

## RESEARCH ARTICLE

# Synchronous activity lowers the energetic cost of nest escape for sea turtle hatchlings

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## ABSTRACT

A potential advantage of group movement in animals is increased locomotion efficiency. This implies a reduced energetic cost for individuals that occur in larger groups such as herds, flocks and schools. When chelonian hatchlings hatch in the underground nest with finite energy for their post-hatching dispersal phase, they face the challenge of minimizing energetic expenditure while escaping the nest. The term ‘social facilitation’ has been used to describe the combined digging effort of sea turtle hatchlings during nest escape. Given that in a normal clutch, a substantial part of the energy reserve within the residual yolk is used by hatchlings in the digging out process, a decreased cohort size may reduce the energy reserve available to cross the beach and sustain the initial swimming frenzy. This hypothesis was experimentally tested by varying cohort size in hatchling green turtles (*Chelonia mydas*) and measuring energy expenditure during the nest escape process using open-flow respirometry. The energetic cost of escaping through 40 cm of sand was calculated to vary between 4.4 and 28.3 kJ per individual, the cost decreasing as the number of individuals in the cohort increased. This represents 11–68% of the energy contained in a hatchling’s residual yolk at hatching. The reduced energetic cost associated with large cohorts resulted from both a lower metabolic rate per individual and a shortened nest escape time. We conclude that synchronous digging activity of many hatchlings during nest escape evolved not only to facilitate rapid nest emergence but also to reduce the energetic cost to individuals.

**KEY WORDS:** Aggregation behaviour, Social facilitation, Metabolic expenditure, Green turtle, Oxygen consumption, Hatchlings

## INTRODUCTION

Grouping of individuals of the same species resulting in mutual benefits is common in animals. Experimental data from a broad range of taxa show that there are at least four mutual benefits that can result from aggregation behaviour: (i) decreased chance of predation (Colbert et al., 2010; Creel et al., 2014; Unglaub et al., 2013), (ii) increased feeding efficiency (Horst, 1995; Jackson et al., 2008; Hsia and Wood-Gush, 1982; Lazarus, 1979), (iii) increased locomotion efficiency (Ebensperger and Bozinovic, 2000; Fish, 1995; Voelkl et al., 2015) and (iv) decreased energy spent on thermoregulation (Gilbert et al., 2008, 2010; Nunez-Villegas et al., 2014; Withers and Jarvis, 1980). However, aggregation behaviour may have more than

one function within a single species (Lazarus, 1979). For example, formation flight by migrating birds might increase their flight range (Portugal et al., 2014; Voelkl et al., 2015) but also decrease the chance of predation by way of a dilution effect as the group size increases (Dehn, 1990).

Although sea turtles are typically considered to be solitary species, they exhibit grouping behaviour (Carr and Hirth, 1961; Hendrickson, 1958) when leaving the underground nest after hatching from a clutch of about 50–150 eggs (Spotila, 2004). Because all eggs within a clutch experience a similar incubation temperature, they hatch almost synchronously with their clutch mates, and at least some species are able to stimulate the hatching of clutch mates if the process has not already begun (Spencer et al., 2001). Synchronous hatching within a clutch results in incidental association among clutch mates and allows simultaneous digging activity of individual hatchlings. The term ‘social facilitation’ has been used to describe this phenomenon in sea turtle hatchlings along with the scramble of hatchlings from the nest to the sea (Carr and Hirth, 1961; Hendrickson, 1958), but we only discuss this concept with respect to the nest escape process. Social facilitation may facilitate nest escape because the synchronous effort of many individuals might be needed to dig successfully through the column of sand above the nest chamber (Hendrickson, 1958; Carr and Hirth, 1961). Sea turtle hatchlings do not dig continuously while escaping the nest; periods of intense digging activity are interspersed with periods of rest (Carr and Hirth, 1961; Drake and Spotila, 2002). At the end of a rest period, the spontaneous digging activity of one individual triggers the individuals around it to also start digging, resulting in cohort-wide synchronous digging (Bustard, 1967; Carr and Hirth, 1961; Gyuris, 1993). Digging activity is also inhibited by high temperatures. Hence, when hatchlings approach the surface during the day when the sun is shining, the hot sub-surface sand inhibits activity (Bustard, 1967; Gyuris, 1993). After the sand cools in the late afternoon or at night, digging activity can resume and this is the reason why most sea turtle hatchling emergence events occur at night or during cool cloudy days (Bustard, 1967; Gyuris, 1993). The group digging behaviour is hypothesized to result in sea turtle hatchlings emerging from the nest in large cohorts, which can lead to a predator swamping phenomenon that may decrease the overall predation rate of hatchlings as they leave the beach and swim out to sea (Bustard, 1972).

Early reports of upward digging activity in sea turtle hatchlings were based primarily on nest excavation during the hatching to surfacing period (Hendrickson, 1958), and observations made through a glass pane inserted into nests (Carr and Ogren, 1959). Bustard (1972) suggested that hatchlings may actively dig upwards through the nest column at any time during the day or night. Recent studies (Baldwin et al., 1989; Dial, 1987; Hamann et al., 2007) have used hatchlings’ blood lactate concentration as an index of digging intensity, but were unable to relate these values to the overall energetics of the nest escape process. Another study addressed the

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energetics of nest escape in olive ridley (*Lepidochelys olivacea*) hatchlings using the doubly labelled water (DLW) technique (Clusella Trullas et al., 2006). These authors estimated that the field metabolic rate of olive ridley hatchlings digging out from 25 cm below the surface in a cohort of 20 individuals was 39.52 kJ per individual. However, this approach required the taking of multiple blood samples, which disturbed the natural digging behaviour of the turtles.

The fixed amount of energy available in the freshly laid egg is the only energy available for embryonic development, nest escape, beach escape and the initial swim frenzy of sea turtle hatchlings. Hence, any energy saved during these progressive stages will ultimately be available for the energetically demanding initial swim frenzy before hatchlings reach oceanic waters where feeding behaviour begins. In sea turtles, it is thought that a substantial proportion of the energy reserve in the residual yolk is used during nest escape (Clusella Trullas et al., 2006; Kraemer and Bennett, 1981), and the social facilitation hypothesis suggests that this energy will vary depending on cohort size. This hypothesis may be tested experimentally by measuring energy expenditure during the digging out process across different cohort sizes. In the current study, we investigated two aspects of the social facilitation hypothesis using green turtle (*Chelonia mydas*) hatchlings: (i) larger cohort size decreases the time taken to dig out of the nest and (ii) the energetic cost per individual decreases as the cohort size increases. We also evaluated the energy used during nest escape so it can be placed in the context of the total energy available in the egg, and the proportion of that energy used during embryonic development, nest escape and the off-shore swim.

## MATERIALS AND METHODS

### Animals

This study was approved by The University of Queensland Animal Ethics Committee (AEC approval number: SBS/133/13/URG). Getting *Chelonia mydas* (Linnaeus 1758) hatchlings to dig in a natural way within respiratory chambers was problematic, and several iterations of different protocols were trialled before a protocol that worked was discovered. In these ‘developmental’ trials, four clutches of green turtle eggs were collected (Department of Environment and Heritage Protection, Queensland, Australia; permit no: WITK12887013) during oviposition from females at Heron Island (Queensland, Australia). These clutches were chilled to 7–10°C for 8 h to retard embryonic development (Harry and Limpus, 1989) before being transported by boat to the mainland and then by car to the laboratory facilities (approximately 10 h travelling distance) at The University of Queensland, St Lucia campus. Upon arrival in the laboratory, eggs were incubated in moist heat-sterilized beach sand at a constant temperature of either 27 or 30°C until they were about to hatch. Hatchlings were killed at the end of these trials by cooling to 3°C then freezing; because of this, we were only permitted to collect four clutches. Some eggs and hatchlings from these trials were also used to determine their energy content so that the energy expended during nest escape could be put into context of the overall egg to hatchling energy budget.

For the major trial of this study, green turtle eggs were collected at Chagar Hutang beach (Redang Island, Malaysia) Marine Turtle Research Station of Universiti Malaysia Terengganu (UMT). Six clutches that had been incubating *in situ* for between 53 and 57 days were transferred periodically (two clutches at a time) to the Institute of Oceanography and Environment (INOS, UMT) laboratory facilities by placing clutches into an icebox (no cooling used) for the 3 h journey by boat and car. Thus, all clutches were expected to

begin hatching with 24–72 h of being collected. A semi-permanent mark in pen was placed on top of each egg as it was removed from the nest to maintain egg orientation during transportation. Hatchling metabolic expenditure during nest escape was measured once and, after surfacing, hatchlings were kept together in darkness on top of moist beach sand (~less than 20 min) prior to being released in a dark section of a nearby beach to complete their crawl to the sea.

### Measuring energy expenditure

Two methods were used to quantify energy expenditure during nest escape. The first used open-flow respirometry (which assumes that ultimately all energy is derived from aerobic metabolism) and the second used differences in the energy content of hatchlings before and after the digging out process.

Open-flow respirometry was used to measure hatchling metabolic rate. Using the clutches collected from Heron Island, we developed by trial and error a protocol that finally resulted in the successful measurement of metabolic rate during the digging out process. This perfected protocol appeared to show similar nest digging out behaviour to that of hatchlings in natural nests and was then used in the trials conducted in Malaysia.

### Preliminary system used in University of Queensland trials

Respirometry chambers were constructed from transparent cylindrical Perspex placed vertically (7.5 cm radius and 80.0 cm height). Chambers were sealed at both ends with the top cover made of clear acrylic so that hatchlings could be seen when surfacing. Newly hatched turtles were randomly selected and buried under a column of beach sand in respirometry chambers. As the total number of hatchlings used differed among chambers, the depth of sand from the uppermost hatchlings to the surface was standardized to 40 cm (the typical depth from the top of an egg chamber to the surface sand of green turtle nests) to ensure that hatchlings dug through the same volume of sand to reach the surface. Air entered through a tube at the base of the chamber and exited via a tube in the lid of the chamber. All preliminary trial experiments were performed in a 28°C constant temperature room with 24 h lights so that webcam images could be recorded continuously.

### Final system used in UMT trials

Respirometry chambers were constructed from light-proof PVC cylindrical pipe placed vertically (10.5 cm radius and 80 cm height). Chambers were sealed at both ends with the top cover made of clear acrylic so that hatchlings could be seen when surfacing. Air entered through a tube at the base of the chamber and exited via a tube in the lid of the chamber. A 5 cm layer of vermiculite was placed at the bottom of the chamber to avoid fluid retention at egg level during egg hatching. Pipping eggs (those in which the hatchling had pipped the shell with their egg tooth prior to hatching) were randomly selected and buried in groups of varying size (10–60 eggs) under a column of beach sand in respirometry chambers. As the total number of pipped eggs differed among chambers, the depth of sand from the uppermost eggs to the surface was standardized to 40 cm. Sand from Chagar Hutang beach, Redang Island, was used, and sieving indicated the sand was a medium sand grade (mean particle size 1.05–1.24  $\phi$ ; Folk and Ward, 1957). In order to determine the time of hatching within the opaque respirometry chambers, a thin strip (~2 mm) of aluminium foil was laid on the uppermost eggs before the sand was added and connected in-line with a 1000  $\Omega$  resistor, 1.5 V alkaline battery and a voltmeter (analog/digital converter, ADInstruments, PowerLab 4/30) placed across the resistor. At hatching, the hatchlings broke

the aluminium foil, causing the electrical potential to fall to zero. Digging duration time was assumed to be the difference between the time when the electric potential fell to zero and the time when hatchlings appeared on the sand surface as recorded by a webcam setup that viewed the sand surface through the chamber's transparent lid. These experiments were performed in UMT laboratory facilities at 28°C with 24 h light so that the emergence event could be monitored continuously.

An open-flow respirometry system was used to measure the rate of oxygen consumption ( $\dot{V}_{O_2}$ ) throughout these experiments (Fig. 1). Outside air was pumped (Cole-Parmer, Air Cadet) sequentially through soda lime (to scrub  $CO_2$ ), Drierite (to absorb water vapour) and a mass flow controller (OMEGA, FMA5400/5500) that regulated the air flow at 1000 ml  $min^{-1}$ . The outflow air from the top end of the chamber was sub-sampled at 100 ml  $min^{-1}$  through scrubbing columns of soda lime and Drierite prior to entering an oxygen analyser (Sable Systems, PA-1B). The voltage output of the oxygen analyser was sampled every 30 s through an analog/digital converter (ADInstruments, PowerLab 4/30) connected to a computer running Lab Chart 7 software (ADInstruments). Calibration of the oxygen analyser was performed every 3 h with  $CO_2$ -free dry air. Oxygen consumption was calculated using eqn 11.1 of Lighton (2008).

#### Calculation of energy expenditure from oxygen consumption

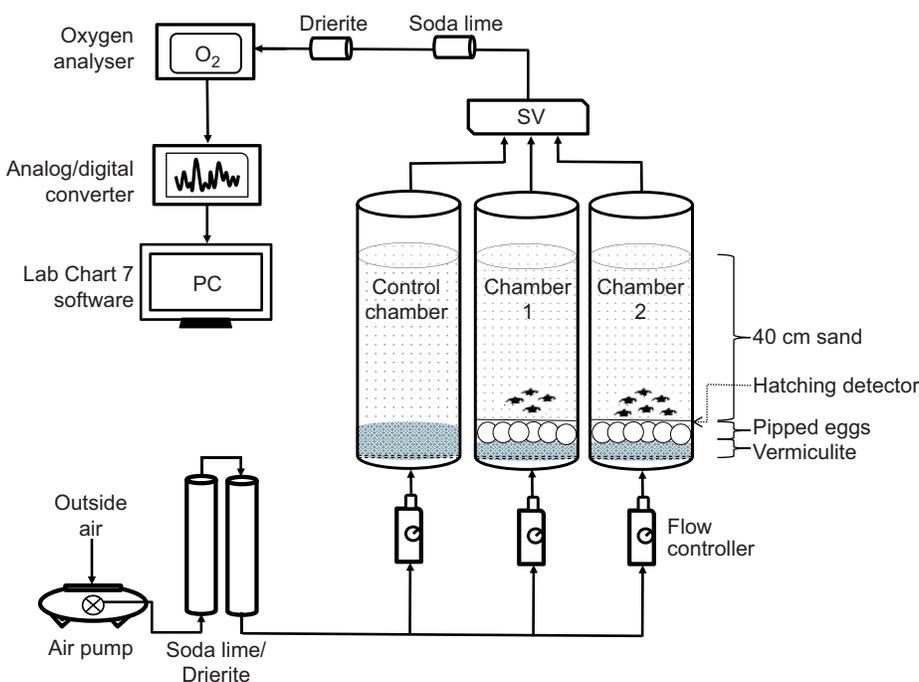
Three respirometry chambers were used simultaneously, with one chamber consisting of vermiculite and 40 cm of sand with no hatchlings (hereafter known as the 'control chamber') to measure background microbial  $\dot{V}_{O_2}$ , while the remaining two chambers contained hatchlings. The  $\dot{V}_{O_2}$  was recorded for 10 min at a time in chambers containing hatchlings in sequence via a series of solenoid valves that were controlled through the 'event manager' module in Chart 7 software. Oxygen consumption of the control chamber was recorded periodically for 10 min once every 3 h. When swapping from one chamber to another, it took 3 min for the gas to completely flush through the system so that only the last 7 min of  $\dot{V}_{O_2}$  measurement in a 10 min cycle were used. The  $\dot{V}_{O_2}$  of hatchlings

was then calculated by subtracting the background microbial  $\dot{V}_{O_2}$ , which was relatively constant and small (0.02–0.03%  $\dot{V}_{O_2}$  of hatchlings), from the raw hatchling chamber  $\dot{V}_{O_2}$ .

The total energy consumed by hatchlings during the digging out process was calculated by first integrating this to units of energy by assuming every litre of oxygen consumed corresponded to the expenditure of 19.7 kJ of energy (Schmidt-Nielsen, 1997). To compare energy expended per individual among trials, the total energetic cost per individual was calculated by dividing the energetic cost for the whole group by the number of individuals within the group. Once on the surface, hatchlings were removed from the chamber and weighed. When emergence onto the surface by hatchlings occurred asynchronously, each individual was removed from the chamber immediately on surfacing and the calculation of individual oxygen consumption adjusted using the number of individuals remaining in the chamber (Table 1). The  $\dot{V}_{O_2}$  trials were terminated 96 h after the last cohort appeared on the surface because our observations indicated it was unlikely that any more hatchlings would make it to the surface after this time. In some trials, some hatchlings failed to make it to the surface, but these hatchlings were alive when the experiment was terminated. None of these hatchlings had progressed upward from the egg level, so it was assumed they did not contribute to the digging effort made by their clutch mates. Hence, the  $\dot{V}_{O_2}$  of these remaining hatchlings after their emerged siblings had been removed from the respirometry chamber was treated in the same way as background microbial  $\dot{V}_{O_2}$  when calculating the  $\dot{V}_{O_2}$  of their siblings that successfully dug up through the sand column.

#### Energy content of eggs and hatchlings

Energy content analysis was performed on eggs and hatchlings from the Heron Island green turtle clutches. Twenty freshly laid eggs (five eggs from each of four clutches) were separated into albumen and yolk, and 26 recently hatched turtles (six or seven hatchlings from each of four clutches) were killed and dissected to separate the residual yolk from the hatchling's body. Each component was



**Fig. 1. Schematic diagram of the open-flow respirometry system.** Flow path: air pump – soda lime – Drierite – mass flow controller – respirometry chambers – solenoid valve (SV) – soda lime – Drierite – oxygen analyser – analog/digital converter – computer.

**Table 1. Calculation of total individual energetic cost for green turtle hatchlings digging through 40 cm of sand when adjusting for varying numbers of individuals remaining in the respirometry chamber**

Elapsed time (h)	No. of hatchlings remaining in chamber	Total O <sub>2</sub> consumed (ml)	Total O <sub>2</sub> consumed per individual (ml)	Total O <sub>2</sub> consumed to reach surface per individual (ml)	Energetic cost of reaching surface per individual (kJ)
0–72.3	50	8415	168.3	168.3	3.316
72.4–87.7	15	503	33.5	201.8	3.975
87.8–135.8	14	1349	96.4	298.2	5.874
135.9–231.8	9				
Clutch average cost per individual				184.95	3.640

Example data are from trial 2B, which had three separate emergence cohorts with 41 hatchlings successfully reaching the surface (size of cohort reaching the surface together was 35, 1 and 5 hatchlings) while nine hatchlings remained at the base of the chamber and never began the journey upwards.

weighed to 0.1 mg prior to being dried to constant mass in a freeze drier. The dried samples of egg components and residual yolk of hatchlings were homogenized using a mortar and pestle, and yolk-free carcasses were ground using a coffee grinder to a homogeneous powder.

The energy density of dried samples was determined using ballistic bomb calorimetry. A sub-sample (0.1–0.2 g) of each component was transferred to a metal thimble and fully combusted in 20 atmospheres of oxygen inside a ballistic bomb calorimeter (Gallenkamp Autobomb, UK). Energy density value was determined in triplicate for each component. Periodically, the calorimeter was calibrated with thermochemical standard benzoic acid (26.442 J g<sup>-1</sup>; Bureau of Analysed Standards Ltd, Middlesbrough, UK). Energy density is reported on a dry mass basis that includes the ash component.

### Data analysis

Spearman's correlation was used to examine the association between clutch size and digging duration and clutch size and total energy expenditure. Pearson's correlation was used to examine the relationship between digging duration and total energetic cost. Statistical significance was assumed if  $P \leq 0.05$ .

## RESULTS

### Emergence pattern

In the initial trials with hatchlings from the Heron Island population, all hatchlings failed to show natural digging and emergence behaviour. Some hatchlings made it to the surface, but they emerged as individuals and not in a group. As a consequence,

the experimental design and protocol went through several different iterations until a protocol that resulted in 80% of eggs producing hatchlings reaching the surface was developed (Table 2). This protocol was used in all trials conducted at UMT in Malaysia. Overall, hatchlings took between 3.7 and 7.8 days to emerge from beneath 40 cm of sand, and emerged onto the surface in one to four cohorts. In seven out of 11 trials, we noticed that hatchlings remained slightly buried with their heads partially protruding from the sand on the surface for 2–4.5 h before completely moving out onto the sand's surface. In all trials, the first cohort to emerge onto the surface contained the largest number of hatchlings. Trials that consisted of a larger number of individuals took the shortest time to dig upward through the 40 cm of sand (Fig. 2A).

### The energetic cost of digging upward

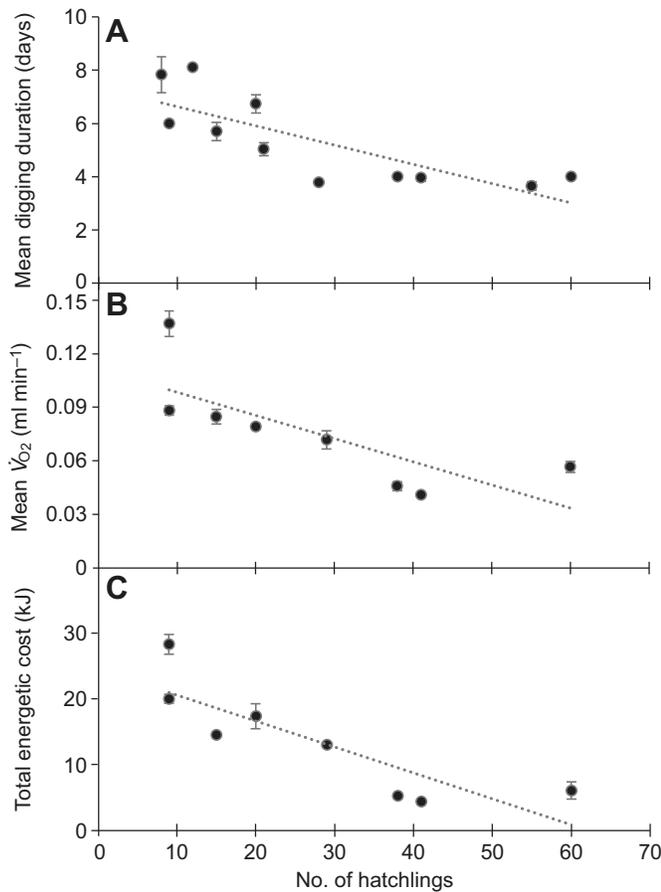
Out of the 11 trials conducted, we recorded  $\dot{V}_{O_2}$  throughout the entire upward digging process from eight clutches. Oxygen consumption data from three trials was lost as a result of file corruption when power to equipment briefly failed. The pattern of  $\dot{V}_{O_2}$  was similar in all trials, with large fluctuations throughout the entire time and occasional sharp peaks that lasted 10–15 h (Fig. 3).

The per-individual  $\dot{V}_{O_2}$  average over the entire digging out period varied between 0.04 and 0.14 ml min<sup>-1</sup>, and decreased as cohort size increased (Fig. 2B). The per-individual total energy expended during the digging period, calculated by integrating respirometry data, varied between 4.4 and 28.3 kJ and decreased as cohort size increased (Fig. 2C). Hatchling individual energetic cost was correlated with digging duration (Fig. 4).

**Table 2. Time to emergence and cohort characteristics of green turtle hatchlings digging up through 40 cm of sand in respirometry chambers**

Clutch no.	Group	No. of eggs placed in chamber	Proportion of hatchlings reaching surface (%)	Mean ( $\pm$ s.e.m.) digging duration (days)	Time between emergence of first and last cohorts (days)	No. of separate cohorts that emerged	Proportion of hatchlings in the first cohort (%)
1	A	10	90	7.83 $\pm$ 0.7	5	3	77.8
	B	20	100	6.74 $\pm$ 0.3	5.7	4	85
2	A	30	93.3	3.78 $\pm$ 0.0	0.6	2	96.6
	B	50	82	3.95 $\pm$ 0.1	2.2	3	85.4
3	A	10	90	6.00 $\pm$ 0.0	0	1	100
	B	47	80.9	4.00 $\pm$ 0.0	0	1	100
4	A	12	100	8.10 $\pm$ 0.0	0	1	100
	B	60	100	4.00 $\pm$ 0.0	0	1	100
5	A	15	100	5.70 $\pm$ 0.3	0	1	100
	B	60	91.7	3.65 $\pm$ 0.2	3.9	2	83.3
6		25	84	5.03 $\pm$ 0.3	4.1	3	85.7

Group A, small; group B, big.



**Fig. 2. Duration and energetic cost of digging.** Variation in (A) digging duration ( $y = -0.072x + 7.36$ ,  $r^2 = 0.64$ ,  $P < 0.01$ ,  $N = 11$ ), (B) mean individual rate of oxygen consumption ( $\dot{V}_{O_2}$ ) throughout the digging period ( $y = -0.001x + 0.11$ ,  $r^2 = 0.58$ ,  $P = 0.008$ ,  $N = 8$ ) and (C) total individual energetic cost ( $y = -0.4x + 24.5$ ,  $r^2 = 0.73$ ,  $P = 0.002$ ,  $N = 8$ ) with clutch size. Vertical bars represent s.e.m.

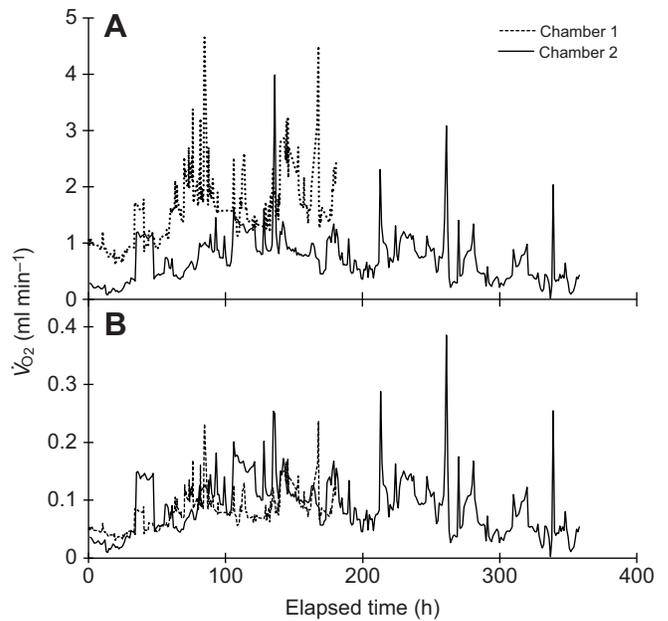
### Energy content

The yolk samples had a much greater energy density and total energy content than the albumen (Table 3). Although residual yolk had a greater energy density than the yolk-free carcass, the greater mass of the yolk-free carcass resulted in there being more energy in the yolk-free carcass than in the residual yolk (Table 3). We calculated that eggs contributed 172.4 kJ of their energy to the embryonic development and hatching process. Based on the energy content of the residual yolk at hatching, 41.7 kJ was available for nest escape and dispersal from the nest.

## DISCUSSION

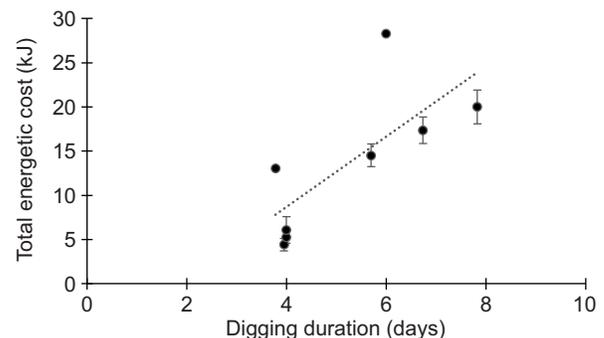
### System troubleshooting and improvement

This is the first study to measure metabolic rate continuously of sea turtle hatchlings during the nest escape process, and we encountered a number of unanticipated problems in our first trials. In our first attempt, active newly hatched turtles were placed under 40 cm of sieved beach sand in a transparent cylindrical Perspex chamber, with the aim of continuously monitoring digging activity visually while also recording  $\dot{V}_{O_2}$ . However, hatchlings remained more or less motionless under the sand column for more than 48 h before this first trial was abandoned. A possible explanation for this unexpected lack of digging behaviour is that hatchlings require some working air space in which to start their digging activity (Carr and Hirth, 1961). In natural nests, there are usually some air spaces



**Fig. 3. Rate of oxygen consumption.** An example of real-time  $\dot{V}_{O_2}$  (A) for all hatchlings combined and (B) per individual. Note the differences in the time taken to reach the surface between chambers one and two. Chamber one contained  $N = 20$  individuals whereas chamber two contained  $N = 10$  individuals.

between the spherical eggs in the egg chamber (Carr and Hirth, 1961; Kraemer and Richardson, 1979), and additional space would be created during the hatching process as fluids drain away from the nest when hatchlings escape their eggs. Hence, burying hatchlings under a solid column of sand with no air spaces may have inhibited the start of digging behaviour. To overcome this problem, in the next iteration of these experiments, we used eggs from a clutch that had just started to pip. During the egg transfer process we meticulously maintained the orientation of the egg so that the top of the egg always remained upwards, by marking the top surface before movement began, because it is known that chelonian embryos at the late stage of incubation orientate themselves into a hatching position prior to the hatching event (Miller, 1985). Therefore, changing an egg's orientation could prolong the hatching process as embryos reposition themselves within the egg if eggs are rotated while being placed in the chamber. We also introduced a layer of vermiculite below the eggs to provide a space for the fluids released during hatching to drain into.



**Fig. 4. Relationship between the energetic cost of digging through 40 cm of sand per individual hatchling and digging duration.**  $y = 3.98x - 7.3$ ,  $r^2 = 0.55$ ,  $P = 0.028$ ,  $N = 8$ . Vertical bars represent s.e.m.

**Table 3. Estimation of the total energy contained within freshly laid eggs and newly hatched turtle components based on energy density (calculated on a dry mass basis including ash) and mean dry mass of green turtles**

	<i>N</i>	Mean ( $\pm$ s.e.m.) dry mass (g)	Mean ( $\pm$ s.e.m.) energy density (kJ g dry mass <sup>-1</sup> )	Calculated total energy (kJ)
Freshly laid eggs				
Albumen	20	0.37 $\pm$ 0.02	13.1 $\pm$ 0.5	4.8
Yolk	20	11.61 $\pm$ 0.40	30.8 $\pm$ 0.4	357.8
Entire egg contents				<b>362.6</b>
Newly hatched turtles				
Yolk-free carcass	26	6.07 $\pm$ 0.1	24.5 $\pm$ 0.2	148.5
Residual yolk	26	1.09 $\pm$ 0.1	38.3 $\pm$ 0.3	41.7
Entire hatchling				<b>190.2</b>
Energy used during embryonic development				<b>172.4</b>

The least-square ANCOVA adjusted means for freshly laid egg and hatchling mass are at 47.8 and 25.1 g, respectively (wet mass).

This experimental protocol resulted in hatchlings starting to dig upwards after escaping the eggs, as would occur in natural nests, providing experimental evidence that hatchling sea turtles do indeed need an air space in order to begin their nest escape digging behaviour. However, we found that rather than concentrating their digging effort in a vertical direction, as would be expected in a natural nest, many hatchlings directed their digging effort sideways and became stuck against the chamber's wall. It would appear that rather than relying solely on gravitational cues to direct their digging effort upwards, as is suspected to occur in natural nests, which are completely dark, hatchlings were attracted to light entering through the transparent chamber walls. It is well known that hatchling sea turtles use light cues to direct their movement once they exit the nest in nature (e.g. Mrosovsky and Kingsmill, 1985; Kawamura et al., 2009), and it appears that they can be attracted to light even while buried underground, but in nature such cues are absent during the nest escape process. Thus, in the final experimental protocol, we used light-proof PVC pipe as our respiratory chambers. In this way, hatchlings remained in the dark and were light naive until they reached the surface of the sand column. To determine the time when hatchlings reached the surface, we used a transparent chamber lid and a 24 h webcam. To determine the time when hatchlings emerged from their eggs and started to dig upwards, we placed a thin strip of aluminium foil on top of the uppermost eggs, connected in series with a resistor and a battery; when the digging out process began and the aluminium strip was broken, the electrical potential fell to zero. This hatching detector was assumed not to interfere with the upward movement of hatchlings.

Sea turtle hatchlings do not dig continuously during their nest escape; periods of active digging are punctuated by periods of rest (Carr and Hirth, 1961; Drake and Spotila, 2002). Originally, we hoped to continuously monitor the digging progression of hatchlings as they moved upward, but discovered that light interfered with this process. Other work from our laboratory also shows that hatchlings are distracted by infrared light, so we did not attempt to use the transparent chambers and infrared video. We did find that by placing an ear direct against the opaque chamber wall, scratching noises presumably generated by digging activity could be heard intermittently. Unfortunately, we were unable to source a surface-mounted microphone system during our experiments, but this would be a useful addition to future experiments. An important observation was that external sound such as talking in close vicinity of the chamber could trigger an activity bout. For this reason, care was taken to minimize external noise during these experiments. Noise may be a natural cue used to trigger synchronous digging

activity amongst clutch mates as it is known that chelonian hatchlings can detect and respond to sound stimuli (Ferrara and Vogt, 2014; Ferrara et al., 2013, 2014; Harms et al., 2014).

Immediately before the emergence event, we noticed that in most trials hatchlings spent some time resting just below the sand surface before finally emerging onto the surface. This behaviour is similar to that described by Dial (1987) for sea turtle hatchlings emerging from natural nests. There are three hypotheses about the cues used by sea turtle hatchlings to trigger the final nest emergence process: (i) the threshold temperature hypothesis (Drake and Spotila, 2002; Hendrickson, 1958; Matsuzawa et al., 2002), (ii) the rapid temperature change in surface sand hypothesis (Witherington et al., 1990) and (iii) the negative thermal gradient hypothesis, when surface sand becomes cooler than the sub-surface sand (Gyuris, 1993). As the current trials were conducted at a constant temperature, none of the temperature change hypotheses can explain our observations. However, it is possible that the delay we observed was caused by a build-up of lactic acid in the blood, and hatchlings waited until blood lactate levels dropped to a lower level before completing the emergence process as suggested by Dial (1987).

#### Metabolic rate and hatchling activity

The spikes in  $\dot{V}_{O_2}$  that occurred throughout the digging out process were presumably caused by bouts of synchronous digging. These spikes were separated by periods of decreased oxygen consumption. During periods of active digging, lactic acid accumulates in hatchling sea turtles (Dial, 1987; Hamann et al., 2007) and causes the hatchlings to stop digging once it reaches an upper threshold (Edwards and Gleeson, 2001). Oxygen consumption would probably remain high for a period after digging in order to pay back an oxygen debt, a period of time when lactate is converted back into pyruvate and pyruvate enters gluconeogenesis (Randall et al., 1997). We intended to continuously observe digging behaviour so that the timing of digging and resting could be correlated with  $\dot{V}_{O_2}$  but this was not possible because hatchlings would not engage in their normal digging behaviour in the transparent chambers.

$\dot{V}_{O_2}$  during 'rest periods' typically varied between 0.03 and 0.05 ml min<sup>-1</sup>, and peak  $\dot{V}_{O_2}$  typically varied between 0.2 and 0.4 ml min<sup>-1</sup> (Fig. 3). For comparison,  $\dot{V}_{O_2}$  is 0.06 ml min<sup>-1</sup> for full-term green turtle embryos just prior to pipping (Booth and Astill, 2001) and 0.55 ml min<sup>-1</sup> for green turtle hatchlings maximally swimming immediately after entering the water during their frenzy swim (Booth, 2009). This indicates that maximum aerobic digging effort is less than maximum aerobic swimming

**Table 4. Summary of the energy budget of green turtle hatchlings from freshly laid egg through to the end of the first day of the frenzy swim**

Component	Energy (kJ)	Fraction of energy in fresh egg (%)	Fraction of energy in residual yolk (%)	Source
Fresh egg	360	100		Current study
Yolk-free hatchling	150	42		Current study
Residual yolk	40	11	100	Current study
Embryonic development	170	47		Current study
Nest escape*	5–25	1.4–7	13–63	Current study
First 24 h of swim	6	2	15	Booth (2009)

\*Varies depending upon clutch size.

Note that this estimation was compared with hatchlings originating from different populations (eggs, embryonic development and the first day of swimming from southern Great Barrier Reef population; nest escape from the mainland Malaysia population), as discussed in Materials and methods.

effort, and is consistent with the suggestion that anaerobic effort during digging is also lower than anaerobic swimming effort as reflected by lower blood lactate concentrations in digging compared with swimming hatchling green turtles (Hamann et al., 2007). Clusella Trullas et al. (2006) also found that digging had a lower metabolic requirement than swimming.

### Digging duration and energetic cost

Digging duration and energetic cost were dependent on cohort size, with larger cohorts requiring shorter digging out times (Fig. 2A) and having a lower per-individual energetic cost (Fig. 2C). The lower energetic cost of individuals digging out in large groups resulted from a combination of a lower absolute mean metabolic rate per individual and a shorter digging out time. The relative importance of both these factors to individual energy expenditure is similar, with an increase from a group size of 10–60 resulting in a ~50% decrease in both digging duration and mean metabolic rate. Hence, in nature, it is likely that hatchlings receive a significant benefit by belonging to a large clutch because of a reduced per-individual energetic cost of nest escape. The role an individual sea turtle hatchling plays during the nest escape process varies depending on its position within the digging cohort: some scratch down the sand roof and others compact the sand underneath the group (Carr and Hirth, 1961). These roles probably have different energetic costs. In theory, moving in a cohort against the resistance (i.e. sand) requires the leading edge individual(s) to incur higher energetic costs than the others (Fish, 1995; Trenchard et al., 2015). However, this added energetic cost to lead individuals can be shared by rotating positions while moving (Voelkl et al., 2015). Hence, in future studies it would be good to be able to identify individuals to see whether they share the work load by rotating positions and roles, or whether certain individuals do more of the digging work than others.

The greater the number of actively digging hatchlings in a cohort, the greater the metabolic heat production, and as a consequence the nest temperature may also be higher, causing an increase in hatchling body temperature. Ectotherm locomotor performance generally increases with an increase in body temperature; for example, swimming performance of newly emerged sea turtle hatchlings is greater in warm water (Booth and Evans, 2011). Hence, sea turtle hatchlings digging in a larger cohort may also indirectly receive a benefit through the effect of increased body temperature on locomotion performance. The total energetic cost per hatchling of digging vertically through 40 cm of sand varied between 4.4 and 28.3 kJ and decreased as cohort size increased (Fig. 2C). Because energy is limited for sea turtle hatchlings as they escape the nest, the energy saved by social facilitation would be allocated to the remaining dispersal activities such as crawling

across the beach and the swimming frenzy. The grouping of individuals during locomotion to save on individual energetic costs is well established (reviewed in Portugal et al., 2014). For example, Lissaman and Shollenberger (1970) estimated that a group of 25 birds flying in a V-formation would have 71% more flight range than a bird flying by itself, and a power saving of up to 14% has been estimated for flocks of pink-footed geese (Cutts and Speakman, 1994). In the current study, we found that variation in group size could cause a 4-fold change in an individual's energetic cost of nest escape.

The energetic cost of nest escape can be put into an ecological context when considering it in the context of available energy in the freshly laid egg, and energetic costs of embryonic development and the off-shore swim (Table 4). Although the energetic cost of nest escape is small compared with the overall energy in a fresh egg, it can be a very large proportion of the energy remaining in the residual yolk at hatching, and is similar to or larger than the energy used during the first day of swimming. Hence, hatching success, which determines the number of hatchlings in a cohort, can have a significant influence on the per-individual cost of nest escape, and as a consequence a large influence on the amount of energy remaining in the residual yolk after nest escape. Hatchlings entering the sea with larger energy reserves are presumably at an advantage as they can survive longer before finding food and therefore escaping the nest in larger cohorts probably results in hatchlings of greater fitness. This finding may have implications for active conservation management of sea turtles in some regions of the world like Malaysia where a common strategy is to split natural clutches into smaller clutches (Mortimer, 1999; Mortimer et al., 1994) when relocating them into hatcheries. Sometimes clutches are also partially harvested before being placed into a hatchery, a practice which also reduces the cohort size (Koch et al., 2007). Reducing the number of eggs in a clutch may ultimately result in the production of hatchlings with reduced energy reserves when they enter the sea.

### Conclusions

Over 50 years ago, Carr and Hirth (1961) suggested that 'social facilitation' within the nest played an important role in the nest escape process of newly hatched sea turtles. However, empirical evidence for the existence of mutual benefits to individual hatchlings during nest escape has been limited. Our study provides new insight into sea turtle hatchling synchronous activity during nest escape, and how it influences the energetic cost of nest escape. We found that an increase in group size from 10 to 60 hatchlings caused a ~50% decrease in both the time taken to escape the nest and mean metabolic rate during this time, resulting in reduced energy expenditure during nest escape. Because a finite amount of energy is available to hatchlings upon hatching, the

energy saved by synchronous digging can be allocated to other activities such as the frenzy off-shore swim.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

M.U.R. and D.T.B. designed the experiments; D.T.B. and J.J. provided materials; M.U.R. performed experiments, analysed the data and wrote the manuscript; D.T.B. and J.J. revised the manuscript.

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