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ORIGINAL ARTICLE

Bigger clutch sizes save offspring energy during nest escapes

Mohd Uzair Rusli[1,2] · David T. Booth[2]

Abstract Hatchling turtles typically emerge from underground nests in groups, so the nest escape process may represent another example of animals sharing a task (in this case, digging out of a nest) to save on individual energy expenditure. Previous studies have reported the energetic cost of embryonic development across chelonian taxa, but none has quantified the extra amount of energy needed to escape the nest. Brisbane river turtle (*Emydura macquarii signata*) hatchlings were found to fuel this activity by using approximately 50 % of their residual yolk energy content. An open-flow respirometry system was used to quantify the effect of clutch size on an individual's energetic cost while digging out of the nest. The energetic cost of nest escaping 15 cm upward in the fine moist sand was calculated to be between 0.34 and 2.32 kJ per individual depending upon the number of hatchlings digging together. The energetic cost decreased as the number of individuals digging together increased and thus supports the 'social facilitation' hypothesis which suggests hatchlings cooperate to share the workload of digging out of the nest amongst clutch mates to reduce individual energy expenditure. The reduced energetic cost associated with large cohorts was chiefly caused by the shorter time taken to dig out of the nest by larger numbers of individuals. We conclude that synchronous digging activity of many individuals during nest escape evolved not only to facilitate quicker nest emergence but also reduce the energetic cost to individuals.

Keywords Energetic expenditure · Social facilitation · Aggregation · Clutch size · Brisbane river turtle

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Significance statement

Turtles typically lay their eggs on land in an underground nest. As the amount of energy is finite upon hatching, the energy used during nest escape will detract from the reserve energy for early life activities. Brisbane river turtle (*Emydura macquarii signata*) hatchlings were found to use approximately 50 % of their residual yolk energy content to escape from the underground nest. However, the energetic cost has decreased as the number of individuals digging together increased. Hatchlings are likely to share the workload of digging out of the nest amongst clutch mates to reduce individual energy expenditure. This finding may have implications for a common strategy to split turtle clutches into half when relocating them into hatchery to increase incubation success. Further, such information would be useful to predict hatchling susceptibility to predation during their early life.

Introduction

Research on social facilitation while moving in a group originated with an observation of competitive cyclists riding faster

by 25 % when accompanied by front line pacemakers (Triplett 1899). In the animal kingdom, at least ten different patterns of moving formation have been identified and described with mathematical models (Eftimie et al. 2007). Some of the most remarkable examples of moving formation can be observed in flying bird flocks and fish schools that have been shown to influence speed and reduce resistance around the adjacent individuals in formation and thus save on the energetic cost of transport (Hansell 1993; Fish 1995; Ebensperger and Bozinovic 2000). The concept of energy saving within group members is not restricted to locomotion effort; it also benefits stationary aggregations such as huddling behaviour in penguins during freezing weather (Gilbert et al. 2008, 2010; Zitterbart et al. 2011).

Energy saving on one aspect of life history such as locomotion allows animals to spend energy on other functions, such as growth and reproduction, thus improving their survival and fitness. As chelonian hatchlings typically emerge from their underground nest simultaneously (as reviewed in Salmon and Reising 2014), the term 'social facilitation' has been used to describe how synchronous hatching and nest emergence might enhance hatchling fitness in this taxa (Carr and Ogren 1959; Carr and Hirth 1961; Koch et al. 2007; Spencer and Janzen 2011; Pignati et al. 2013). Sharing the work required to dig out of the nest across nest mates might also be a strategy to reduce the energy expended by individual hatchlings during the nest escape process. In this hypothesis, the larger the clutch size, the smaller the per individual energetic cost will become.

Prolonged intense physical activity results in high energy demands, and this could be challenging to vertebrate neonates if it happens immediately after hatching. Chelonian hatchlings are lecithotrophic (i.e. they have a relatively large amount of yolk in their eggs), and the energy in the residual yolk after hatching provides sufficient energy to engage in post hatching activities such as digging, crawling and swimming (Kraemer and Bennett 1981; Booth and Astill 2001; Clusella Trullas et al. 2006). Given that the amount of energy in the residual yolk is finite, the energy used during nest escape will detract from energy available to hatchlings for post-nest activities such as crawling to water or swimming once the water is reached. However, the nest escape process has been reported to last about a week in turtle hatchlings, during which periods of intense digging activity are separated by rest periods (Bustard 1967; Mrosovsky 1968; Moran et al. 1999; Pignati et al. 2013).

Theoretically, hatchlings have to dig upward against gravity and they are not morphologically specialized as a digger, so their energetic cost of digging is likely to be greater than specialist burrowers (for a review, see Dorgan 2015). Hence, the nest escape process in chelonian hatchlings might be energetically expensive, being fuelled principally by residual yolk which typically protrudes through the plastron in a newly

hatched turtles but is absorbed into the abdomen during the nest escape process. Because the cost of digging out from a nest is potentially expensive, females have to allocate energy to offspring beyond that required for embryonic development to fuel this and other post hatching activities.

This study investigated the proportion of energy in residual yolk that is used during nest escape in the Brisbane river turtle *E. m. signata* by comparing the size and energy content of residual yolk in newly hatched hatchlings to that of hatchlings that have dug their way through a column of sand similar in depth to that of natural nests. We hypothesized that the fuel for nest escape comes from the residual yolk and as a consequence the mass of residual yolk is reduced during nest escape. A second aim was to explore the effects of clutch size on an individual's energetic cost of nest escape. Because of the number of eggs laid in a clutch of the Brisbane river turtle can vary between 9 and 25 (Booth 1999), we hypothesized that the greater the number of hatchlings in a nest, the greater the effect of the benefits of social facilitation would be, so that the individual energetic cost of nest escape would be reduced in larger clutches. This hypothesis was tested by comparing the time required to escape the nest and combined metabolic cost of nest escape across different sized groups of digging hatchlings. The combined metabolic cost from each group size was divided by the number of hatchlings in each group to determine the average individual energetic cost of nest escape.

Materials and methods

Obtaining hatchlings

Ten gravid Brisbane river turtles (*E. m. signata*) were captured from The University of Queensland (St. Lucia Campus) Lakes during December 2013 and induced to lay eggs by intramuscular inject of synthetic oxytocin (activity = 10 iu ml^{-1}) at a dose of 2 ml kg^{-1}. Females were then placed in a plastic bin container (80 cm × 30 cm × 40 cm) in 10 cm depth water. Once eggs were laid, they were immediately removed from the water and labelled with a pencil before buried in moist river sand for incubation. Females were returned to the lakes after oviposition. Eggs were incubated at a constant temperature of 28 °C, but some clutches were initially incubated for 1 to 2 weeks at 24 °C to ensure that different clutches hatch at different times. The newly hatched hatchlings were weighed and marked prior to experiments and individuals marked by notching peripheral scutes with a nail clipper.

Measuring energy expenditure during nest escape

The newly hatched turtles (6–8 h after hatching) were buried under a column of fine moist sand in a clear perspex cylindrical respiratory chamber 2.85 cm in radius and 30 cm in height.

The cylinder was placed vertically as hatchlings naturally dig upward to escape from their nest. Because the total number of hatchlings used in each digging out trial differed, the depth of sand from the uppermost hatchling to the surface was standardized to 15 cm to ensure that hatchlings dug through the same volume of sand to reach the surface. Open-flow respirometry was used to measure the rate of carbon dioxide production (V_{CO_2}, ml h^{-1}) throughout the experiment. Outside air was pumped sequentially through a series of absorbent tubes (soda lime and drierite; to scrub CO_2 and water vapour, respectively) and a mass flow controller (OMEGA, FMA5400/5500) regulated at an air flow of 100 ml min^{-1}. The dry CO_2-free air was supplied through the base of the respirometry chamber containing a group of hatchlings. The outflow air from the top end of the chamber was then directed through another drying column of drierite before entering a CO_2 analyser (PP Systems, SBA-5). The voltage output of the CO_2 analyser was connected to a computer via an analogue/digital converter (ADInstrumens, PowerLab 4/30). The ADInstruments Lab Chart 7 data acquisition software was used to sample the voltage output every 30 s. The CO_2 analyser was calibrated every 3-h with CO_2 free air and a precision CO_2 gas mixture. The CO_2 production was calculated using equation 10.5 of Lighton (2008) by using a respiratory quotient of 0.72 because lipid was assumed to the substrate metabolized during respiration. To record hatchling activity and their emergence time, two webcams, on opposite sides of the respirometry chamber, were utilized so that hatchlings could be observed whenever they were near the clear wall of the respirometer. To minimize observer bias, blinded methods were used when all behavioural data were recorded and analysed. Once individuals reached the surface, hatchling/s were removed from the chamber immediately and weighed. All experiments were performed in a 28 °C constant temperature room with 24-h lights so that webcam imagery could be obtained continuously.

Three respirometry chambers were used simultaneously with one chamber consisting of sand with no hatchlings (hereafter known as 'blank chamber') to measure background microbial carbon dioxide production, while the remaining two chambers contained hatchlings. Carbon dioxide production measurements were recorded for 10 min at a time in each chamber in sequence via a series of solenoid valves that were controlled through the "event manager" module in Chart 7 software. When swapping from one chamber to another, it took 3 min for the gas to flush completely through the system so that only the last 7 min of V_{CO_2} measurement in a 10-min cycle was used for that measurement period. Carbon dioxide production of hatchlings was then calculated by subtracting the background microbial V_{CO_2} from the raw chamber V_{CO_2}. Total energy expended by all hatchlings during the digging out process was calculated by first integrating the area under the V_{CO_2} versus time curve and converting this to units of

energy by assuming a respiratory quotient of 0.72 and a CO_2 calorific equivalent of 1 ml $CO_2 = 25.6$ J (Withers 1992). The rate of digging upward (m h^{-1}) was calculated by dividing the distance dug (0.15 m) by the time required to reach the surface (h). The total energetic cost per individuals was calculated by dividing the energetic cost for the entire group by the number of individuals within the group. In the cases of hatchlings reaching the surface at different times, each individuals was taken out immediately once on the surface, and the calculation was adjusted using the remaining number of individuals still in the respirometer. Therefore, hatchlings that reached the surface at different times would have different total individual energetic cost as shown in Table 1.

Calculating residual yolk energy utilization

The total energy within residual yolk and the yolk-free carcass of hatchlings were determined. Hatchlings were divided into two groups, newly hatched (NH) and post digging (PD). NH hatchlings were collected and euthanized immediately after hatching from the egg, and the PD hatchlings were euthanized after they had gone through a digging trial as described in metabolic rate measurement section. Each hatchling was weighed to 0.1 mg before being euthanized by cooling to 3 °C and then freezing. Hatchlings were dissected while still frozen to separate the residual yolk from the hatchlings' body, and these components weighed separately to 0.001 mg. Both samples were dried to constant mass using a freeze drier. Dried samples of residual yolk were homogenized using a mortar and pestle, and the yolk-free carcasses were ground to a homogenous powder using a coffee grinder.

The energy density of dried samples of yolk and yolk-free carcasses was determined using ballistic bomb calorimetry. Triplicate sub-samples (0.1–0.2 g) of residual yolk and yolk-free carcasses of individuals were transferred to a metal thimble and fully combusted in 20 atm of oxygen within a ballistic bomb calorimeter (Gallenkamp auto bomb, England) to determine their energy density. The calorimeter was calibrated with thermochemical standard benzoic acid (26.442 J g^{-1} Bureau of Analysed Standards Ltd, Middlesbrough, UK) periodically throughout these analyses. Energy density is reported on a dry mass basis that includes the ash component. The energy used during the nest escaping process was calculated by subtracting the total energy in the residual yolk of PD hatchlings from NH hatchlings.

Statistical analysis

Spearman's correlation was used to explore the relationship between the group size and digging duration, while Pearson's correlation has been used to investigate correlation between duration of the digging out process (independent variable) with energy expenditure and mass loss of hatchlings.

Table 1 Example from Clutch 8 showing how total individual energetic cost of digging out was calculated. First total CO_2 production was calculated and then this value was converted to joules

Elapsed time (h)	Remaining hatchlings (n)	Total CO_2 produced (ml)	Total CO_2 produced adjusted per individual (ml)	Total CO_2 produced to reach surface per individual (ml)	Energetic cost of reaching surface per individual (kJ)
0.00–33.5	10	263.38	26.34	26.34	0.674
33.6–51.7	7	159.44	22.78	49.12	1.257
51.8–56.8	4	14.89	3.72	52.84	1.353
Clutch average cost per individual				42.57	1.094

ANOVA or ANCOVA with hatchlings mass upon hatching as the covariate were used to explore effects on the dependent variables hatchling components (yolk-free carcass and residual yolk), energy density and fractional water content (%). The clutch was included as a random factor in ANOVA and ANCOVA. Statistical significance was assumed if $p \leq 0.05$.

Results

Observations of digging activity

The digging behaviour of hatchlings could be monitored when individuals were digging near the respirometry chambers' wall. At the beginning of the experiment, newly hatched (6–8 h after hatching) turtles were placed horizontally plastron down and stacked one on top of another on sand near the bottom of the chamber, and 15 cm of sand placed on top of the topmost hatchlings. When digging began, hatchlings moved their head upwards until their body was turned vertically (~90° from their starting position) and they retained this orientation throughout the digging process (see supplementary video). Most of the time, both front and rear feet were used in intermittent asynchronous movements. Hatchlings used the ventral surface of their front feet to scratch for short periods (see supplementary video) to scratch down the sand ceiling. Meanwhile, the rear feet were involved in two distinct movements (i) stomping their feet in a downward motion to compact the sand under them and (ii) to push their body upward by stretching the hind limbs against the floor of the cavity they were in. While they stretched their limbs in this manner, their head was moved from side to side creating space within the sand column. The head was regularly seen to rest in this space. During these resting periods, hatchlings remained still, their limbs and neck motionless. Even though hatchlings were placed in the respirometry chambers at the same time, after they started to dig, hatchlings formed discrete groups containing several individuals while moving upward. The formation of these discrete groups was clearly established by the time they reached half-way to the surface. As a consequence of forming discrete groups, synchronous digging activities only occurred between individuals within the same group. On reaching the surface hatchlings rapidly broke through and moved continuously on top of the sand surface until they were removed from the chamber which in these trials occurred within 5 min of them surfacing.

Digging duration

In general, intermittent digging activities were characterized by two to three strokes of front limb digging (mean ± SD 5.4 ± 1.3 s, $n = 52$) and followed by a period of rest (35.8 ± 9.7 s, $n = 52$). The nature of this digging behaviour was not consistent amongst trials with some hatchlings digging almost continuously, while in others, the resting period lasted 6–15 min. Hatchlings took between 12.2 and 162.8 h to dig up through the 15 cm of sand (Table 2). In most trials, the period between first emergence and the last emergence to the surface was less than 12 h, and in only one trial (Clutch no. 7) was this time interval greater than 24 h.

An average digging duration per clutch was used to investigate the relationship with clutch size. Spearman's correlation analysis found a negative correlation between clutch size and digging duration with larger groups having shorter digging durations (Fig. 1a).

Because hatchlings formed discrete groups that were separate from each other during the digging out process, and at any moment in time the activity of these groups was asynchronous (i.e. one group could be resting and another group actively digging), it was not possible to determine a "resting" V_{CO_2} and an actively digging V_{CO_2} as was originally planned. For this reason, only the average V_{CO_2} over the entire digging out trial (Fig. 2) was calculated and these values converted to energy units.

Energy expenditure during nest escape

The total energy expenditure obtained from the respirometry method throughout the digging out process varied between 0.34 and 2.32 kJ per individual and was dependent on clutch

Table 2 Time taken for the fastest, slowest and average individual to dig upwards through 15 cm of moist sand and average digging rate during this process

Clutch no.	Clutch size (n)	Fastest individual (h)	Slowest individual (h)	Difference between fastest and slowest (h)	Average digging duration ± SE (h)	Average digging rate (mm h^{-1})
1	10	37.00	49.77	12.8	41.5 ± 1.4	3.62
2	14	39.94	49.94	10.0	47.7 ± 1.0	3.14
3	11	12.20	12.20	0.0	12.2 ± 0.0	12.30
4	6	57.18	74.00	16.8	67.7 ± 3.3	2.22
5	6	49.67	55.67	6.0	50.7 ± 1.0	2.96
6	12	28.75	31.00	2.3	30.6 ± 0.3	4.90
7	4	112.25	162.77	50.5	137.5 ± 14.6	1.10
8	10	33.17	56.83	23.7	48.2 ± 3.3	3.11
9	4	59.33	59.67	0.3	59.5 ± 0.1	2.52
10	10	33.83	34.22	0.4	34.1 ± 0.1	4.40

size (Fig. 1b). The duration of the digging out process was positively correlated with energy expenditure (Fig. 1c). However, there was no significant influence of hatchling mass on the total energy expenditure (ANOVA $F_{1, 9} = 0.063$, $p > 0.05$). Hence, these data show that higher energetic cost can be attributed to the longer time spent in the nest digging as opposed to differences in body mass.

Mass loss during the nest escapes process

Hatchlings lost less than 10 % of their initial mass during the nest emergence period (mean ± SD 5.2 ± 0.3), and there was a positive correlation between digging duration and mass lost during this digging process (Fig. 1d).

Residual yolk utilization

Wet mass of residual yolk from NH and PD hatchlings was compared by ANCOVA in which the whole body initial mass of hatchlings was the covariate. Newly hatched hatchlings had larger residual yolks compared to post digging hatchlings (ANCOVA, $F_{1, 36} = 7.656$, $p = 0.009$, Fig. 3).

Water fraction within residual yolk (49.0 ± 12.2 %) and yolk-free carcass (72.5 ± 1.7 %) was similar in newly hatched and post digging hatchlings (yolk-free carcass ANOVA, $F_{2, 36} = 0.144$, $p = 0.707$; residual yolk ANOVA, $F_{2, 36} = 0.349$, $p = 0.559$). However, yolk-free carcass water fraction was independent of body mass, while the water fraction of residual yolk increased with hatchling wet mass (Fig. 4).

There were no differences in energy density of yolk-free carcasses (ANOVA, $F_{2, 18} = 1.618$, $p = 0.212$) or residual yolks (ANOVA, $F_{2, 18} = 0.026$, $p = 0.873$) between newly hatched and post digging hatchlings (Table 3). However, there was a difference (ANOVA, $F_{2, 72} = 175.817$, $p < 0.05$) in the energy density of residual yolk and yolk-free carcass. Dry mass of yolk-free carcass was similar in newly hatched and post digging hatchlings (ANCOVA, $F_{1, 36} = 3.72$, $p = 0.062$), while

residual yolk dry mass was greater in newly hatched compared to post digging hatchlings (ANCOVA, $F_{1, 36} = 7.791$, $p = 0.009$). There was no difference in the calculated total energy in the yolk-free carcass (ANCOVA, $F_{1, 36} = 3.72$, $p = 0.062$); conversely, there was a significant difference in residual yolk (ANCOVA, $F_{1, 36} = 7.69$, $p = 0.009$) between NH and PD hatchling groups. Further, the PD group was not classified according to the number of hatchlings involved in digging trials as no significant difference were found (ANCOVA, $F_{2, 18} = 1.106$, $p = 0.358$); hence, data were pooled and represented by average value. Therefore, by assuming that hatchlings only relied on their residual yolk to fuel their nest escape, an average of 3.22 kJ (50 % of residual yolk energy) of energy was used during the nest escape process (Table 3).

Discussion

Digging activity

Hatchlings did not dig continuously; relatively short bouts of digging were separated by relative long breaks of inactivity. According to Seymour (1973), there are two advantages of intermittent digging: (i) this process is more economic in terms of energy usage and (ii) keeping bouts of intense activity which are presumably powered in part by anaerobic metabolism, to short bursts of digging prevents the build-up of a larger oxygen debt, and the inactive period allows sufficient time to pay back the oxygen debt before the next digging bout begins. In this study, both small and large groups of hatchlings used the intermittent digging strategy, but there was a difference in terms of time needed to dig the 15 cm to the surface. It appeared that the smaller groups spent a longer time resting between digging bouts so they may have accrued a larger oxygen debt during the digging bout compared to larger groups. This larger oxygen debt might result from more
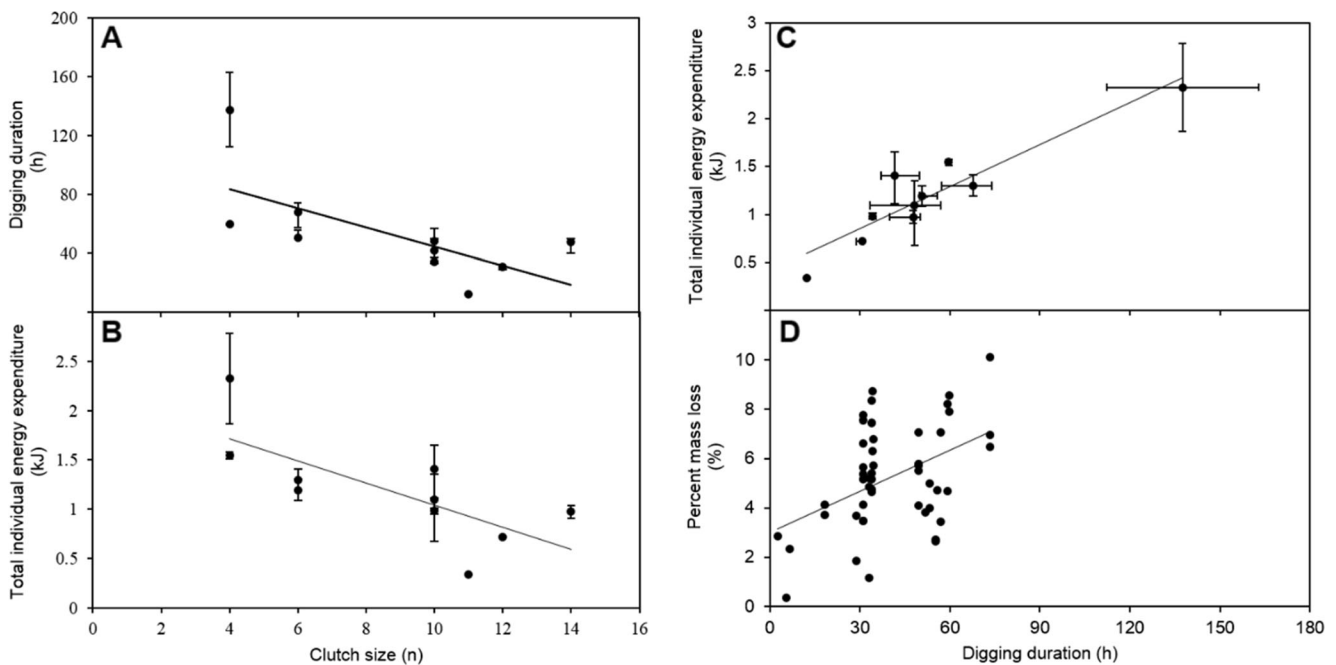
Fig. 1 a Relationship between mean digging duration and clutch size ($y = -6.499x + 109.51$, $r^2 = 0.45$, $p < 0.001$, $n = 10$). *Vertical bars* = range of digging times amongst individuals from within a clutch. **b** Relationship between total individual energy expenditure during the digging out process and clutch size ($y = -0.112x + 2.162$, $r^2 = 0.54$, $p < 0.001$, $n = 10$). *Vertical bars* = range of total individual energetic expenditure amongst individuals from within a clutch. **c** Relationship between digging duration (hours) and the energetic cost (kJ) of nest escape ($y = 0.015 + 0.41$, $r^2 = 0.86$, $p < 0.001$, $n = 10$). *Vertical bars* = range of total individual energetic expenditure amongst individuals from within a clutch, while *horizontal bars* = range of digging duration per clutch. **d** The relationship between a hatchlings' digging duration (h) and mass loss (%) from four clutches ($y = 0.056x + 2.99$, $r^2 = 0.20$, $p < 0.001$, $n = 49$)

intense digging as the digging work is shared across fewer individuals. The net effect is that it takes larger groups a shorter time to dig out of a nest not because they dig faster, but because they have shorter rest periods between bouts of digging.

Presumably, the reason why turtle hatchlings do not dig continuously during nest escape is that the muscles fatigue due to the accumulation of lactic acid. Compared to mammal and bird species, reptiles have a lower aerobic capacity because of the limitation in respiratory and cardiovascular systems

(Schmidt-Nielsen 1997). In particular, chelonian hatchlings would accumulate lactate in blood during periods of intense activity (Dial 1987; Baldwin et al. 1989; Hamann et al. 2007; Pereira et al. 2012), but blood lactate was not measured in the present study. Our data suggest that the bigger group size most probably required less resting time because they accumulated less lactate in their blood during digging bouts. This hypothesis needs to be experimentally tested in the future.

Webcam recordings showed that the digging movement of one hatchling typically triggered the start of digging in other

Fig. 2 An example of an individual CO_2 production from Clutch 8 throughout the entire digging trial. The total energy consumption was calculated by integrating the area under the rate of CO_2 production versus time curve before convert to units of energy (J) as described in the 'Materials and methods' section. *Letters* in the middle of each integrated area represents the total CO_2 produced adjusted per individual within that time frame ($A = 26.34$ ml, $B = 22.78$ ml, $C = 3.72$ ml)
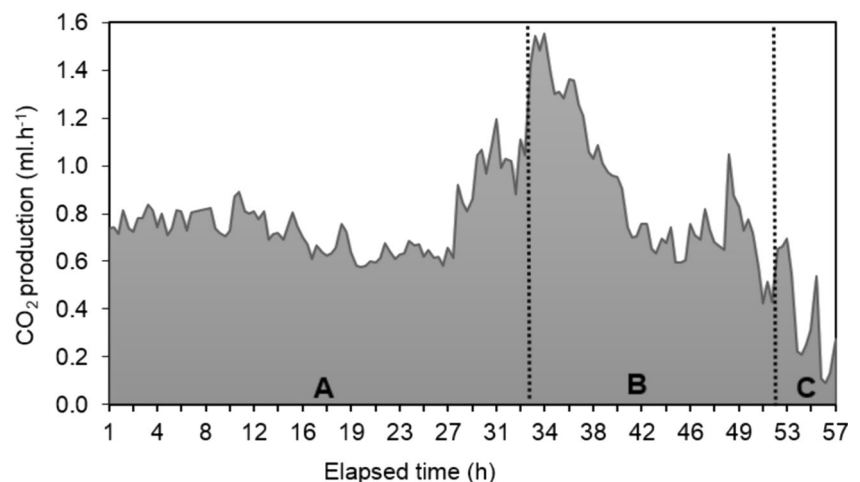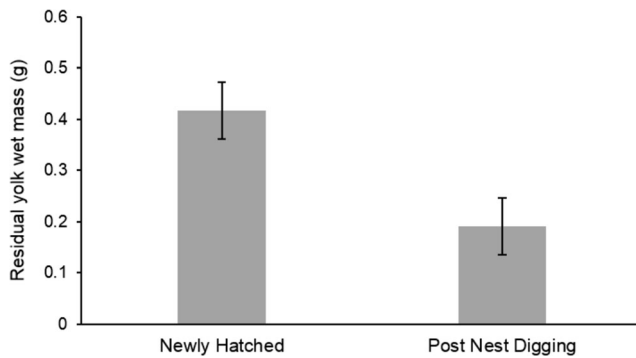
Fig. 3 Comparison of least square means residual yolk wet mass between the newly emerged and post-nest digging hatchling Brisbane river turtles. *Error bars* represent standard error

adjacent hatchlings so that several hatchlings dug simultaneously during a digging bout. The same behaviour has been reported for sea turtle hatchlings during nest escape (Mrosovsky 1968; Moran et al. 1999). The lead hatchlings that triggered the activity were not necessarily positioned on the top of their group. The group response could also be initiated by hatchlings in the middle or on the edge of their group. However, the cue used by a hatchling to start a digging bout is unclear but may be related to the fall of blood lactate concentration to below a threshold level.

The emergence pattern in terms of the number of hatchlings reaching the surface almost simultaneously varied amongst trials and was not correlated with clutch size. Hatchlings generally emerged onto the surface together in a few separate small groups, and typically all hatchlings within a clutch emerged within 12 h of the first individual emerging (Table 2). This indicates that hatchlings moved up through the sands column together in the general close proximity of each other.

Typically, chelonian hatchlings tend to emerge from the nest synchronously, and it has been hypothesized that this serves to swamp predators (Bustard 1972) and thus decrease

the probability of an individual being predated (Dehn 1990). However, the present study reveals that there are two other advantages to digging out of the nest in a group synchronously. Larger groups took less time to dig out of the nest (Fig. 1a) and also expended less energy per individual while escaping the nest (Fig. 1b). A previous study on *E. macquarii* also found that groups of 10 hatchlings escaped the nest faster compared to single hatchlings (Spencer et al. 2001). Given that in respirometry analysis hatchlings spent between 0.34 and 2.32 kJ per individual (Fig. 1b) to escape, this constitute approximately 5.3–36 % from their residual yolk reserved energy (as calculated in Table 3). From these findings, it can be inferred that hatchlings emerging from nests of a larger clutch size have more energy reserves to enter the next stage of their life cycle compared to hatchlings emerging from nests with small clutch size.

Social facilitation

The individual hatchling energy expenditure obtained in this study was calculated as an average using the numbers of hatchlings within in a clutch. Theoretically, in moving forward as a bunch against a resistance such as soil, the leading edge animals would spend more energy than other group members (Fish 1995). This strategy is used in human bicycle races in which pacemaker riders often sacrifice their energy in order to conserve the energy of the following riders (Trenchard et al. 2015). Similarly, northern bald ibis (*Geronticus eremita*) were found to share the workload of being the lead bird in a flying formation by swapping positions while flying in echelon formation (Voelkl et al. 2015). In the current experiment, the leading edge individual(s) was not identifiable, but it would be interesting to see if this position was maintained by the same individual throughout nest escape or swapped around between different individuals to share the workload.

Fig. 4 Relationships between percent water content of residual yolk and yolk-free carcass and hatchling wet mass. *Regression lines* represent relationship between residual yolk percent water content and hatchling mass in newly hatched hatchlings (NH) ($y = 6.409x + 25.666$, $r^2 = 0.584$, $p < 0.001$, $n = 18$) and post digging hatchlings (PD) ($y = 5.489x + 29.284$, $r^2 = 0.820$, $p < 0.001$, $n = 18$). Yolk-free water content was not correlated with hatchling mass ($r^2 = 0.007$, $p = 0.62$, $n = 36$)
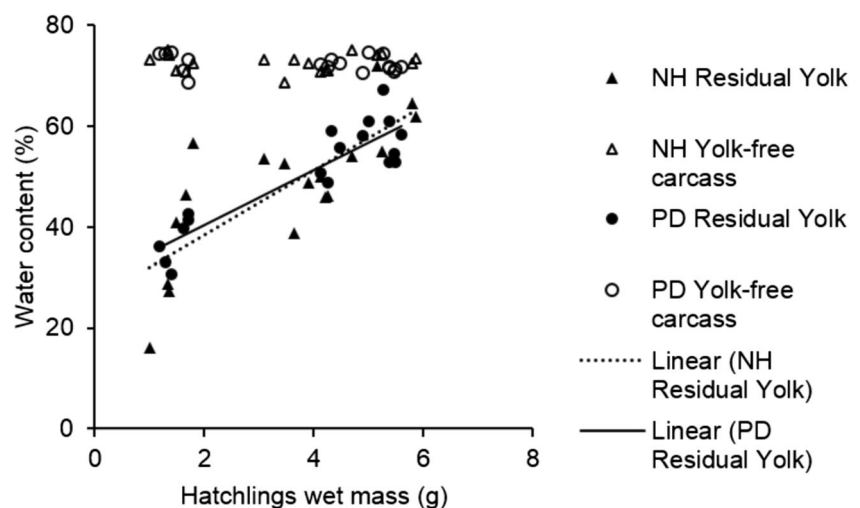
Table 3 Total energy contained within hatchlings components based on energy density (calculated on a dry mass basis including ash) and dry mass. Data are mean ± SE (energy density) and least square ANCOVA adjusted means for a hatchling mass of 5.21 g

Group	N	Energy density (kJ g^{-1})	Dry mass (g)	Calculated total energy (kJ)
Yolk-free carcass				
Newly hatched	18	22.74 ± 0.30	1.183 ± 0.02	26.90
Post digging	18	23.23 ± 0.24	1.239 ± 0.02	28.78
Residual yolk				
Newly hatched	18	31.81 ± 0.57	0.203 ± 0.03	6.46
Post digging	18	32.02 ± 1.18	0.101 ± 0.03	3.23
Energy used during digging				3.23

Previous studies have claimed the reduction of an individual's total energetic cost in subterranean animals moving through sand when they shared the workload of burrowing between several individuals (Hansell 1993; Ebensperger and Bozinovic 2000). One of the most remarkable examples occurs in naked mole rats (*Heterocephalus glaber*) which form a chain of diggers while constructing burrows. *Heterocephalus* often live in colonies of more than 40 individuals huddling behaviour while building tunnels reduces an individual's metabolic cost (Withers and Jarvis 1980; Lovegrove 1989). In chelonian, social behaviour begins earlier in the subterranean nest when synchronous hatching occurs amongst sibling eggs. Remarkably, despite thermal gradients in nests causing differences in embryonic development, embryo use multiple cues, namely vocalizations, physical disturbance, hypoxia and temperature change to induce synchronous hatching (Spencer et al. 2001; reviewed in Doody 2011). It appears that this synchronous hatching behaviour could facilitate communal digging and ease digging effort amongst siblings out from the nest.

E. m. signata typically construct their nest within 2–10 m of the water's edge in either soil or clay medium (Booth 2010). As egg incubation usually takes 2–3 months (Booth 1998b), nesting soil would become compacted during this period. As shown by Horrocks and Scott (1991), hawksbill sea turtle (*Eretmochelys imbricata*) hatchlings had a decreased emergence success with an increase in soil compaction. Hence, another plausible explanation for synchronous hatching is that it may facilitate digging through compacted soil. An experimental approach is needed to provide more definitive information about hatchling energetic cost and fitness consequences of female nest site selection with respect to soil type and compactness.

The current study found that it is an advantage having a larger group in the nest as it decreases the energetic cost of digging out of the nest for individuals, suggesting that females should attempt to maximize their clutch size during a reproductive bout. On the other hand, it is thought that larger offspring are generally fitter than smaller offspring (Smith and Fretwell 1974). Hence, there may be a trade-off between clutch size and hatchlings size if a female has a fixed amount of resources to allocate to reproduction, with many smaller individuals saving on the energy needed to dig out of a nest, but smaller individuals having lower fitness once they leave the nest. This aspect warrants further investigation because physically larger individuals may be more efficient diggers than smaller individuals, so data on relative digging efficiencies in terms of energy expenditure over a range of hatchling sizes is needed. Freshwater turtles would make good models to study this question because the same populations of breeding females can produce a broad range of egg and clutch sizes (Booth 1998a).

While having a larger group might be energetically beneficially for chelonian hatchlings during nest escape, asynchronous nest emergence is widespread amongst chelonian species (Witherington et al. 1990; Hays et al. 1992; Houghton and Hays 2001). Asynchronous nest emergence is thought to be due to asynchronous hatching caused by thermal gradients within nests, which are more likely to affect shallow nesters (i.e. freshwater species compared to the sea turtles). For example, temperature differences in the Muray river turtle, *Emydura macquarii*, nests were found to reach 6 °C between top and bottom eggs (Thompson 1989). Accordingly, Hays et al. (1992) proposed that early hatched hatchlings may exhibit a 'waiting period' in order to take advantage from synchronous digging effort, but this waiting also incurs an energetic cost in the form of maintenance metabolism during the waiting period. An estimate of the energetic cost of the dig alone as soon as you hatch strategy compared to the wait and dig together with your clutch mate strategy can be made. A hatchling digging alone would consume approximately 2.05 kJ, while a hatchling in a group of 14 would consume just 0.59 kJ (Fig. 1b), a difference of 1.46 kJ. A full-term Brisbane river turtle embryo consumes approximately 0.2 kJ per day (calculated from data in Booth 1998b), so an embryo could wait 7.3 days until its clutch mates also hatched and spend the same amount of energy in nest escape. Hence, if the wait period is less than 7.3 days, from an energy expenditure point of view, a hatchling is better off waiting for its clutch mates before commencing the nest escape process.

The utilization of residual yolk

Mass was lost from hatchlings when digging out of the nest, and this loss was greater when the digging out period was

longer (Fig. 1d). Bennett et al. (1986) explain that mass loss in chelonian hatchlings can be attributed to the utilization of yolk and to water loss. Thus, water loss could possibly be a major contributor to the mass loss. However, in the current study, there was no change in hydration state between the newly hatched and the post-emergence groups, but there was a decrease in dry yolk matter (0.14 g) between newly hatched and post digging hatchlings indicating that the observed mass loss was most likely caused by yolk metabolism with mass loss due to respiratory gas exchange and loss of metabolically produced water.

Despite the fact that a significant amount of residual yolk was metabolized while digging, the chemical constituents of this yolk did not appear to change significantly as indicated by no change in its energy density (Kraemer and Bennett 1981; Booth 2003). The difference in energy density between residual yolk and yolk-free carcass can be explained by differences in chemical composition between these two components. Previously, it was postulated that residual yolk has a higher proportion of energy-rich lipid compared to yolk-free carcass, and that the inorganic mineral component which has no combustible energy content is greater in the yolk-free carcass because of the skeleton (Speake and Thompson 2000; Speake et al. 2003).

In the context of the reproductive energy allocations of this species, Booth (2003) estimated that the amount of energy in a fresh egg is 4.17 kJ g^{-1}. Thus, for a typical 8.0 g egg (size range 5.0–10.6 g), egg energy content would be 33.4 kJ. While according to Booth (1998b), the total energy expended during embryo development is 11.52 kJ (~35 % of initial energy content), and in current study, we found that the digging process consumes 3.23 kJ (approximately 10 % of initial energy content and approximately 50 % of the energy remaining in the residual yolk at hatching). Thus, hatchlings have 55 % of the initial egg energy content for post-nest activities such as predator avoidance, foraging and early growth.

Conclusions

The estimated energetic cost of individuals by digging for *E. m. signata* hatchlings are 0.34–2.32 kJ per individual depending upon the number of hatchlings digging together. Although their energetic cost consumed at least 50 % of the remaining energy in the residual yolk, the amount of energy consumed is reduced by synchronous digging of many individuals during nest escape. The present study concludes that the clutch size and the time spent digging within the nest column are important determinants of the energetic cost while digging out of the nest. Future study should be focused on higher resolution data to estimate the individual net cost of transport (NCOT) so that this can be compared the energetic cost of nest escape of other chelonian species with that vary greatly in clutch size.

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