constraints. To date, there are only three studies that have investigated the field metabolic rates of marine turtles (Wallace et al., 2005; Clusella Trullas et al., 2006; Southwood et al., 2006) using the doubly labelled water method (DLW) (Lifson et al., 1955; Speakman, 1997). These studies concerned hatchling olive ridley turtles (*Lepidochelys olivacea*) (Clusella Trullas et al., 2006), juvenile green turtles (*Chelonia mydas*) (Southwood et al., 2006) and adult leatherback turtles (*Dermochelys coriacea*) (Wallace et al., 2005). However, there has been some uncertainty as to whether the DLW method is appropriate to use with marine turtles. The combination of high water turnover rates and low metabolic rates, typically found in marine turtles, might render this method unreliable. A recent validation study with juvenile green turtles that used DLW in combination with respirometry found that the DLW method produced valid results in certain circumstances (fed animals), while it did not in others (fasted animals), and therefore advised a cautionary use with marine reptiles (Jones et al., 2009). Furthermore, the DLW method gives an estimate for the overall energetic costs of an animal during the measurement period, but it does not provide more detailed information (i.e. activity-specific metabolic costs).

Apart from field studies, there have been a considerable number of investigations into the energetics of marine turtles in captive settings during the last decades using respirometry. While these studies have increased our understanding of marine turtle energetics tremendously (Wallace and Jones, 2008), there has been a strong bias towards hatchlings and immature animals. For most species,

we know little about the energy requirements of adult turtles during the different phases of their oceanic life. Furthermore, in respirometry studies, marine turtles have typically been confined to small tanks with little opportunity for swimming, except hatchlings and small juveniles (Wyneken, 1997; Butler et al., 1984). Hence, most of these measurements reflect conditions either during rest (i.e. motionless) or during routine activity (e.g. turtles slowly moving along the tank bottom), while respirometry measurements of actively swimming turtles, apart from hatchlings and small juveniles, are missing.

Recently, a promising new method for estimating activity-specific metabolic rates of animals in the field has been developed. It has become known as 'accelerometry', as it involves the recording of body acceleration of animals (Wilson et al., 2006). Accelerometry is based on the principle that animal movement, realised through muscular contraction, requires energy, so a relationship between movement patterns and energy expenditure can be expected. By attaching accelerometers to animals, we can accurately quantify their overall movement through space over time (referred to as 'ODBA', overall dynamic body acceleration) (*sensu* Wilson et al., 2006) and this might serve as a proxy for energy expenditure. Accelerometry requires calibration with captive animals under controlled conditions, to relate the proxy variable to metabolic rate, which is typically measured as oxygen consumption rate using respirometry. Such calibration studies have been conducted over a range of species, typically involving treadmill exercise and, hence, terrestrial locomotion (Halsey et al., 2009). Interestingly, a significant relationship between body acceleration and metabolic rate was also found during diving in a mammalian diver, the Steller sea lion (*Eumetopias jubatus*) (Fahlman et al., 2008a). Most recently, accelerometry was successfully used with another ectotherm species, the cane toad (*Bufo marinus*) (Halsey and White, 2010). These calibration studies provide predictive equations, allowing estimation of metabolic rate from the recording of body acceleration. The error estimates associated with these predictive equations are generally low, with mean algebraic errors typically ~±5% and mean absolute errors ~10% (Halsey et al., 2009). They are therefore similar to the error estimates associated with a more established method that records the heart rate of animals as a proxy of metabolic rate (Butler et al., 2004). However, unlike accelerometry, the heart rate method requires surgical implantation, and while it has been used successfully to investigate the energetics of avian divers over the annual cycle (Green et al., 2009a; White et al., 2011), it has not yet been used to estimate marine turtle energy expenditure. Such investigation might be complicated by the fact that turtles posses a functionally undivided ventricle, so that shunting of blood between the systemic and pulmonary circulation might occur, especially during diving. When diving to depth, the increase in pressure will compress the lungs, so that the functional gas exchange might be altered, further complicating the relationship between heart rate and oxygen consumption. Accelerometry might therefore be the method of choice to investigate marine turtle energetics.

The south-western Indian Ocean hosts important nesting and feeding areas for marine turtles, especially green turtles (e.g. Comoros Islands, Mayotte Island, Scattered Islands) (Bourjea et al., 2007; Lauret-Stepler et al., 2007). For example, at Mayotte Island juvenile and adult green turtles exploit seagrass meadows on a year-round basis (Taquet et al., 2006; Ballorain et al., 2010). Similarly, at Reunion Island foraging juvenile and adult green turtles are present in coral reef zones throughout the year (Jean et al., 2010). Given their year-round presence, turtles are exposed to the seasonal changes in environmental conditions (e.g. water temperature, light conditions, food availability), which might have profound effects on behavioural patterns and energetics (Southwood et al., 2003; Hochscheid et al., 2004; Ballorain, 2010). Hence, this setting provides a great opportunity to study energy acquisition and allocation of these animals during the different phases of their life cycle in great detail and under natural conditions. A prerequisite for such investigation, however, is to establish a reliable method that can be used to measure the energetic state of turtles in the field.

The aim of our study was (1) to investigate the energy expenditure of adult green turtles during rest and when swimming in a 13 m-long channel; (2) to investigate the effect of seasonal changes in water temperature on turtle energy expenditure; and (3) to test the hypothesis that turtle body acceleration can be used as a proxy for energy expenditure. If verified, the ultimate goal of our study was to establish a predictive equation that could be used to estimate energy expenditure of turtles in the wild from the recording of acceleration.

MATERIALS AND METHODS

Turtles

Six adult female green turtles (*Chelonia mydas* L. 1758) with a body mass (M_b) range of 98.4–170.2 kg and a curved carapace length (CCL) of 103.7±6.4 cm were used in this study (Table 1). All turtles were captured as hatchlings on Tromelin Island in the Western Indian Ocean (15°53′S, 54°31′E) during the late 1980s. They were raised and maintained at the former Ferme Corail, which is now a public aquarium (Kélonia), Reunion Island (21°06′S, 55°36′E). Turtles were kept communally inside an uncovered concrete pool (13×15 m, 1–2.2 m deep, 300 m^3 volume), adjacent to a shallow lagoon, which continuously supplied sea water to the pool at a rate of 150 $m^3 h^{-1}$. Water temperature (T_w) in the turtle pool matched the temperature of the adjacent lagoon and underwent the same seasonal changes (Fig. 1). Given the shallow depth of the adjacent lagoon and the typically strong solar radiation during the day, T_w also underwent considerable daily oscillations, with differences often of ~2–3°C between day and night. Turtles were exposed to the natural light cycle, with ~13.5 h light (10.5 h dark) during the austral summer and ~11 h light (13 h dark) during the austral winter. Turtles were fed 5 times a day with a diet consisting of vitamin-supplemented fish pellets (Ecolife 16F N9, BioMar SAS, Nersac, France; ~70 g daily) and plant leaves (tree heliotrope, *Heliotropium foertherianum*; ~300 g daily). All experimental procedures were approved by the Comité National pour la Protection de la Nature of the French Ministry in charge of Environmental Affairs and adhered to all institutional guidelines and the legal requirements of the country in which the work was carried out.

Experimental set-up

We constructed a swim channel inside the concrete pool where turtles were housed, by subdividing an area (13 m long, 2 m wide) using PVC tubing and plastic fencing (Fig. 2). The depth of the channel was ~1 m at one end, from which it sloped gradually to ~2.2 m at the opposite end. At the shallow end of the channel a gate was left open, so that turtles could enter and leave the channel of their own volition. Over the course of 3 months the surface of the channel was gradually covered with elements (2×2 m) constructed with PVC tubing and plastic-coated metal fence, securely fastened at a water depth of ~10 cm, until only an area of ~2×2 m at the shallow end remained open. In parallel with channel construction, we installed an underwater video array that allowed continuous observation of turtles inside the swim channel (Fig. 2). Four black and white video cameras (Model CVC6990, Lorex, MBrands,

Table 1. Oxygen consumption rates and respiratory frequency of adult green turtles when resting and swimming at different water temperatures

				Resting			Swimming		
Turtle	M_b (kg)	CCL (cm)	T_w (°C)	$\dot{V}_{O_2}$ (ml min^{-1})	s$\dot{V}_{O_2}$ (ml min^{-1} kg$^{-0.83}$)	f_R (breaths h^{-1})	$\dot{V}_{O_2}$ (ml min^{-1})	s$\dot{V}_{O_2}$ (ml min^{-1} kg$^{-0.83}$)	f_R (breaths h^{-1})
Austral winter									
Elisabeth	120.9±0.3	96	25.8±0.6	21.55±1.33	0.40±0.02	3.7±0.5	43.96±5.52	0.82±0.10	7.8±2.1
Monique	170.2±0.5	108	25.0±0.3	36.17±4.52	0.51±0.06	5.6±0.7	59.80±4.10	0.84±0.06	8.3±1.5
Delphine	156.1±0.4	109	24.5±0.4	32.49±4.63	0.49±0.07	3.7±0.8	50.13±6.63	0.76±0.10	5.2±0.8
Sandy	156.1±1.2	107	25.7±0.9	36.82±7.27	0.56±0.11	6.1±1.9	102.82±13.75	1.55±0.20	18.1±4.4
Nadia	151.0±0.0	107	26.6±0.9	24.96±1.62	0.39±0.03	3.2±0.6	44.32±6.34	0.69±0.10	5.7±0.6
Karima	98.4±1.5	95	27.0±1.0	28.61±3.47	0.63±0.08	5.0±1.0	39.50±4.04	0.88±0.09	5.9±1.1
Grand mean	142.1±26.9	103.7±6.4	25.8±1.0	30.10±6.15	0.50±0.09	4.5±1.2	56.76±23.63[b]	0.92±0.32[b]	8.5±4.9[b]
Austral summer									
Elisabeth	114.7±0.3	96	30.2±0.4	42.62±3.12	0.83±0.06	8.6±1.3	60.30±4.33	1.18±0.09	11.6±1.3
Monique	163.6±1.6	108	29.2±0.9	44.41±4.57	0.65±0.07	7.1±1.6	54.13±5.00	0.79±0.07	7.3±0.9
Delphine	160.2±0.0	109	29.9±0.7	46.61±4.92	0.69±0.07	5.3±1.1	60.96±3.32	0.90±0.05	6.1±0.6
Nadia	148.3±0.7	107	29.2±0.5	33.14±4.19	0.52±0.07	5.0±0.8	48.14±4.34	0.76±0.07	8.5±1.5
Grand mean	146.7±22.3	103.7±6.4	29.6±0.5	41.69±5.93[a]	0.67±0.13[a]	6.5±1.7[a]	55.88±6.01[a,b]	0.91±0.19[a,b]	8.4±2.4[a,b]

Data were obtained during the austral winter (Jun.–Nov. 2009) and austral summer (Jan.–Apr. 2010). Values are means ± s.d. Grand means were established from individual turtle means. Values were averaged from 7–11 trials conducted with each turtle during both seasons. Trial duration was between 3.5 and 4 h, with each trial being split into two periods of contrasting activity status (resting/swimming) based on behavioural observation and acceleration recordings. Each of these periods per trial, over which mean values were calculated, lasted on average ~1.5 h and consisted of multiple dive cycles (on average ~8 dive cycles, with a miimum and maximum of 3 and 26 dive cycles, respectively).

M_b, body mass; CCL, curved carapace length; T_w, water temperature; $\dot{V}_{O_2}$, oxygen consumption rate; s$\dot{V}_{O_2}$, mass-specific oxygen consumption rate; f_R, respiratory frequency.

[a]Significant difference from the austral winter; [b]significant difference from resting conditions.

Scarborough, ON, Canada) were positioned alongside the channel and connected to a multiplexer (EverFocus Electronics Corp., Taipei, Taiwan) and video monitor inside a small observation hut. This hut was built adjacent to the shallow end of the channel and also housed all respirometry equipment. During these months all turtles entered and left the swim channel on a daily basis and habituated to surface at the increasingly smaller open end of the channel to breathe. Food pellets were used to stimulate turtles to enter the channel and to attract them to the breathing area.

Training and experimental trials

Turtles were weighed before and after participation in each round of experimental trials. To cover the annual range in T_w experienced by wild turtles in this region of the Indian Ocean, we subjected turtles to two rounds of trials. The first round was conducted with six turtles during the austral winter between June and November 2009 (trial T_w: 25.8±1.0°C). This was followed by trials with four of these six turtles during the austral summer between January and April 2010 (trial T_w: 29.6±0.5°C). Body mass changes during each experimental period were negligible (–0.26±2.18% and 0.08±0.89% during austral winter and summer, respectively) but changes between the seasons were larger (–2.04±3.40%, from winter to summer). The experimental period for a turtle was about 4 weeks per season. It started with a training period of 2 weeks, during which each turtle became accustomed to the exact protocol used during the recording period that followed.

Measuring oxygen consumption rates in diving animals requires them to breathe inside a chamber, from which gas samples can be taken. Each turtle therefore had to firstly ‘learn’ the exact position of this chamber, located at the shallow end of the 13 m-long swim channel (Fig. 2), so that she would consistently find it and lift her head into it for breathing. In the morning of a training/recording day a surface area of 2×2 m, at the shallow end of the channel (where the Plexiglas® chamber would eventually be positioned), was still open. A turtle was enclosed inside the swim channel and closely monitored from within the hut. Once inside the swim channel, a turtle typically swam back and forth, interrupted by periods spent resting at the bottom. Over the course of a few hours, the rest of the surface area was gradually covered and the Plexiglas® chamber set in place. With increasing cover, a turtle typically became less active and settled at the bottom to rest. Turtles showed a good capacity to ‘learn’ the position of the chamber, so that after a few

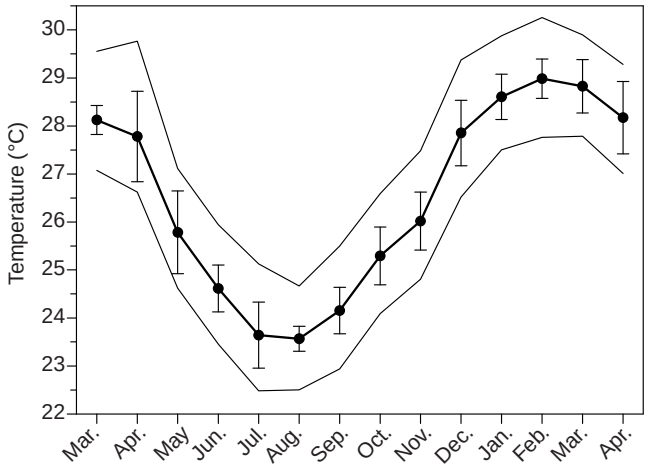

Fig. 1. Seasonal changes in water temperature (T_w, °C) inside the turtle pool between March 2009 and April 2010. The middle line indicates the monthly mean T_w (±s.d.), while the upper and lower lines indicate the mean maximum and minimum temperatures, respectively. Values were calculated from daily means and are based on continuous recordings of a temperature logger.

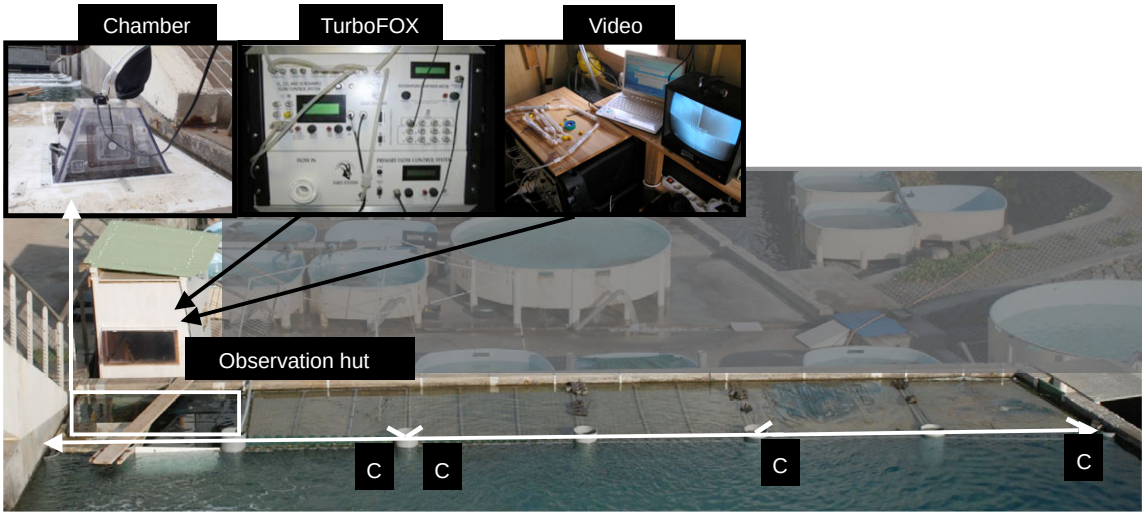

Fig. 2. Experimental set-up, showing the 13 m-long swim channel with a covered surface except in a small area in front of the observation hut (housing respirometry/video equipment and an observer), which was covered with a PVC board during trials, and into which the respirometer chamber was fitted. Positions and orientation of the underwater video cameras (C) are also indicated.

days of training, they all consistently breathed into the chamber without difficulty or apparent excitement. Given the size of our channel, this is remarkable, as the ability of sea turtles to find breathing holes has been questioned for pools of far lesser size (Lutz et al., 1989; Hochscheid et al., 2004).

A recording trial started with a turtle typically settled to rest at the bottom of the channel, just below the chamber. Turtles often rested motionless for long periods (maximum dive duration 44.2 min), only periodically floating upward into the chamber to breathe. Investigating the relationship between oxygen consumption rate ($\dot{V}_{O_2}$) and body acceleration (partial dynamic body acceleration, PDBA) required the recording of sufficiently long periods with different activity levels (i.e. resting/low activity periods and swim periods; see below). Therefore, if turtles did not start to swim spontaneously after resting for 1.5–2 h, a diver entered the pool (outside the channel) and encouraged the turtle to swim by gently tapping her carapace with a stick. Such motivation was sufficient to initiate swimming. During the swim period the diver swam alongside the turtle (but outside the channel), to closely monitor behaviour and encourage maintained swimming activity. After initial motivation turtles swam completely at their own volition, only occasionally requiring further encouragement.

We made every effort to maintain conditions between trials as stable as possible, especially with respect to T_w, which was monitored continuously using a temperature logger (Minilog-T 8K, Vemco Ltd, Halifax, NS, Canada; submerged at a depth of ~1 m; sampling interval 5 min; resolution 0.1°C; accuracy ±0.5%). Given the daily T_w fluctuations, all recordings were conducted during the early afternoon (12–17 h), when T_w conditions were most stable (mean maximum T_w variations during trials 0.5±0.1°C). Recording duration for each trial was between 3.5 and 4 h, split into two periods with contrasting activity levels of similar duration (see below). For each season, 7–11 trials were recorded per turtle. During the experimental period turtles were maintained on their regular feeding regime, so that all turtles participating in trials were in the absorptive state. To ensure a comparable nutritional status for all turtles, food delivery was discontinued 1 h before a trial until its completion.

Respirometry system

Open-circuit respirometry was used to measure $\dot{V}_{O_2}$ of turtles when resting and swimming within the channel. Turtles were trained to breathe within a transparent Plexiglas® chamber in the shape of a truncated pyramid (bottom 38×38 cm, top 19×19 cm, height 24 cm, volume 20 l) with its bottom submerged. Given the forceful exhalation of turtles upon surfacing, their large tidal volume and the relatively small size of our chamber, the chamber was fitted with a latex breathing bag (6.1 l; Deltalyo & Valmy, Roanne, France) to receive overflow expired gas, while a unidirectional breathing valve (Smiths, Rungis, France) was fitted to the inflow opening, ensuring that expired gas would not escape. During exhalation the breathing bag inflated but as soon as exhalation stopped it deflated and its contents were pushed back into the chamber. A small fan inside the chamber provided rapid mixing of expired gas. Air was pulled through the chamber at a rate of 20 l min^{-1} [automatically corrected to standard temperature and pressure (STP), 273 K and 101.3 kPa] and led into the adjacent hut, where it entered the primary flow control unit (FK-100) of a TurboFOX-RM integrated field respirometry system (Sable Systems International, Las Vegas, NV, USA). A subsample (200 ml min^{-1}) was pulled through a humidity meter (RH-300) and scrubbing columns (Drierite, soda lime, Drierite; Xenia, OH, USA) to remove water vapour and CO_2, before entering the fuel cell oxygen analyser of the TurboFOX-RM unit. Oxygen concentration within the chamber, main flow rate through the chamber, humidity of the gas sample and barometric pressure were recorded every second onto a laptop computer using Expedata (Sable Systems). As all measurements were conducted over seawater, which has a great affinity for CO_2, we did not measure the latter but scrubbed it from the subsample using soda lime. All connections between the various components of the respirometry system were made using gas-impermeable Bev-a-Line or Tygon tubing. The O_2 analyser was calibrated before each trial using outside air scrubbed of water vapour and CO_2 (set to 20.95% O_2; the zero point was fixed and not subjected to drift). The humidity meter was calibrated weekly according to the recommendations of the manufacturer. We used dry 99.995% pure N_2 (Air Liquide, Le Port, Reunion) and a water-saturated air sample to calibrate the water vapour pressure reading (kPa). Before a trial the entire system was tested for leaks by infusing pure N_2 gas. The time delay between an exhalation

into the chamber and a change in oxygen concentration being registered by the gas analyser was ~25 s, while it took ~4 min for the O_2 signal to return to baseline after a disturbance (i.e. exhalation).

Body acceleration

In parallel with respirometry, we deployed a bi-axial acceleration data logger (M190L-D2GT; length 53 mm, diameter 15 mm, mass 17 g; 12-bit resolution; recording range ±3 g; Little Leonardo, Tokyo, Japan) to record body acceleration of turtles. The logger was set to record heave and surge at a frequency of 16 Hz. Before deployment, the logger was fitted snugly into a custom-made base (made from rubber and lined with Velcro™). A Velcro patch was glued to the centre of the second vertebral scute of a turtle in anterior–posterior direction, before a series of trials started. This allowed for an easy attachment of the streamlined logger base to a turtle before a trial and for its quick removal afterwards, while the turtle remained inside the water channel. Care was taken to attach the logger in the same position during all trials. After a trial, data from the logger were downloaded onto a PC.

Data analysis

Respirometry data were analysed using Expedata. During analysis, O_2 analyser drift and lag time of the respirometry system were corrected for. Main flow rate was corrected to STP dry (STPD) using equation 8.6 in Lighton (Lighton, 2008):

$$FR_c = FR\,(BP - WVP) / BP\,, \quad (1)$$

where FR_c is the dry-corrected flow rate, FR is the uncorrected flow rate, BP is the barometric pressure (kPa) and WVP is the water vapour pressure (kPa). $\dot{V}_{O_2}$ was calculated using equation 4b in Withers (Withers, 1977), which is nearly independent of the respiratory quotient (RQ):

$$\dot{V}_{O_2} = FR_e\,(F_{I_{O_2}} - F''_{E_{O_2}}) / [1 - F_{I_{O_2}} + RQ\,(F_{I_{O_2}} - F''_{E_{O_2}})]\,, \quad (2)$$

where FR_e is the excurrent flow rate, $F_{I_{O_2}}$ is the fractional concentration of the incurrent O_2, and $F''_{E_{O_2}}$ is the fractional concentration of the excurrent O_2 (scrubbed of water vapour and CO_2). We assumed a RQ of 0.85.

Our analysis of turtle $\dot{V}_{O_2}$ was based on multiple dive cycles and considered entire periods with contrasting activity levels (see below). We chose this approach despite the fact that the dive duration of turtles was typically sufficiently long (i.e. >5 min) and our respirometry system sufficiently fast to allow calculation of $\dot{V}_{O_2}$ on the level of single dive cycles. The rationale behind this was that preliminary analysis of $\dot{V}_{O_2}$ during resting trials returned highly variable values when calculated on the basis of single dive cycles, suggesting that turtles might not have reloaded their oxygen stores to identical levels during each surface interval. Consequently, oxygen uptake (V_{O_2}) after a dive might not necessarily reflect oxygen consumption rate ($\dot{V}_{O_2}$) during that particular dive. During these trials, turtles exhibited a wide range of dive durations but typically only took a single breath between dives. While there was a significant increase in oxygen uptake after a dive as dive duration increased (Fig. 3A), this increase was so small, considering the range of dive durations, that reloading of the oxygen stores to identical levels between dives could not have occurred. Consequently, $\dot{V}_{O_2}$ would be underestimated during long dives and overestimated during short dives. A plot of dive duration *versus* $\dot{V}_{O_2}$ suggested that $\dot{V}_{O_2}$ during short dives (5 min) was 4–5 times higher than during long dives (20 min), while turtles rested motionless at the bottom in both cases (Fig. 3B). By contrast, oxygen uptake after a 20 min dive was only ~40% greater than after a 5 min dive. We are therefore convinced that the large differences in $\dot{V}_{O_2}$ we observed

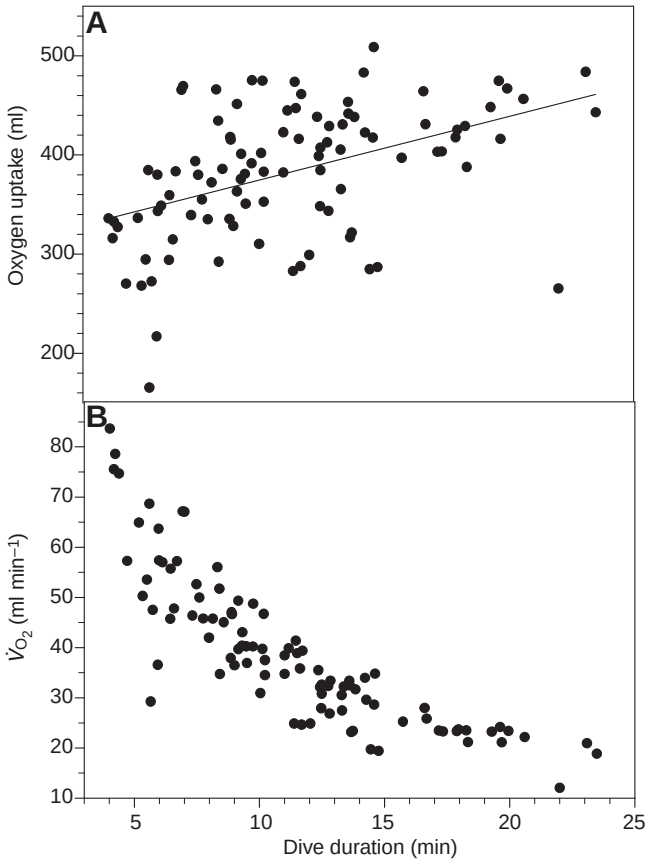

Fig. 3. (A) Oxygen uptake (V_{O_2}) after resting dives and (B) oxygen consumption rate ($\dot{V}_{O_2}$) during these dives against dive duration for one turtle [Elisabeth, body mass (M_b) 117.7 kg, T_w 29.9±0.9°C, 97 dives during 8 trials; single dive cycle resolution]. While there was a significant increase in V_{O_2} after a dive with increasing dive duration (y=6.42x+310.25; r^2=0.19, F=23.78, P<0.0001), this increase was so small, considering the range of dive durations, that reloading of the oxygen store to identical levels between dives could not have occurred. Hence, the fourfold to fivefold difference in $\dot{V}_{O_2}$ between long and short dives (B) is an artefact, related to the breathing pattern displayed by our turtles (single breaths), habituated to dive in a shallow pool. Consequently, the $\dot{V}_{O_2}$ values plotted here do not reflect the true energetic costs of these dives and we therefore based our analysis on multiple dive cycles (see Materials and methods).

between short and long resting dives are an artefact related to the particular breathing pattern displayed by our turtles (single breaths), habituated to dive in a shallow pool. Interestingly, incomplete reloading of the oxygen store during brief periods at the surface has also been observed in mammalian divers. For example, during foraging dives conducted in quick succession, Steller sea lions ran down their oxygen store, which was only completely reloaded during longer surface intervals (Fahlman et al., 2008b). Highly variable oxygen consumption rates were also observed in juvenile and adult loggerhead turtles (*Caretta caretta*) diving in a small/shallow tank, when $\dot{V}_{O_2}$ was calculated over single dive cycles (Hochscheid, 2003). Consequently, if oxygen reloading is incomplete during single dive cycles in our turtles, calculation of $\dot{V}_{O_2}$ requires consideration of multiple dive cycles, when this is more likely to be achieved.

Each trial was split into two periods of contrasting activity level based on behavioural observations and acceleration recordings: a resting/low activity period and a swim period (Fig. 4). Experimental design and turtle behaviour allowed us to clearly separate these

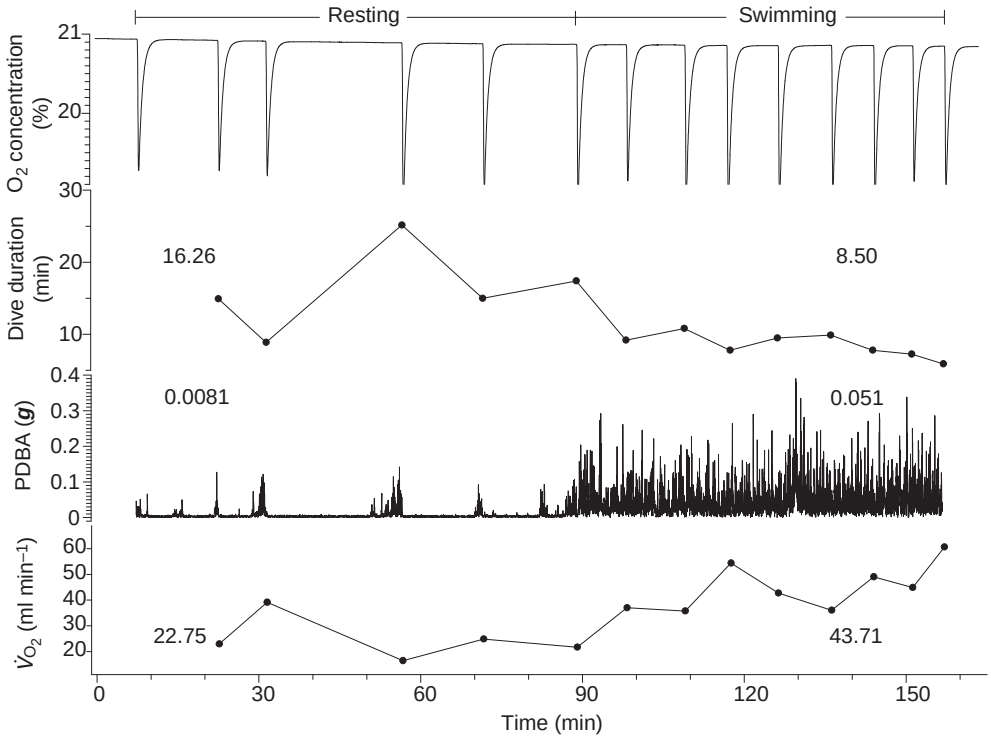

Fig. 4. Example of combined respirometry and accelerometry recording during a typical trial with one turtle. Traces (from top to bottom) show the O_2 concentration inside the chamber, dive duration, partial dynamic body acceleration (PDBA) and $\dot{V}_{O_2}$. The downward deflections in the top trace reflect breathing events. Dive duration was calculated as the time elapsed between two consecutive breathing events. PDBA was calculated from turtle body acceleration (surge and heave), recorded in parallel with respirometry using a bi-axial acceleration logger. $\dot{V}_{O_2}$ for single dive cycles was calculated from the area under the curve associated with each breathing event in a continuous plot of apparent $\dot{V}_{O_2}$ against time. However, in our analysis, we did not use a single dive cycle resolution but divided each trial into two periods with contrasting activity level (indicated by the bars at the top) and calculated $\dot{V}_{O_2}$ over these periods. For each trace two numbers are given, representing the calculated values for the resting and swimming period of that particular trial, included in the analysis.

periods. If brief periods of mixed activity occurred during a trial (i.e. a turtle alternated resting and swimming), they were excluded from further analysis. Periods from each trial included in the analysis were of similar duration (~1.5 h on average) and were used to calculate $\dot{V}_{O_2}$ (ml min^{-1}), PDBA (***g***) and respiratory frequency (f_R, breaths h^{-1}) (Fig. 4). $\dot{V}_{O_2}$ was calculated as the total oxygen uptake during a period divided by period duration. To account for the variation in $\dot{V}_{O_2}$ between individuals associated with differences in M_b, we also report $\dot{V}_{O_2}$ mass independently as $s\dot{V}_{O_2}$ (ml min^{-1} kg$^{-0.83}$). The scaling exponent of 0.83 has commonly been used in the turtle literature (Prange and Jackson, 1976; Southwood et al., 2003) and is similar to the exponent we found during separate resting trials with nine turtles (0.87; M_b range 9.7–166.2 kg; mean T_w 29.2±1.4°C; M.R.E., S.C. and J.-Y.G., unpublished). f_R was determined as the number of breaths divided by the length of the measurement period.

From the recorded acceleration data (surge and heave) we calculated a trace of PDBA for each trial following Wilson and colleagues (Wilson et al., 2006). Briefly, recorded data from both axes were first converted into absolute acceleration values (***g***) using our calibration equations. We removed the static/low frequency component of acceleration (due to animal posture) by smoothing each axis using running means over 4 s, based on observed flipper beat cycle durations (Fig. 5) (Shepard et al., 2008), which were then subtracted from the unsmoothed data of both axes, to produce an approximation of dynamic acceleration (high frequency component; due to the active movement of the animal). After converting these derived values into absolute positive units, values from both axes were summed to produce a trace of PDBA. Logger and respirometry time were synchronised and mean values of PDBA were calculated for the periods identified as 'resting/low activity' and 'swimming' for all trials. Each period consisted of multiple dive cycles (on average ~8 dive cycles for both activity levels, with a minimum and maximum of 3 and 26 dive cycles, respectively).

Statistical analysis

All statistical analyses were conducted using JMP (v.8.0.2.2, SAS Institute Inc., Cary, NC, USA). Differences in oxygen consumption rates ($\dot{V}_{O_2}$ and $s\dot{V}_{O_2}$) and respiratory frequency (f_R) during different activity states (resting *versus* swimming) and during different seasons (austral winter *versus* summer) were tested using a linear mixed-effects model (standard least-squares regression fitted by REML) (Table 1). Activity status and season were included as fixed effects, while turtle ID was included as a random effect. A linear mixed-effects model (LME) was also used to investigate the relationship between $s\dot{V}_{O_2}$ and PDBA during diving. Because our goal was to produce a predictive equation for $s\dot{V}_{O_2}$ that could be used to investigate the energetics of green turtles in the wild and as T_w strongly affects turtle $s\dot{V}_{O_2}$, we also included T_w in the model. Hence, we included PDBA and T_w as fixed effects, while turtle ID was included as a random effect. We further tested whether the relationship between $s\dot{V}_{O_2}$ and PDBA varied with activity status (PDBA × activity status) and between individuals (PDBA × turtle ID). Finally, we ran the model without the interaction terms to produce a common predictive equation for $s\dot{V}_{O_2}$ based on PDBA and T_w.

We used a jack-knife procedure to validate the common predictive equation calculating $s\dot{V}_{O_2}$ from PDBA and T_w (Sokal and Rohlf, 1981; Halsey and White, 2010). Data for one individual were removed from the data set and a new predictive equation was calculated from the remaining data set. PDBA and T_w recorded during trials of the excluded individual were fed into the new predictive equation to estimate its $s\dot{V}_{O_2}$. These $s\dot{V}_{O_2}$ estimates were compared with the $s\dot{V}_{O_2}$ values measured concurrently with PDBA and T_w for that individual. This procedure was conducted sequentially for all individuals and mean algebraic error, mean absolute error and the range of errors for individuals were computed as percentage errors [(estimated value − observed value)×100/observed value].

Significance for all statistical tests was accepted at $P<0.05$. All mean values are presented ±1 s.d.

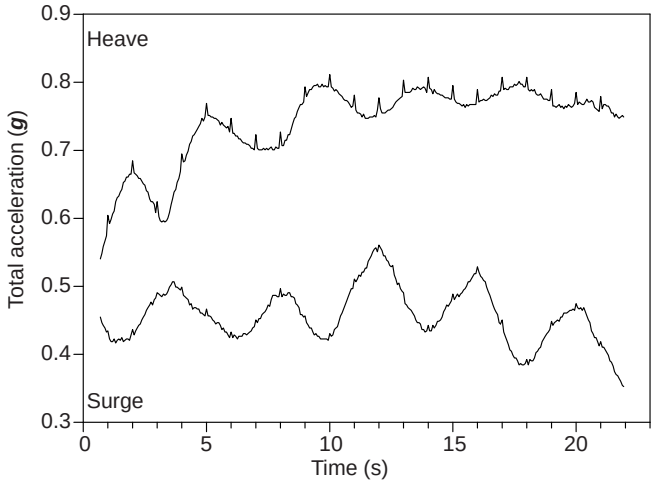

Fig. 5. Typical acceleration trace recorded from one turtle swimming in the channel. Shown is the total acceleration in the dorso-ventral direction (heave, top) and in antero-posterior direction (surge, bottom). Individual cycles, visible especially in the surge trace, correspond to flipper beat cycles.

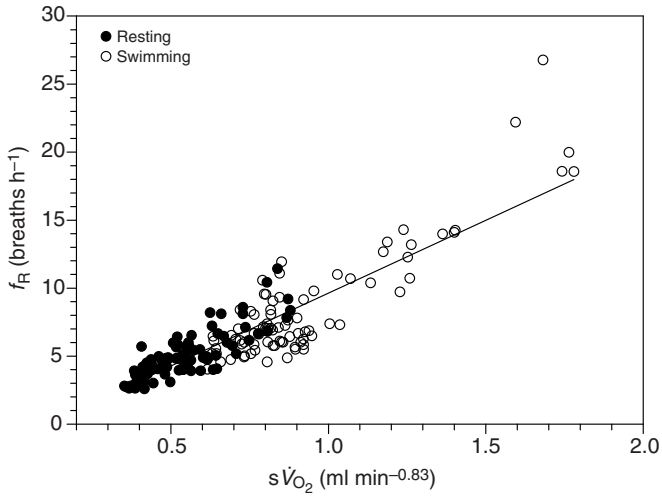

Fig. 6. Relationship between respiratory frequency (f_R) and mass-specific oxygen consumption (s$\dot{V}_{O_2}$) during resting (filled circles) and swimming (open circles) in six adult green turtles (n=165 observations). Linear mixed-effects (LME) analysis revealed that the slope of the relationship was not affected by activity level (P=0.44) or T_w (P=0.76). The solid line indicates the common relationship, established from LME analysis and is best described by: y=10.712x–1.084; F=727.19, P<0.0001, r^2=0.88.

RESULTS

$\dot{V}_{O_2}$, s$\dot{V}_{O_2}$ and f_R during resting and swimming and the effect of T_w

Mean $\dot{V}_{O_2}$ of resting turtles during the austral winter (mean T_w 25.8±1.0°C) was 30.10±6.15 ml min^{-1}, corresponding to 0.50±0.09 ml min^{-1} kg$^{-0.83}$ (Table 1). LME model analysis revealed that both water temperature (for $\dot{V}_{O_2}$: F=21.27, P<0.0001; for s$\dot{V}_{O_2}$: F=28.87, P<0.0001) and activity level (for $\dot{V}_{O_2}$: F=194.92, P<0.0001; for s$\dot{V}_{O_2}$: F=204.02, P<0.0001) significantly affected oxygen consumption rates (Table 1). A mean increase in T_w of 4.1±1.2°C during the austral summer over winter conditions resulted in a mean increase in s$\dot{V}_{O_2}$ during rest of 52.2±36.6% (N=4). Similarly, s$\dot{V}_{O_2}$ during swimming was increased on average by 86.4±50.5% during winter and by 34.8±10.7% during summer, when compared with resting dives. f_R of resting turtles during the austral winter was 4.54±1.19 breaths h^{-1} and increased significantly with an increase in T_w (F=17.17, P<0.0001) and activity level (F=77.51, P<0.0001; Table 1). We found a significant linear relationship between f_R and s$\dot{V}_{O_2}$ (Fig. 6; F=727.19, P<0.0001, r^2=0.88), the slope of which was not affected by activity level (P=0.44) or T_w (P=0.76). Breathing patterns in both resting and swimming turtles typically consisted of single breaths during the short surface periods between dives. Only occasionally would turtles take multiple breaths during a surface period and this usually occurred after long dives.

Using body acceleration to estimate $\dot{V}_{O_2}$ during diving

Activity levels during the swim periods differed between trials and between turtles, resulting in a wide range of $\dot{V}_{O_2}$ and PDBA values (Fig. 7) for these periods. The LME model indicated a significant relationship between s$\dot{V}_{O_2}$ and PDBA (P<0.0001) as well as T_w (P<0.0001). There was no interaction between PDBA and activity status (resting *versus* swimming; P=0.69), indicating that the relationship between s$\dot{V}_{O_2}$ and PDBA did not vary with activity status. However, the slope of the relationship between s$\dot{V}_{O_2}$ and PDBA varied between individuals, as indicated by the significant interaction between PDBA and turtle ID (P<0.0001). After removing the interaction terms, the same model (s$\dot{V}_{O_2}$=PDBA+T_w+turtle ID [random]) was used to generate a common predictive equation, which is given by:

$$s\dot{V}_{O_2} = 12.17\,\mathrm{PDBA} + 0.03\,T_w - 0.46\,, \quad (3)$$

where s$\dot{V}_{O_2}$ is in ml min^{-1} kg$^{-0.83}$, PDBA is in ***g*** and T_w is in °C (r^2=0.83, N=6 turtles, n=170 observations during 89 trials; for PDBA: F=519.57, P<0.0001; for T_w: F=48.98, P<0.0001). The relationship between s$\dot{V}_{O_2}$ and PDBA for six adult green turtles over a range of activity levels is illustrated in Fig. 7. For the purpose of this plot, s$\dot{V}_{O_2}$ values were adjusted to the mean T_w during trials (27.2±2.1°C, range 23.9–31.3°C), using Q_{10} values established for each individual in separate resting trials (T_w range 22.0–32.1°C) (M.R.E., S.C. and J.-Y.G., unpublished). The validation exercise (Table 2) returned a mean algebraic error of 3.3% (range: –20.0% to 22.9%) and a mean absolute error of 17.1% (range 7.9% to 23.0%).

DISCUSSION

This is, to the best of our knowledge, the first study using respirometry (1) to investigate the energetic costs associated with diving in resting and actively swimming adult sea turtles, and (2) to assess the suitability of the accelerometry method to study sea turtle energetics. Resting metabolic rates of our adult green turtles during submergence were low when compared with literature values concerning adult green turtles resting on the beach and juveniles routinely active in small tanks (see below). The scope of the increase in metabolic rate over the resting rate that we observed during swimming was relatively small (Table 1), underlining the cost efficiency of underwater locomotion in marine turtles, when compared with terrestrial locomotion. Water temperature significantly affected the metabolic rate of turtles during resting dives and when swimming (Table 1). We found a tight relationship between body acceleration (PDBA) of green turtles and associated oxygen consumption rates (s$\dot{V}_{O_2}$) during diving (Fig. 7), indicating that PDBA might serve as a reliable proxy of turtle metabolic rate. The resulting predictive equation is a starting point in our endeavour to investigate the energetic requirements of adult green turtles in the wild.

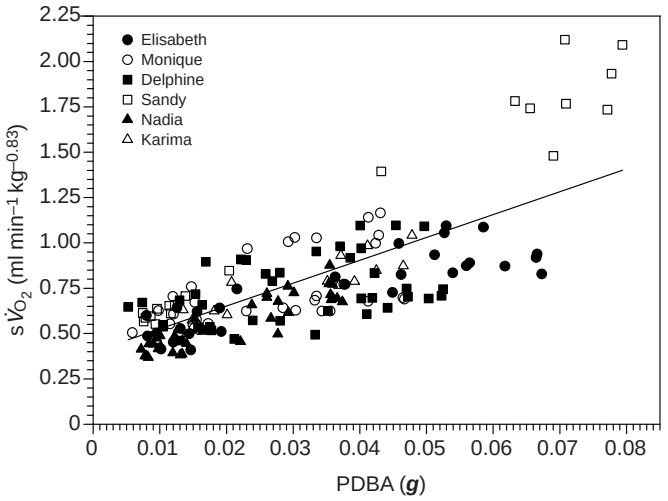

Fig. 7. Rate of $s\dot{V}_{O_2}$ *versus* PDBA for six adult green turtles over a range of activity levels (n=170 observations during 89 trials). For the purpose of this plot, $s\dot{V}_{O_2}$ values were adjusted to the mean T_w during trials (27.2±2.1°C, range 23.9–31.3°C), using Q_{10} values established for each individual in separate resting trials (T_w range 22.0–32.1°C). The line indicates the common relationship, established from a LME model and is best described by y=12.61x+0.40 (r^2=0.75, F=313.51, P<0.0001).

Resting metabolism

Metabolic rate measurements for adult green turtles during submergence do not exist in the literature. The only measurements concerning adult green turtles (mean M_b 128 kg) available in the literature were realised with females after completion of nesting activity (Prange and Jackson, 1976; Jackson and Prange, 1979). In these studies, average metabolic rate of four nesting females resting on the beach (air temperature, T_a 23–27°C) was reported as 0.93 ml min^{-1} kg$^{-0.83}$. A further five females were captured and moved to the laboratory, where their oxygen consumption when resting in air (T_a 26–30°C) was considerably higher (2.28 ml min^{-1} kg$^{-0.83}$) and increased about fourfold during exercise in air to 8.79 ml min^{-1} kg$^{-0.83}$ (Jackson and Prange, 1979). While not elaborating on this substantial difference between resting measurements, the authors suggested that the lower value measured on the beach probably approximates the 'true standard metabolism for nesting turtles'. The $s\dot{V}_{O_2}$ we measured during resting dives in our turtles (0.50 and 0.67 ml min^{-1} kg$^{-0.83}$ during the austral winter and summer, respectively; Table 1) is below this suggested standard metabolic rate. However, our turtles were non-reproductive, so differences in the physiological state of these two groups of turtles might explain the difference in metabolic rate. Certainly, reproduction is a physiologically demanding time for turtles (migration, mating, nesting), where hormonal status will be altered (Hamann et al., 2003), with potential consequences for overall metabolism. In addition, being on land, without the structural support provided by seawater, might be physically challenging for sea turtles. Consequently, $\dot{V}_{O_2}$ levels from nesting turtles (in air) may not be good approximations of $\dot{V}_{O_2}$ for turtles in water.

Our resting measurements are also lower than values reported for juvenile green turtles. In a seasonal acclimation study, $s\dot{V}_{O_2}$ of fasting and routinely active juvenile green turtles (mean M_b 24.1–32.5 kg) ranged between 0.58 ml min^{-1} kg$^{-0.83}$ in winter (T_w 17°C) and 0.89 ml min^{-1} kg$^{-0.83}$ in summer (T_w 26°C) (Southwood et al., 2003). Similarly, Jones and colleagues reported values of 0.80 and 1.63 ml min^{-1} kg$^{-0.83}$ for routinely active juvenile green turtles when fasted and fed, respectively (mean M_b 22.0–22.4 kg; T_w 25.1–25.8°C) (Jones et al., 2009). While part of the difference between our measurements on adults and these studies on juvenile turtles may be related to the different life history stages, most may be explained by the different activity levels of turtles. During our resting measurements turtles generally rested motionless at the bottom of the swim channel, only periodically floating up to exchange gases within the chamber, before sinking again to the bottom. By contrast, in previous studies juvenile turtles were routinely active, either resting at the bottom of a small tank or slowly paddling/shuffling along (Southwood et al., 2003; Jones et al., 2009). Figure 2 in Southwood et al. (Southwood et al., 2003) clearly illustrates the effect of activity on turtle $s\dot{V}_{O_2}$, where $s\dot{V}_{O_2}$ increases significantly with activity level. It also shows that $s\dot{V}_{O_2}$ values reported for juvenile turtles in that study corresponded to activity levels of ~40% (i.e. turtles were active for ~40% of a trial). Only during winter conditions did turtles reduce their activity level to below 20%, with correspondingly lower $s\dot{V}_{O_2}$ values (Southwood et al., 2003). Nevertheless, the metabolic rates of our resting turtles remain low in comparison, as our turtles continued feeding between daily trials (absorptive), while the metabolic rates reported for juveniles mostly concern unfed turtles.

In the absence of diving metabolic rate measurements on adult green turtles, Hays and colleagues (Hays et al., 2000) constructed a bioenergetics model to estimate turtle oxygen stores and oxygen consumption during resting dives. Their model was based on time–depth data recorded from green turtles during the internesting period at Ascension Island (Atlantic Ocean) and literature values concerning oxygen storage capacity. It was also assumed that turtles

Table 2. Results of validation exercise

Turtle	Observed $s\dot{V}_{O_2}$ (ml min^{-1} kg$^{-0.83}$)	Estimated $s\dot{V}_{O_2}$ (ml min^{-1} kg$^{-0.83}$)	Estimated–observed $s\dot{V}_O$ (ml min^{-1} kg$^{-0.83}$)	Algebraic error (%)	Absolute error (%)
Elisabeth	0.84	0.86	0.02	6.9	22.0
Monique	0.70	0.72	0.01	2.0	12.0
Delphine	0.71	0.76	0.05	5.5	17.5
Sandy	1.00	0.74	–0.26	–20.0	20.0
Nadia	0.59	0.70	0.11	22.9	23.0
Karima	0.76	0.77	0.02	2.5	7.9
Grand mean	0.77±0.14	0.76±0.06	–0.01±0.13	3.3	17.1

Shown are the observed and estimated mass-specific oxygen consumption rates ($s\dot{V}_{O_2}$) of adult green turtles during diving and associated error estimates (see Materials and methods for details).
Values are means calculated from 170 observations during 89 trials (7–20 trials per turtle).
A grand mean (±s.d.) is the mean of the individual turtle means.
The mean algebraic error takes the difference of the sign into account, while the mean absolute error does not.

would alter lung inflation to control their buoyancy so as to be slightly negative at resting depth. They estimated the $s\dot{V}_{O_2}$ during resting dives for two green turtles with a M_b of 150.6 and 236.5 kg to be 0.63 and 0.84 ml min^{-1} $kg^{-0.83}$, respectively. While their estimates rest on a great number of assumptions (most importantly, the depth at which negative buoyancy is reached with fully inflated lungs), they are similar to the values measured in our turtles during the austral summer (Table 1). Hays and colleagues did not report T_w in their study (Hays et al., 2000) but given it was carried out during the austral summer, T_w was presumably within a similar range to that of our study.

Effect of activity on metabolism

During swimming, $s\dot{V}_{O_2}$ of turtles was significantly elevated when compared with resting. The mean factorial increase in $s\dot{V}_{O_2}$ during swimming was 1.9 (maximum 3.5) in the austral winter and 1.3 (maximum 1.7) in the austral summer. While these values are certainly much lower than those reported from nesting green turtles crawling on the beach (8.9 times resting) (Prange and Jackson, 1976), they are similar to values obtained from juvenile green turtles during swimming (Prange, 1976; Butler et al., 1984). Prange reported an increase in oxygen consumption of 1.6–3.3 times the resting values in juvenile turtles swimming at a speed of between 0.14 and 0.35 m s^{-1} (Prange, 1976). Similarly, Butler and colleagues observed an increase of 1.9–2.8 times resting oxygen consumption in juvenile turtles swimming at a speed of between 0.4 and 0.6 m s^{-1} (Butler et al., 1984). We did not determine the swim speed of our turtles systematically but turtles generally swam at a low pace, rarely exceeding 0.3 m s^{-1}. As they were not swimming in a flume but in a still-water channel, they did not maintain a constant speed throughout. They also had to turn at the end of the channel and frequently engaged in other activities, like investigating the channel bottom. It is therefore not surprising that, on average, we observed a relatively low factorial increase in $s\dot{V}_{O_2}$ during swimming. However, at times turtles swam at an increased speed (albeit for short periods) and in these cases $s\dot{V}_{O_2}$ was increased to a much greater degree, as indicated by the observed maximum factorial increase of 3.5 times resting. Certainly, adult green turtles might be able to increase $s\dot{V}_{O_2}$ during swimming to a much greater degree than what we typically observed within our set-up. Nevertheless, our results demonstrate that swimming in marine turtles is far less demanding than terrestrial locomotion (Prange and Jackson, 1976). This is underlined by the relatively low field metabolic rate (FMR) values found in internesting leatherback turtles (35 kJ kg^{-1} day^{-1}) (Wallace et al., 2005). By contrast, FMR values of juvenile green turtles foraging in Australia were found to be relatively high (142 and 81 kJ kg^{-1} day^{-1} during summer and winter, respectively) (Southwood et al., 2006). The difference between the FMR values of adult leatherback and juvenile green turtles might, at least partially, be explained by the great size difference between species and differences in feeding status during investigation. Nevertheless, the FMR values for juvenile green turtles in Australia are 8.5 to 10 times the metabolic rates of routinely active juvenile green turtles of slightly greater mass in captivity, reported by the same authors (Southwood et al., 2003). While it is not surprising to find higher metabolic rates in the field than in the laboratory, as activity levels might be greater in the field, the amplitude of this difference is remarkable. However, differences in the feeding status of turtles in the studies concerned might again be responsible for some of this, while methodological uncertainties with the derivation of metabolic rates from DLW studies might have led to an overestimation of turtle FMR values. In their validation study, Jones and colleagues argued that a different equation should be applied to determine the metabolic rates of marine turtles from DLW studies (Jones et al., 2009). Doing so would lower the above FMR values of foraging juvenile green turtles by 37% (Jones et al., 2009).

Effect of temperature on metabolism

$s\dot{V}_{O_2}$ of turtles was significantly affected by T_w (Table 1). In the four turtles measured during both seasons, a mean temperature increase of 4.1°C between the austral winter and summer resulted in an average increase in resting $s\dot{V}_{O_2}$ of 52%. Clearly, temperature strongly influences biochemical reaction rates and, hence, physiological processes. The effect of temperature on ectotherm metabolism, including sea turtles, has been documented in the literature. Early investigators found that oxygen consumption rates of juvenile green turtles in the laboratory, exposed to acute temperature changes between 15 and 35°C, changed fourfold to fivefold (Kraus and Jackson, 1980; Davenport et al., 1982). A similar thermal dependence of metabolism was also reported for juvenile and adult loggerhead turtles during both acute (Lutz et al., 1989) and seasonal temperature changes (Hochscheid et al., 2004). In a recent study on juvenile green turtles, Southwood and colleagues simulated seasonal changes and, when accounting for feeding and activity status, found a rather moderate decrease in their metabolism (24–27%) when temperature declined from 26 to 17°C (Southwood et al., 2003). They concluded that the thermal dependence of green turtle metabolism might be relatively low over the temperature range they naturally encounter. Nevertheless, FMR of juvenile green turtles foraging on the Great Barrier Reef was shown to be considerably increased (75%) during summer (T_w 25.8°C) when compared with the winter (T_w 21.4°C) (Southwood et al., 2006). Some of this difference, of course, might be explained by different activity levels and feeding status during the two seasons. The temperature effect on metabolism of our turtles was less pronounced during swimming (Table 1). The $s\dot{V}_{O_2}$ of four turtles swimming during winter and summer changed on average by 17%. While this could be related to a difference in exercise intensity between seasons, we did not find evidence for this, as mean PDBA of turtles (indicating swimming effort) was similar during the two seasons.

Respiratory frequency (f_R)

Our turtles displayed a breathing pattern that typically consisted of single breaths during the short surface intervals between dives, regardless of activity status. Such a pattern has been observed in other captive marine turtles, typically confined to shallow tanks. For example, Lutz and Bentley found that single breaths were the norm in juvenile green and loggerhead turtles, and observed multiple breaths only occasionally after long dives (Lutz and Bentley, 1985). By contrast, Lutcavage and Lutz reported breathing episodes in immature loggerhead turtles that consisted mostly of two breaths and observed an increase in the number of breaths per episode with dive duration (Lutcavage and Lutz, 1991). An increase in the number of breaths per episode with dive duration was also observed in captive juvenile and adult hawksbill turtles (*Eretmochelys imbricata*) (M.R.E., S.C. and J.-Y.G., unpublished). Within our set-up, green turtles would only occasionally take multiple breaths, typically after long resting dives. However, when diving to depth, green turtles around Reunion Island and Mayotte Island are frequently seen to take multiple breaths at the surface before descending again (M.R.E., unpublished; K. Ballorain unpublished). Hence, the single breath pattern observed in captive turtles might be related to the shallow depth of their holding facilities, where air can be accessed easily, often by simply lifting the head. Mean f_R of our turtles during rest (4.5–6.5 breaths h^{-1}; Table 1) and when swimming (8.5 breaths h^{-1}) was lower than f_R reported for routinely active juvenile green turtles (9.1 and 12.3 breaths h^{-1} for

winter and summer, respectively) (Southwood et al., 2003), which might be related to the size difference of turtles in the two studies. In our study, f_R increased significantly with increases in T_w and activity level (Table 1). A number of marine turtle studies have found a marked change in f_R with T_w and activity level (Prange and Jackson, 1976; Jackson and Prange, 1979; Butler et al., 1984; Hochscheid et al., 2004). In most marine turtles, such an increase in f_R seems to be the major mechanism to increase ventilation that parallels the increase in $\dot{V}_{O_2}$, while tidal volume changes little (Prange and Jackson, 1976; Jackson and Prange, 1979; Lutcavage and Lutz, 1997). The significant relationship between f_R and $s\dot{V}_{O_2}$ during resting and swimming that we found (Fig. 6) indicates that this is also the case in adult green turtles.

Using PDBA to estimate $s\dot{V}_{O_2}$ during diving

We found a highly significant positive relationship between PDBA and $s\dot{V}_{O_2}$ in our turtles during diving that was modulated by T_w (Fig. 7), suggesting that body acceleration of turtles might serve as a good proxy of energy expenditure during diving. From this it was possible to generate a significant predictive equation (Eqn 3) that allows estimation of turtle $s\dot{V}_{O_2}$ from the recording of PDBA and T_w. A validation exercise returned mean algebraic and absolute errors that are small (Table 2) and similar to those of other studies deploying both the accelerometry and the heart rate method to estimate the energy expenditure of animals (Halsey et al., 2009). This makes accelerometry a promising tool to investigate the at-sea metabolic rates of marine turtles.

Since its first application to animal biology (Wilson et al., 2006), the accelerometry method has been deployed in a wide range of species and its validity to estimate energy expenditure has been assessed (Halsey et al., 2009; Green et al., 2009b). While most studies originally centred on terrestrial locomotion in mostly endotherm species, there are now a number of studies on semi-aquatic/aquatic species and ectotherms (Halsey et al., 2011; Fahlman et al., 2008a; Payne et al., 2011; Halsey and White, 2010). The majority of these studies suggest that dynamic body acceleration can serve as a reliable proxy of energy expenditure in active animals, especially in the context of terrestrial locomotion. Error estimates typically associated with the accelerometry method are similar to those of the heart rate method (Halsey et al., 2009). In fact, the accuracy of both methods is such that both estimate more accurately the energy expenditure of a group of animals, rather than that of individuals, as the algebraic error is typically considerably lower than the absolute error. However, the accelerometry method has its limitations that arise from the fact that it quantifies energy-consuming movements of animals, assuming that energy expenditure increases with movement intensity and duration. Accordingly, it works best if the energy expended during activity accounts for the majority of the overall energy budget of an animal. Periods of inactivity, when animals move little but might engage in energetically costly physiological processes (e.g. digestion, thermoregulation), will most likely not be picked up by the accelerometry method. Despite this, body acceleration was shown to be a significant predictor of energy expenditure in bantam chickens (*Gallus gallus*), even during periods of inactivity, albeit with much less accuracy than provided by the heart rate method (Green et al., 2009b). While this is perhaps surprising, significance was achieved by pooling data from various periods of inactivity (i.e. digestion and thermoregulation) during which there must have been sufficient movement, correlating with both PDBA and $\dot{V}_{O_2}$.

Considering the implications of this for our study with marine turtles, it is clear that thermoregulatory costs are of little importance to most of these reptiles. Apart from behavioural means (Hochscheid et al., 2010), chelonid sea turtles generally do not thermoregulate and, hence, do not spend energy for thermoregulatory purposes. Leatherback turtles, on the other hand, might use the metabolic heat generated by vigorous swimming activity to maintain an elevated and stable body temperature (Bostrom and Jones, 2007; Bostrom et al., 2011). This, however, does not pose a problem for the accelerometry method. By contrast, energetic costs associated with the process of digestion would most likely go undetected by the accelerometry method. However, our turtles were not in a post-absorptive state during experimentation but were instead maintained on their normal feeding schedule, so that digestive costs are included in our respiratory measurements. We decided on this approach for two reasons. Firstly, it would have been impossible to maintain the animals in an unfed state over such a long period within our set-up, without causing severe disturbance. Secondly, green turtles at their foraging grounds, the main target of our future investigations, will most likely be in a comparable nutritional state (i.e. absorptive). One possible complication in this context is the basking behaviour occasionally observed in marine turtles. For loggerhead turtles, it was suggested that periods spent basking at the surface have a re-warming function to compensate for decreased body temperatures when diving in cooler water, possibly to enhance digestive function (Hochscheid et al., 2010). While ambient temperature is monitored by the acceleration data logger and was included in our model, it is not clear how well such temperature measurements will represent turtle body temperature (affecting turtle metabolism) under these circumstances. The relationship between PDBA and $\dot{V}_{O_2}$ that we found in adult green turtles was not affected by activity status, indicating that body acceleration can be used to estimate energy expenditure even during periods of rest. Similar to the case of the bantham chickens (Green et al., 2009b), there must have been sufficient movement during resting periods of turtles (ascending into the chamber to breathe and descending again) for a correlation between PDBA and $\dot{V}_{O_2}$.

In contrast to most accelerometry studies to date, Halsey and colleagues (Halsey et al., 2011) found that body acceleration (ODBA) did not correlate with $\dot{V}_{O_2}$ during single dive cycles of double-crested cormorants (*Phalacrocorax auritus*) diving to a depth of 5 m. A number of potential explanations were advanced for this missing correlation. One possibility is rooted in the methodological constraint of calculating metabolic rate in diving animals by measuring the amount of oxygen taken up between dives. If divers do not always replenish their oxygen stores to identical levels, then metabolic rate calculations based on single dive cycles will be erroneous, so that it is necessary to consider entire dive bouts (Fahlman et al., 2008b). Clearly, our turtles did not replenish their oxygen stores to identical levels with their single breaths between dives (Fig. 3; see Materials and methods). Hence, it was not possible to investigate dive costs and their association with dynamic body acceleration on the basis of single dive cycles. Accordingly, we considered periods as long as possible (typically ~1.5 h for each activity level) for our investigation. By doing so, we prevented erroneous metabolic rate calculations that might have concealed the correlation between PDBA and $\dot{V}_{O_2}$. Another potential explanation for the missing correlation in cormorants is linked to a difference in density of the two media that the birds moved in. Movement of the birds during diving might have been dampened by the greater resistance in water, so activity at the surface might have disproportionately affected ODBA. Marine turtles, by contrast, spend the majority of their time underwater with only brief periods at the surface and even then remain effectively submerged. They only break the surface with their head (and potentially the top of their carapace), which makes them less susceptible to problems associated with differences in the resistance of water and air, potentially affecting ODBA/PDBA (Halsey et al., 2011). In this

context it is of interest to note that a recent study successfully used accelerometry to estimate FMR in a fully aquatic species, the Australian cuttlefish (*Sepia apama*) (Payne et al., 2011). Hence, while the suitability of the accelerometry method to estimate energy expenditure during diving in endotherm divers awaits further investigation, our results suggest that accelerometry works well in aquatic reptiles.

LIST OF ABBREVIATIONS

FMR	field metabolic rate
f_R	respiratory frequency (breaths h^{-1})
LME	linear mixed effects model
M_b	body mass (kg)
ODBA	overall dynamic body acceleration ($\boldsymbol{g}$)
PDBA	partial dynamic body acceleration ($\boldsymbol{g}$)
$s\dot{V}_{O_2}$	mass-specific oxygen consumption rate (ml min^{-1} $kg^{-0.83}$)
T_w	water temperature (°C)
V_{O_2}	oxygen uptake (ml)
$\dot{V}_{O_2}$	oxygen consumption rate (ml min^{-1})

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Inside JEB

TURTLE ENERGY EXPENDITURE FROM ACCELERATION

Gliding through the warm south-western Indian Ocean, green turtles live a peaceful life feasting on seagrass meadows before returning to their deep coral reef homes. But their idyllic lifestyle is under threat from human activity; so understanding how these enigmatic creatures stay in tune with their environment is becoming increasingly important. 'If they don't balance their energy budget there is going to be a problem for the individual and later on at a population level,' explains Manfred Enstipp from the DEPE/CNRS Laboratory in Strasbourg, France. However, conventional methods for measuring energy expenditure may be unreliable in turtles, so Enstipp and Jean-Yves Georges decided to try another approach: measuring the animals' movements with accelerometers to estimate their energy use. First, the team had to discover whether there was a measurable relationship between the turtle's acceleration and their oxygen consumption in order to extrapolate the animals' energy use from their activity (p. 4010).

However, when Enstipp and his colleagues embarked on the study they weren't even sure that they could measure the turtle's oxygen consumption rate. 'They had to find this hole that is 40×40 cm to breathe so that we could measure the oxygen consumption rate,' explains Enstipp, but many of his colleagues thought that the freely swimming animals could not be trained to surface repeatedly at a single point in long channel.

Travelling to Stephane Ciccione's Kelonia Turtle Aquarium on Reunion Island, Enstipp isolated a 13 m-long swim channel in the turtles' enclosure and then, over a period of months, he and Benoit Gineste slowly covered the channel's surface with sections of metal fence until the turtles were content to surface and breathe at the respirometry dome, which covered the channel's only remaining open surface. Despite his colleagues' misgivings, Enstipp found that the turtles successfully learned to surface and breathe at the respirometry dome. Then, Enstipp velcroed a two-axis accelerometer onto a turtle's shell before recording her oxygen consumption and movements as she swam to and fro.

Returning to Reunion Island with Gineste, Myriam Milbergue and Virginie Plot in the summer and winter, Enstipp successfully measured the oxygen consumption rates of six adult turtles at temperatures ranging from 24.8 to 30.1°C while also measuring their acceleration patterns. But could he use the acceleration traces to accurately estimate the turtles' oxygen consumption?

Developing an equation to calculate the turtles' oxygen consumption based on their acceleration and the temperature of the surrounding water – which determines turtle metabolic rate – Enstipp calculated the turtles' oxygen consumption rates and compared them with the animals' measured oxygen consumption rates. They agreed well: the team could use the turtle's acceleration pattern to calculate their oxygen consumption, which they could use as a proxy for the animal's energy consumption.

But how does the captive turtle's activity level compare with that of free-ranging green turtles grazing their seagrass meadows? 'We are trying to apply this to Katia Ballorain's data from turtles foraging around Mayotte Island,' Enstipp reports. However, early evidence suggests that the captive turtles were no couch potatoes and were every bit as active as their free-ranging cousins, offering Enstipp and his colleagues hope that accelerometery could provide much needed measurements of green turtles' energy consumption in the wild.

10.1242/jeb.067074

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Kathryn Knight

HONEY BEE LARVAE NEED IRS AND TOR TO BECOME QUEENS

All honey bee larvae are equal when they start out. But then, in a twist of fate, some develop into fertile queen bees, while others are relegated to a life of worker drudgery: and that twist is simply their diet. Larvae fed a nutritious gel – royal jelly – develop into queens, while larvae provided with a plainer diet develop into workers. However, the mechanism for this drastic diet-induced switch of fate wasn't clear. Adam Dolezal from Arizona State University, USA, explains that two metabolic pathways – the insulin-like signalling pathway and TOR (target of rapamycin) pathway – are known to sense the nutritional status of most creatures, adjusting their metabolism and development to make the most of an individual's diet. He adds that a key hormone – juvenile hormone – is also known to throw the switch from worker to queen development. So, Dolezal, his supervisor Gro Amdam and a team of collaborators from Arizona State University and Washington State University decided to find out whether these

signalling pathways affect juvenile hormone levels in response to diet to turn workers into queens (p. 3977).

Knowing that both pathways are triggered by specific signalling proteins [insulin-like receptor substrate (IRS) and TOR], the team decided to inactivate IRS and TOR individually, and simultaneously, while feeding larvae on a diet of royal jelly. If the signalling pathways were carrying the message that the royal jelly-fed bees should develop into queens, then short circuiting the pathways should force the bees to develop into workers, despite the regal diet.

Dolezal and Navdeep Mutti became worker bee nurses to thousands of vulnerable honey bee larvae as they fed the insects a royal jelly diet laced with specially tailored double stranded mRNA molecules, to prevent the larvae from producing the IRS and/or TOR molecules and so inactivate the signalling pathways' responses to the diet. Then the team monitored how the larvae developed without the signalling pathways.

Teaming up with Florian Wolschin, Jasdeep Mutti and Kulvinder Gill, Dolezal and Nardeep Mutti found that the larvae developed into workers, despite their royal jelly diet. And when Dolezal monitored the larvae's juvenile hormone levels, instead of being high – which is what you would expect for larvae fed on royal jelly – the larvae had low levels of the hormone, which had forced them to develop into workers despite their diet.

Switching off the pathways prevented larvae on a royal jelly diet from producing juvenile hormone and developing as queens, but could the insects develop into queens if they received a juvenile hormone supplement? Sure enough, when Dolezal and Mutti gently applied juvenile hormone to the skins of royal jelly fed larvae that had lost insulin and/or TOR signalling, they developed into queens. So, the IRS and TOR signalling molecules are key links between the larvae's diet and their developmental fate.

However, Dolezal points out that another researcher, Masaki Kamakura, tested the link between diet and development at a different point in the insulin signalling pathway. Publishing his work in *Nature*, Kamakura found the opposite result: his royal jelly-fed larvae successfully developed into queens even though the signalling pathway had been short circuited at the signal's receptor. But Dolezal explains that the IRS trigger is also known to activate the epidermal growth factor (EGF) signalling pathway, in addition to the insulin pathway, by interacting with other receptors. The larvae could still develop into queens if the insulin receptor was inactivated because the IRS signal could trigger the alternative pathway, and the team is now keen to test the connection between IRS and EGF signalling in larval development.

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Kathryn Knight

DIAPAUSE TERMINATION PINPOINTED

Some insects only have one annual crack at the whip. Emerging from a state of suspended animation during winter – known as diapause – many insects have a single season to successfully complete development and reproduce. And when your life-cycle is tightly synchronised with other seasonal events, such as fruit production, accurate timing is essential. Yet little was known about how pupae reactivate development when they terminate diapause ready for a new season.

According to Gregory Ragland from the University of Florida, USA, larvae of the apple maggot fly (*Rhagoletis pomonella*) are a major apple pest in the US. However, *R. pomonella* larvae infested indigenous hawthorn fruits – which fruit later than apples – long before settlers brought apples to North America, so the flies that switched host had to evolve rapidly to adjust when they emerge from diapause to catch early fruiting apples. Knowing that *R. pomonella* is studied by scientists intrigued by rapid species evolution, Ragland, Jeffery Feder, Stewart Berlocher and Daniel Hahn decided out find out how the *R. pomonella* population that persists in attacking hawthorn fruits initiate emergence from diapause (p. 3948).

Collecting maggot infested hawthorn fruits from a field in Michigan, Ragland and Hahn transported them to Florida and collected the larvae surfacing from the fruit. Next, the duo simulated the onset of winter by cooling the larvae to 4°C as they transformed into pupae and entered diapause. Then, after 20 weeks in the cold, the team warmed the pupae to 24°C and monitored their metabolic rate to track when it rose and they began to emerge from diapause. Collecting pupae before their metabolic rate began rising, when their metabolic rate had risen by 40% from the diapause level over 24 h, and 48 h after their metabolic rate began to rise, the team analysed how the insect's gene expression patterns changed using a custom-built gene chip containing between 7000 and 8000 unique *R. pomonella* genes. The team compared how the expression of groups of genes changed over the first 48 h after the larvae's metabolism began rising to find out how they terminate diapause and reinitiate development and growth.

'The punch line of the analysis is that almost all the patterns had this large change in gene expression that happened right when we had the metabolic rate increase,' says Ragland. And when Ragland and Scott Egan took a closer look at the genes that were strongly activated, they found several genes involved in cell cycle control, which regulates when cells divide. 'The cell cycle is arrested during diapause and we see marked changes in activity in some of the important cyclins and cyclin-dependent kinases [that regulate the cell cycle] concordant with the up-tick in metabolism. That suggests to us that they are getting ready for, or they are going through, mitosis [cell division] at that point and it is roughly concordant with the increase in metabolic rate, so we feel we have pinpointed pretty well when development starts up and diapause termination is complete,' says Ragland.

The team also found changes in heat shock protein expression, metabolic gene expression, genes involved in development, and genes that are known to participate in diapause termination in other organisms. They are now keen to find genes that have different regulation patterns in the late emerging hawthorn maggots and early emerging apple maggots to identify other key components of the diapause termination process.

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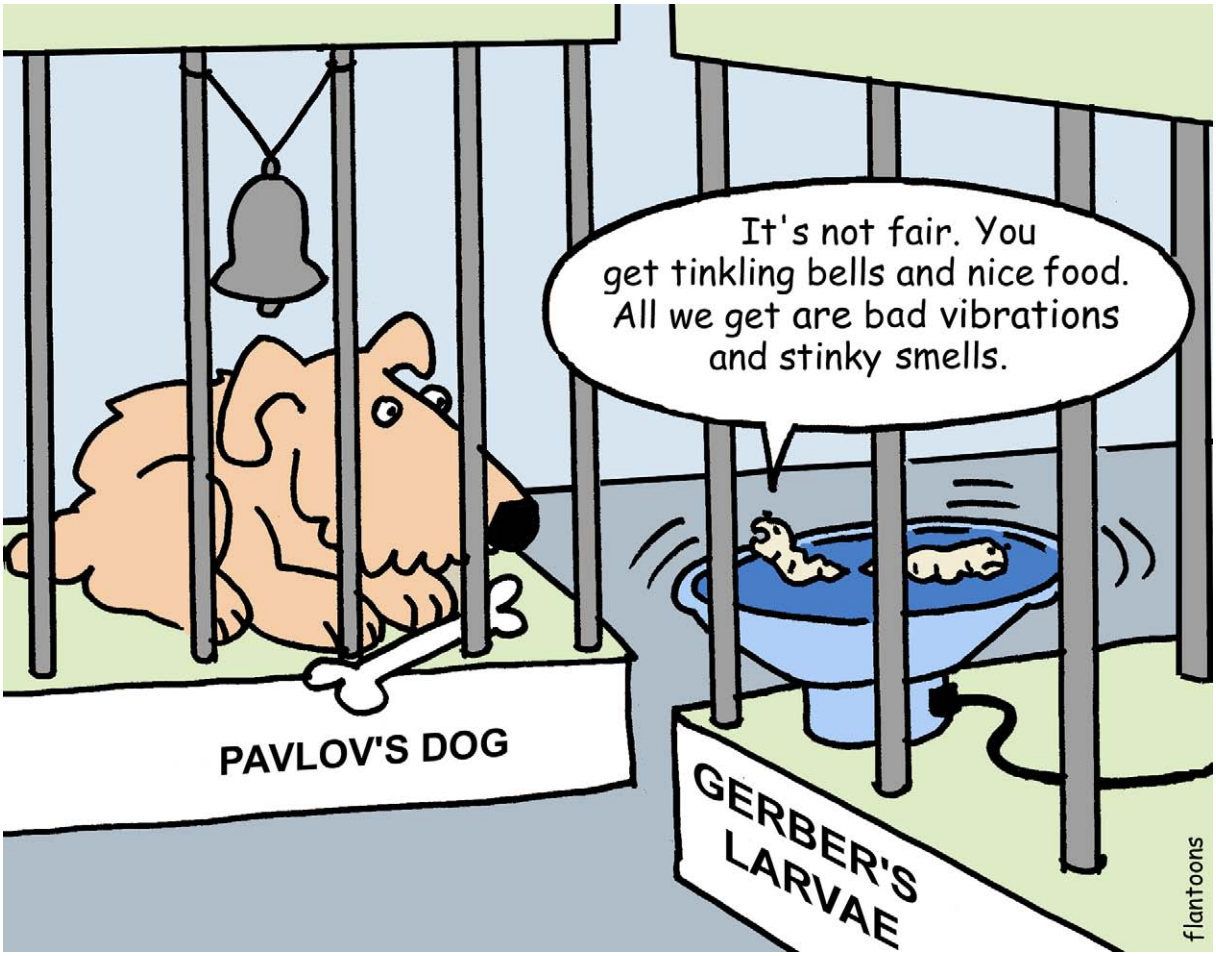
Kathryn Knight

LARVAE ASSOCIATE BAD VIBRATIONS WITH ODOURS



Understanding the neural basis of memory and learning is one of the Holy Grails of neurobiology, but untangling convoluted memory networks in human brains is currently impossible. So neurobiologists have turned their attention to animals that learn and form memories in much simpler brains. Bertram Gerber and colleagues from Germany explain that *Drosophila* larvae can learn to associate odours with punishment – unpleasant tastes and mild electric shocks – but no one had tested whether they could associate odours with disturbing vibrations. Given that vibration can be more finely controlled than other aversive stimulants, the team decided to find out whether the larvae could be trained to associate disagreeable vibrations with specific odours (p. 3897).

Training 50 larvae in a Petri dish by exposing them to 200 ms bursts – a buzz – of 100 Hz vibration from a loudspeaker in the presence of 1-octanol odour and then exposing them to *n*-amyl acetate with the loudspeaker turned off, the team then tested which odour the larvae avoided in the presence of the vibration threat: the larvae wriggled away from the 1-octanol odour. And when the team repeated the experiments, this time associating the vibration with *n*-amyl acetate odour and no vibration with 1-octanol, the vibrated larvae avoided n-amyl acetate when presented with a choice.

After characterising the larvae's behaviour as an escape response, the team also found that the strength of the association between the vibration and odour increased when they increased the number of buzzes and the length of the training period. However, the team point out that the larvae only take evasive action when under threat from the vibration, and they say, 'Aversive memories are behaviourly expressed in the presence of punishment but not in its absence, and… are embedded into a conditioned "escape routine" which is employed only when escape is warranted.'

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Eschbach, C., Cano, C., Haberkern, H., Schraut, K., Guan, C., Triphan, T. and Gerber, B. (2011). Associative learning between odorants and mechanosensory punishment in larval *Drosophila*. *J. Exp. Biol.* **214**, 3897-3905.

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