

## RESEARCH ARTICLE

# The effects of extended crawling on the physiology and swim performance of loggerhead and green sea turtle hatchlings

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## ABSTRACT

Following emergence from the nest, sea turtle hatchling dispersal can be disrupted by artificial lights or skyglow from urban areas. Misorientation or disorientation may increase exposure to predation, thermal stress and dehydration, and consume valuable energy, thus decreasing the likelihood of survival. In this study hatchlings were run on a treadmill for 200 or 500 m to investigate the physiological impacts of disorientation crawling in loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtle hatchlings. Oxygen consumption, lactate production and blood glucose levels were determined, and swim performance was measured over 2 h following crawls. Crawl distances were also determined for hatchlings that disoriented on the Boca Raton beach in Florida, with plasma lactate and blood glucose sampled for both properly oriented and disoriented hatchlings. Green and loggerhead hatchlings rested for 8–12% and 22–25% of crawl time, respectively, both in the laboratory and when disoriented on the beach, which was significantly longer than the time spent resting in non-disoriented turtles. As a result of these rest periods, the extended crawl distances had little effect on oxygen consumption, blood glucose or plasma lactate levels. Swim performance over 2 h following the crawls also changed little compared with controls. Plasma lactate concentrations were significantly higher in hatchlings sampled in the field, but did not correlate with crawl distance. The greatest immediate impact of extended crawling as a result of disorientation events is likely to be the significantly greater period of time spent on the beach and thus exposure to predation.

**KEY WORDS:** Disorientation, Frenzy crawl, Oxygen consumption, Energetics, *Caretta caretta*, *Chelonia mydas*

## INTRODUCTION

For sea turtle hatchlings, the first 24 h following nest emergence, called the frenzy period, is a time of high activity, characterized by a scramble across the beach to reach the surf (Wyneken and Salmon, 1992) and then a long swim to offshore developmental habitats (Carr, 1986; Smith and Salmon, 2009). This frenzy period moves hatchlings quickly through the nearshore waters where there is a predation risk from fishes and small sharks (Stancyk, 1982; Stewart and Wyneken, 2004; Salmon et al., 2009); hatchlings are also vulnerable to predation by birds and terrestrial animals such as raccoons and ghost crabs as they cross the beach (Tomillo et al., 2010; Peterson et al., 2013). Hatchling emergence *en masse* during the cover of night and the 24 h frenzy period are thus thought to be

evolutionary adaptations that increase the survival rate of these animals during the crucial first day of life (Dial, 1987; Wyneken and Salmon, 1992; Santos et al., 2016).

Along with natural threats, the commercial development of nesting beaches for human habitation also has an impact on turtles through poaching, beach use and habitat destruction such as the building of seawalls (Witherington et al., 2011); along the Atlantic coast of the United States, the highest density of nesting sea turtles are often on beaches altered for human use (Weishampel et al., 2003; Antworth et al., 2006). One result of this residential and commercial development is an increase in the amount of artificial lighting on sea turtle nesting beaches that competes with the natural light by which hatchlings navigate the beach upon emergence (Tuxbury and Salmon, 2005). Under natural conditions, hatchlings move towards the brighter horizon of the open ocean and away from the dark silhouettes of sand dunes and beach vegetation that may surround a nest (Fritz et al., 1992; Godfrey and Barreto, 1995; Fuentes-Farias et al., 2011), but they become misdirected or confused by artificial sources of light (Witherington and Bjorndal, 1991; Tuxbury and Salmon, 2005; Lorne and Salmon, 2007). This misdirection of a hatchling towards an artificial point source of light, such as a street light, is termed misorientation. In urban areas hatchlings are also disoriented by skyglow, causing them to wander on the beach without direction; this is termed disorientation (Sella et al., 2006). Misorientation or disorientation can cause hatchlings to travel inland where they face a host of hazards such as roadway automobile traffic and predators (McFarlane, 1963; Tomillo et al., 2010). Over 64,000 sea turtle hatchlings were disoriented in Florida in 2007 for example (Shudes, 2011), although the City of Boca Raton reports that over 50% of disoriented hatchlings eventually make it to the water (K. Rusenko, Boca Raton Turtle Conservation and Research Program, unpublished data, 2015). Disoriented hatchlings crawl longer distances, slow down, and pause more frequently (Triessnig et al., 2012). They can ultimately succumb to dehydration and exhaustion; the increased crawl distances constitute an increased exposure to terrestrial threats (McFarlane, 1963), and may deplete the hatchlings' limited energy (Hamann et al., 2007). Thus mitigation of disorientation and misorientation from light pollution has become a large component of conservation efforts for sea turtles (Witherington and Martin, 2000).

Energy use during misorientation or disorientation is of interest as the emergence and frenzy period is highly energy intensive, requiring hatchlings to dig out of the egg chamber (which can be 45–75 cm deep) (Miller and Dinkelacker, 2008), crawl from the nest to the sea, and swim offshore. These activities use both aerobic and anaerobic metabolism (Bennett, 1982; Dial, 1987), although mass-specific oxygen consumption varies greatly between activities and by species. In general, digging for nest emergence and crawling are energetically more expensive than swimming, with higher rates of both specific oxygen consumption and lactate accumulation (Dial, 1987; Wyneken and Salmon, 1992; Booth, 2009). Glucose, fatty

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acids and proteins may all be used as energy sources at various phases of hatchling dispersal (Hamann et al., 2007).

Upon reaching the water, hatchling dispersal and survival then depend heavily upon their swim performance. The mortality rate of hatchlings due to predation can be high during the first 24 h (Stewart and Wyneken, 2004), but rather than evade predators residing near the shore, hatchlings rely on speed and larger numbers for safety. Therefore, any hindrance to the swimming ability of a hatchling could greatly diminish its chance of survival. The additional energy used during increased crawl distances from misorientation or disorientation may negatively impact the efficiency and speed of the hatchling's swim offshore, as stroke rate and strength both decrease with fatigue (Booth, 2009). Extended crawl times would also cause an individual to lose the benefit of predator protection as a result of encounter dilution (from moving in large numbers), while reducing the amount of fuel available for the following frenzy swim; fuel depletes as activity duration increases (Hamann et al., 2007). To date, the effects of extended crawl distances upon the swim performance of sea turtle hatchlings have not been investigated. Although oxygen consumption in hatchlings during emergence from the nest, direct crawls to the ocean, and the following swim frenzy have been previously explored (Prange and Ackerman, 1974; Lutcavage and Lutz, 1986; Dial, 1987; Wyneken, 1997; Booth, 2009), and metabolic studies have measured the energy used for normal dispersal swimming (Hamann et al., 2007; Pereira et al., 2012), respirometry studies have not addressed the energetic cost to hatchlings of prolonged periods on land, nor have studies considered how hatchling performance during the dispersal swim may be affected by extended crawl distances. This project was thus designed to quantify some of the physiological effects of extended crawl distances such as might occur as a result of disorientation events, and measure the impact on post-crawl swim performance. To verify that the laboratory simulations on treadmills were a good proxy for actual disorientation effects, normal and disoriented hatchling behavior and physiology on the beach were also quantified. The objectives were thus to (1) characterize the energetics of extended crawl distances on hatchlings, (2) determine whether extended crawl distances have an impact on initial dispersal swim performance, and (3) compare behavior and metabolic markers in the field with those from laboratory experiments.

## MATERIALS AND METHODS

All experiments were approved by the Florida Atlantic University Institutional Animal Care and Use Committee and performed in accordance with the Florida Fish and Wildlife Conservation Commission (FWC) Marine Turtle Conservation Guidelines (FWC permit number MTP-053).

### Hatchling collection

Nests deposited between 1 May and 31 August that had incubated for 45–60 days were surveyed nightly on Boca Raton beach, FL, USA in 2013 and 2014. Hatchlings were collected upon emergence from 27 loggerhead, *Caretta caretta* (Linnaeus 1758), and 18 green turtle, *Chelonia mydas* (Linnaeus 1758), nests. Ten hatchlings were collected from each nest and transported to a laboratory on the Florida Atlantic University campus in Boca Raton. A heated pad was placed underneath the sand in a cooler to keep the hatchling turtles quiescent prior to experimentation. Hatchlings were randomly selected for control and experimental trials. Hatchling mass was recorded prior to every trial. All trials were performed the night of emergence, and the order of trials each night was randomized to distribute the times from emergence to trial start. In order to reduce the total time elapsed between emergence and

each trial to <16 h, not all trials could be run for every nest. Individual hatchlings were subjected to only one trial run (control, 200 m crawl, 500 m crawl, 2 h swim control, 200 m crawl +2 h swim, or 500 m crawl +2 h swim).

### Oxygen consumption

To determine resting oxygen consumption ( $\dot{V}_{O_2}$ ), lactate and plasma glucose levels, control subjects were placed in a dark 1 liter jar for 1 h, including a 30 min period for acclimation.  $\dot{V}_{O_2}$  was determined by closed circuit respirometry for the second 30 min period and a blood sample was drawn at the end of the hour. Air flow through the chamber was controlled using a FOX oxygen analyser (version 1.01, Sable Systems International, Las Vegas, NV, USA) at 50–75 ml min<sup>-1</sup>, through Drierite® to absorb moisture and soda lime to absorb CO<sub>2</sub>. A Qubit Systems S102 oxygen sensor calibrated with 99.99% pure nitrogen (Air Gas, Miami, FL, USA) and room air was used to measure the level of oxygen to 0.01%. Per cent oxygen was monitored continuously and  $\dot{V}_{O_2}$  was calculated from oxygen values at the beginning and end of each trial. Negative controls (no turtle) were run to account for any oxygen consumption from bacteria and to ensure that the chamber was airtight.

### Frenzy crawl

Experimental crawl subjects were placed on a treadmill enclosed in an airtight (11.680-liter) respirometry chamber (Williams, 2012) to crawl for 200 or 500 m, simulating likely distances for disorientation on the beach. A dim lamp at the east end of the treadmill in an otherwise darkened room provided the visual orientation cue for hatchling crawling; the other three sides of the chamber were blacked out. Hatchlings were observed continuously through the clear lid of the chamber. The treadmill motor and controls were placed outside the box to prevent heating of the respiratory chamber and were connected via an airtight marine gasket. The treadmill operated at a speed of 2.4 m min<sup>-1</sup> (Williams, 2012). Each turtle was observed through the top lid of the chamber for the entire length of the experimental trial. The treadmill was switched off manually whenever the hatchling paused for rest and was restarted when the animal began to crawl again. The time spent crawling and belt rotation speed were used to calculate the distance travelled. Length and frequency of rest periods were recorded. Mean oxygen consumption for the entire experimental period was calculated from beginning and end oxygen levels as described above.

### Frenzy swimming

Swim trials were performed as in Wyneken (1997); hatchlings were harnessed to a 20 cm monofilament line attached to the center of the lid of a 26-liter sealed glass aquarium filled with 15 liters of autoclaved seawater. The harness allowed the hatchling to swim continuously in any direction without hitting any boundaries. Hatchlings swam for 2 h (the maximum allowed on our permit) immediately following either 200 m or 500 m crawls on an unenclosed treadmill otherwise identical in set-up to the experimental treadmill, or directly from resting (control swim trials). A light positioned on the east side of the swim chamber provided the stimulus for the frenzy swim. During swim trials the number of power strokes (Wyneken, 1997) and breaths were counted for 1 min at 20 min intervals for the duration of the swim. Closed circuit respirometry was used to measure  $\dot{V}_{O_2}$  as described above. Negative controls were run to account for oxygen consumption from bacteria.

At the end of each independent trial (control, crawling or swimming), a 100 µl blood sample was drawn to determine hematocrit, plasma glucose and plasma lactate levels.

### Blood sampling

Blood was drawn from the external jugular vein using sodium-heparinized 1 ml syringes with a 1 cm 27-gauge needle. Hematocrit was determined following centrifugation of a microhematocrit tube as packed cell volume/total sample volume. Blood glucose concentration was determined to the nearest mg dl<sup>-1</sup> with a standard blood glucose monitor (FreeStyle Lite, Abbott Diabetes Care). The remainder of drawn blood was centrifuged at 1398 g for 10 min. Plasma (50 µl) was stored at -20°C in 150 µl of 7% perchloric acid to inhibit lactate dehydrogenase isoenzymes until plasma lactate levels were determined via colorimetric lactate assay (procedure #735, Trinity Biotech) and spectrophotometer with UV detection (Shimadzu UV-1601).

### Field study site

Nests in Palm Beach County, Florida, which had incubated for between 45 and 60 days were surveyed nightly from July to September 2015. As hatchlings emerged, behavioral observations and physiological measurements were taken in accordance with FWC permit number MTP-15-053B. For a broader distribution of the population, a maximum of two hatchlings per nest were observed per night.

### Crawl behavior in the field

Total crawl distances were measured with a meter tape for hatchlings crawling directly to the water (control group), and for hatchlings that were disoriented (experimental group). Disoriented or misoriented animals were defined as those that crawled landward within a 180 deg arc from the nest relative to a baseline drawn parallel to the water line at the nest. Crawling and resting periods were timed and frequency of rests recorded. Plasma glucose and lactate levels were measured as described above from a blood sample taken when the hatchling either reached the water or stopped crawling for more than 5 min if away from the water. All hatchlings observed were taken to Gumbo Limbo Nature Center, Boca Raton, FL, and held until release.

### Data analysis

Statistical analyses were carried out with SigmaPlot 11.0. A Kruskal–Wallis non-parametric test was used for differences between the medians of all laboratory treatments for both species to compare oxygen consumption rates, blood glucose concentrations, and plasma lactate values. Dunn's method for pairwise *post hoc* comparison was used for comparisons of statistically significant differences between

ranked data in treatment groups. ANOVA was used for differences between the means of laboratory swim treatments for both species to compare power stroke and breath rates, and to compare field lactate and glucose measures between control and disoriented hatchlings. Linear regressions were used to test for relationships between crawl distances of control versus disoriented turtles in the field, and glucose and lactate concentrations as well as rest to crawl time ratio and glucose and lactate concentrations. A Kruskal–Wallis non-parametric test was used for differences in crawl times between control and disoriented turtles in field observations. Mann–Whitney tests were used to determine whether differences existed in total rest time between the first and last 100 m of distance during the laboratory crawl treatments.

## RESULTS

### Hatchlings

A total of 150 hatchlings were used in the laboratory studies. Loggerhead hatchlings ( $N=91$ ) averaged 18.56 g in mass and ranged from 14.65 to 23.5 g. Green turtle hatchlings ( $N=59$ ) ranged from 21.94 to 29.23 g in mass, with a mean mass of 24.92 g.

### Oxygen consumption

Median oxygen consumption was significantly higher in crawling hatchlings of both species compared with resting animals. However, there was no significant difference between 200 m and 500 m crawl distances within each species (loggerheads,  $Q=0.423$ ,  $P>0.05$ ; greens,  $Q=0.302$ ,  $P>0.05$ ) or between species within the same treatment group (200 m  $Q=0.230$ ,  $P>0.05$ ; 500 m  $Q=0.192$ ,  $P>0.05$ ) (Table 1). Rates of oxygen consumption during swimming were intermediate between resting and crawling values, thus median swimming  $\dot{V}_{O_2}$  did not differ significantly from resting or crawling in either species, nor were there any differences between hatchlings swimming for 2 h directly from resting compared with those that had crawled either distance prior to the swim trial (Fig. 1).

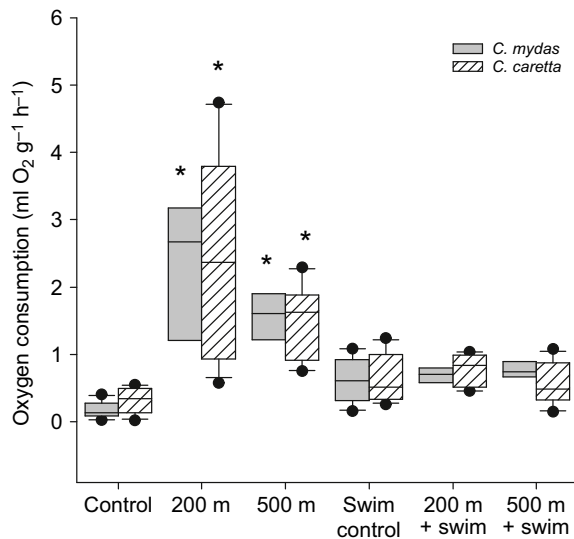
### Blood glucose concentrations

Median±median absolute deviation (MAD) plasma glucose levels did not change significantly from resting concentrations with crawling or swimming between treatment groups or species ( $H=18.862$ , d.f.=11,  $P=0.0064$ ) (Table 1), as individuals showed a high degree of variation. Although there was a trend for glucose concentrations in *C. caretta* to decrease as crawl distance increased, and an increase in plasma glucose in swimming *C. mydas* as the crawl distance prior to swim trials increased, the high variability of

**Table 1. Median±median absolute deviation (MAD) oxygen consumption rates, blood glucose and plasma lactate concentrations, and hematocrit (Hct) following laboratory treatments in *Caretta caretta* and *Chelonia mydas***

Treatment	<i>N</i>	Blood glucose (mg dl <sup>-1</sup> )	Plasma lactate (mg dl <sup>-1</sup> )	Hct (%)	Median oxygen consumption (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )
<i>C. caretta</i> , resting	22	80±8	36.11±17.00	22±4	0.34±0.17
<i>C. caretta</i> , 200 m crawl	18	67±20	35.26±10.11	24±3	2.36±1.26*
<i>C. caretta</i> , 500 m crawl	11	70±5	26.88±8.13	27±2	1.63±0.32*
<i>C. caretta</i> , 2 h swim	16	72±6	44.55±14.79	25±5	0.52±0.19
<i>C. caretta</i> , 200 m crawl, 2 h swim	11	67±7	43.93±18.93	25±3	0.84±0.18
<i>C. caretta</i> , 500 m crawl, 2 h swim	13	75±18	36.53±15.01	23±4	0.49±0.22
<i>C. mydas</i> , resting	12	91±25	18.22±9.32	28±1	0.14±0.05
<i>C. mydas</i> , 200 m crawl	13	85±27	33.43±9.13	29±3	2.67±0.50*
<i>C. mydas</i> , 500 m crawl	8	66±19	40.48±23.29	30±3	1.60±0.21*
<i>C. mydas</i> , 2 h swim	10	63±22	48.83±29.84	30±1	0.61±0.27
<i>C. mydas</i> , 200 m crawl, 2 h swim	8	100±28	17.96±17.96	25±1	0.72±0.07
<i>C. mydas</i> , 500 m crawl, 2 h swim	8	120±34	72.77±35.72	28.1±1.02	0.70±0.15

\*Significantly different from resting controls,  $P<0.05$ .



**Fig. 1. Median oxygen consumption of *Chelonia mydas* and *Caretta caretta* hatchlings was significantly higher in hatchlings of both species crawling either 200 m or 500 m compared with control turtles.** The boxes indicate median  $\pm$ 25th and 75th percentiles; error bars above and below the box indicate the 90th and 10th percentiles, respectively; outliers are represented by dots. \*Significantly different from resting controls,  $P < 0.05$  [Kruskal–Wallis ( $H=83.541$ ,  $d.f.=11$ ,  $P \leq 0.001$ ) with Dunn's test; 200 m loggerheads:  $Q=6.001$ ,  $P < 0.05$ ; greens  $Q=5.287$ ,  $P < 0.05$ ; and 500 m loggerheads:  $Q=4.995$ ,  $P < 0.05$ ; greens  $Q=4.435$ ,  $P < 0.05$ ]. Swimming did not significantly increase  $V_{O_2}$  above resting values for either species.

the data meant that neither trend was significant. However, as the length of time each animal took to crawl 200 or 500 m varied by individual, we compared glucose concentrations standardized to minutes of crawling (Fig. 2), which showed that hatchlings of both species that crawled 500 m had a significant decrease ( $H=65.047$ ,  $d.f.=5$ ,  $P \leq 0.001$ ) in plasma glucose levels compared with hatchlings that crawled 200 m (loggerheads  $Q=6.001$ ,  $P < 0.05$ ; greens  $Q=5.287$ ,  $P < 0.05$ ).

### Plasma lactate concentrations

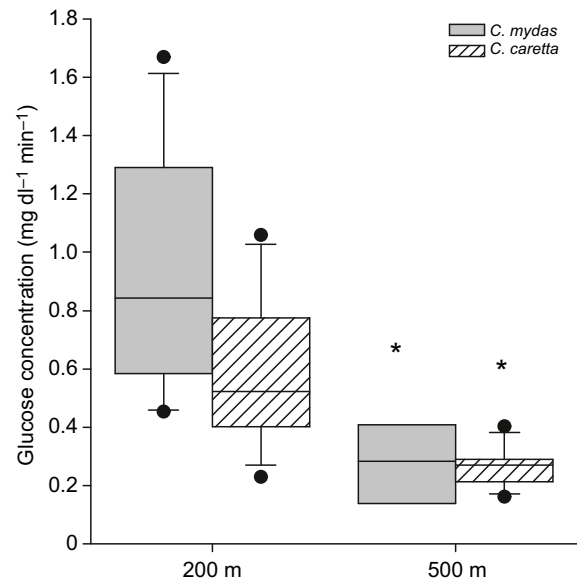
Because of a large degree of individual variability, we found no significant change in median plasma lactate concentrations between resting and post-crawl hatchlings, nor did lactate levels increase in swimming animals (Table 1). As crawl distance increased there was a trend for lactate to increase in greens and decrease in loggerheads, but this was not significant (Kruskal–Wallis  $H=10.296$ ,  $d.f.=11$ ,  $P=0.504$ ). When the data were standardized by time for the extended crawl treatments, however (Fig. 3), there were significant differences ( $H=18.457$ ,  $d.f.=3$ ,  $P \leq 0.001$ ); hatchlings of both species that crawled 500 m had significantly lower concentrations of plasma lactate than those that crawled 200 m (loggerheads  $Q=3.234$ ,  $P < 0.05$ ; greens  $Q=2.706$ ,  $P < 0.05$ ). Between species within the same treatment group there were no significant differences (200 m  $Q=0.707$ ,  $P > 0.05$ ; 500 m  $Q=0.512$ ,  $P > 0.05$ ).

### Hematocrit

Hematocrit was determined as a measure of possible dehydration during the extended crawls. Hematocrit increased slightly as distance increased, but this was not a statistically significant trend (Table 1).

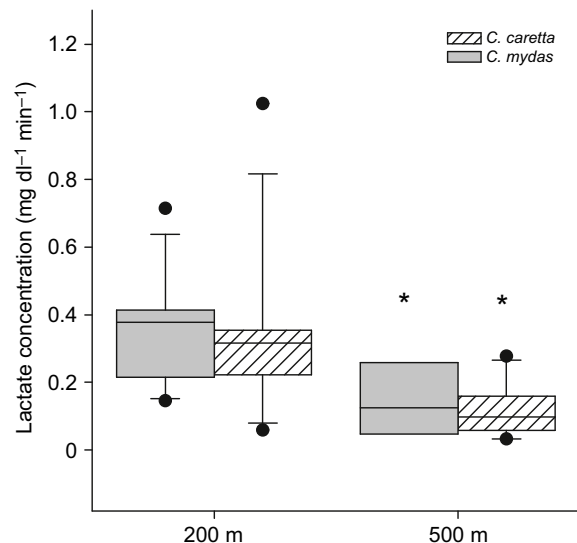
### Behavior during treadmill crawl trials

Crawl trials of 200 m took loggerhead hatchlings a median of 112.6 min total to complete, and green hatchlings a median of



**Fig. 2. Median glucose concentrations standardized to minutes of crawl time declined with crawl distance in *C. mydas* and *C. caretta* hatchlings.** Box boundaries indicate the 25th and 75th percentiles. Error bars above and below the box indicate the 90th and 10th percentiles, respectively; outliers are represented by dots. Resting control glucose concentrations not standardized for time were  $80 \pm 8$   $\text{mg dl}^{-1}$  for loggerheads and  $91 \pm 25$   $\text{mg dl}^{-1}$  for greens. \*Significant difference from 200 m treatment,  $P < 0.05$  (Kruskal–Wallis with Dunn's test).

91.3 min to complete, with mean speeds of 1.78 and 2.19  $\text{m min}^{-1}$ , respectively. Loggerheads took a median of 257.9 min to complete 500 m crawl trials and greens completed 500 m trials in 228.5 min, with mean speeds of 1.94 and 2.19  $\text{m min}^{-1}$ , respectively. Both species thus took significantly longer to crawl 500 m than 200 m, although the difference between mean total crawl times for the same distance was not statistically significant between species (200 m



**Fig. 3. Median plasma lactate concentrations standardized to minutes of crawl time declined with crawl distance in *C. mydas* and *C. caretta* hatchlings.** Box boundaries indicate the 25th and 75th percentiles. Error bars above and below the box indicate the 90th and 10th percentiles, respectively; outliers are represented by dots. \*Significant difference from 200 m treatment,  $P < 0.05$  (Kruskal–Wallis with Dunn's test).



$Q=2.487$ ,  $P>0.05$ ; 500 m  $Q=1.027$ ,  $P>0.05$ ). As the treadmill speed was constant for all trials, the differences in mean velocity were due to differences in how much each species rested during the crawl trial; total crawl times included periods of activity interspersed with periods of rest for all but one green hatchling (which crawled continuously without stopping). The fraction of time spent resting (25–28%) was a significantly greater percentage of the total crawl time for loggerheads than greens (8–9%) at both 200 and 500 m distances (Kruskal–Wallis results,  $H=26.937$ , d.f.=3,  $P\leq 0.001$ ; 200 m  $Q=4.304$ ,  $P>0.05$ ; 500 m  $Q=2.716$ ,  $P>0.05$ ), although there were no differences in the percentage of time spent resting between conspecifics in different treatment groups (loggerheads  $Q=1.353$ ,  $P>0.05$ ; greens  $Q=0.229$ ,  $P>0.05$ ). To determine whether rest period frequency or length increased from the beginning to the end of the crawl trial, we compared the last 100 m and the first 100 m of crawl trials. Loggerheads rested for significantly longer periods in the second 100 m of their 200 m crawls ( $16\pm 2$  versus  $10\pm 2\%$  of total crawl time), and greens rested for longer periods in the last 100 m of their 500 m crawls when compared with the initial 100 m ( $9\pm 2$  versus  $2\pm 1\%$  of total crawl time; Mann–Whitney rank sum tests, *C. caretta* 200 m,  $t=783.000$ ,  $P=0.007$ ; *C. mydas* 500 m,  $t=274.500$ ,  $P=0.005$ ). The number of times each animal rested varied by individual, hence there was no significant difference in mean rest frequency between treatment groups or between the first and last 100 m of other trials.

### Behavior during swim trials

Loggerhead and green hatchlings swimming for 2 h directly from resting (swim controls) had identical mean power stroke rates of 112 strokes per minute; except for greens swimming after crawling 200 m, these power stroke rates did not change significantly for either species or treatment group (Table 2). In general, swimming green control and treatment groups had higher breathing rates than control and treatment loggerheads, but the differences were not statistically significant.

### Field observations

Thirty-three total hatchlings from 13 loggerhead nests and seven green nests were followed on the beach from emergence to the shoreline, or to the dunes for misoriented/disoriented hatchlings. Out of 21 loggerhead hatchlings observed, 12 travelled directly to the waterline and were sampled as controls, and nine disoriented loggerheads were sampled as the experimental group after crawling variable distances. Twelve green hatchlings were observed: seven controls and five animals that disoriented. Fewer green than loggerhead hatchlings were observed as the nest density for green turtles is lower in Boca Raton when compared with loggerhead nest numbers, and also because in general green turtle hatchlings on this beach tend to disorient less readily.

**Table 2. Swim performance over 2 h for loggerhead and green sea turtle hatchlings from resting or following treadmill crawling**

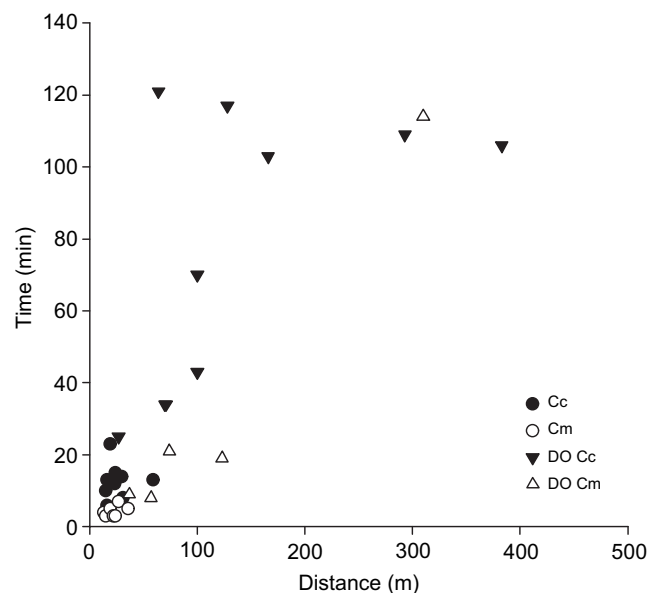
Treatment	N	Power strokes ( $\text{min}^{-1}$ )	Breaths ( $\text{min}^{-1}$ )
<i>C. caretta</i> control	16	115 $\pm$ 12	5.2 $\pm$ 1.3
<i>C. mydas</i> control	10	113 $\pm$ 19	5.9 $\pm$ 1.8
<i>C. caretta</i> 200 m crawl	11	119 $\pm$ 13	4.7 $\pm$ 1.4
<i>C. mydas</i> 200 m crawl	8	88 $\pm$ 12*	5.3 $\pm$ 1.1
<i>C. caretta</i> 500 m crawl	13	103 $\pm$ 22	4.4 $\pm$ 1.1
<i>C. mydas</i> 500 m crawl	8	109 $\pm$ 21	5.5 $\pm$ 1.8

Data are means $\pm$ s.d. \*Significantly different from resting (control) loggerhead turtle (*C. caretta*) hatchlings (Holm–Sidak  $t=3.099$ ,  $P=0.003$ ) as well as loggerheads (*C. mydas*) in the same treatment group ( $t=3.372$ ,  $P<0.001$ ).

For both green and loggerhead turtles, non-disoriented hatchlings travelled shorter distances and spent less time on the beach than disoriented animals (Kruskal–Wallis; loggerheads  $H=12.923$ , d.f.=1,  $P\leq 0.001$ ; greens  $H=8.077$ , d.f.=1,  $P=0.003$ ) (Table 3). Disoriented animals also rested for a larger fraction of the time ( $H=11.005$ , d.f.=1,  $P\leq 0.001$ ), which decreased mean crawl speeds. The percentage of time disoriented hatchlings of each species rested was significantly longer than controls, and loggerheads took significantly longer rests ( $H=3.887$ , d.f.=1,  $P=0.049$ ) and consequently a greater time to crawl similar distances compared with green turtles ( $H=9.233$ , d.f.=1,  $P=0.002$ ; Table 3; Figs 4 and 5). The percentage of time spent resting for both species versus crawling ranged from 0 to 70%. As non-disoriented hatchlings (especially green turtles) rarely rested while crawling to the waterline, crawl speeds with or without including rest periods in the calculation were similar within a species, although greens always crawled faster than loggerhead turtles. Green crawl speed without rests was significantly faster than loggerhead crawl speed without rest, for both oriented and disoriented groups (Kruskal–Wallis  $H=10.928$ , d.f.=1,  $P\leq 0.001$ ).

### Plasma metabolites

Although these field studies did not measure  $\dot{V}_{O_2}$ , blood was taken at the end of each crawl for metabolic measures. Blood glucose concentrations were similar to those obtained in the laboratory portion of the study (Table 3). While end-of-crawl glucose levels were similar between species as well as between disoriented and non-disoriented animals (ANOVA loggerheads  $F=1.623$ ,  $P=0.219$ ; greens  $F=1.154$ ,  $P=0.314$ ), and were not significantly higher than laboratory measurements, plasma lactate levels obtained from hatchlings in the field were 2- to 8-fold higher than laboratory values, although they varied greatly between individuals. A linear regression applied to the distance and glucose data (Fig. 6) showed that crawl distance did not have a significant effect on blood glucose concentrations for loggerheads (power 0.109,  $P=0.462$ ). There was a slightly stronger



**Fig. 4. Time and distance crawled by individual loggerhead and green sea turtle hatchlings that headed directly towards the surf (controls) were less than in misoriented or disoriented hatchlings on Boca Raton beach, Florida.** Cc, *Caretta caretta* (loggerhead); Cm, *Chelonia mydas* (green); DO, disoriented.

**Table 3. Loggerhead and green turtle hatchling behavior and physiology on the beach, Boca Raton, FL, USA**

Treatment (N)	Distance mean or median (m) (DO)	Time on beach (min)	% Time resting	Crawl speed without rests (m min <sup>-1</sup> )	Crawl speed with rests (m min <sup>-1</sup> )	Blood glucose (mg dl <sup>-1</sup> )	Plasma lactate (mg dl <sup>-1</sup> )
<i>C. caretta</i> control (12)	25.4	13.0±6.4	4±5	1.8±0.6	1.8±1.2	91.08±17.74	92.76±26.14
<i>C. mydas</i> control (7)	22.3	4.3±1.5	2±3	5.4±1.8*	5.4±1.8	93.17±24.14	189.32±86.16
<i>C. caretta</i> DO (9)	100 (range 64–383)	81.9±36.7	22±25‡	3.0±1.8	1.8±1.2	81.14±24.70	102.20±28.34
<i>C. mydas</i> DO (5)	74 (range 37–310)	32.2±42.8	12±21‡	5.4±1.2*	4.8±1.8	78.25±16.21	98.04±46.01

Time and crawl speed data are means±s.d.; blood glucose and plasma lactate data are medians±MAD. \*Significant difference between species within a treatment group ( $P\leq 0.001$ ); ‡significant difference compared with controls within same species ( $P\leq 0.05$ ). DO, disoriented; MAD, median absolute deviation.

relationship for greens, although this was also not significant (power 0.325,  $P=0.128$ ). There were also no significant differences in glucose concentrations between control (non-disoriented) and disoriented hatchlings (ANOVA loggerheads  $F=1.623$ ,  $P=0.219$ ; greens  $F=1.154$ ,  $P=0.314$ ). For plasma lactate, linear regression also indicated no relationship in either species between plasma lactate concentrations and distance (Fig. 7), while an ANOVA identified no differences in lactate levels between control and disoriented hatchlings (loggerheads  $F=0.149$ ,  $P=0.704$ ; greens  $F=2.968$ ,  $P=0.123$ ).

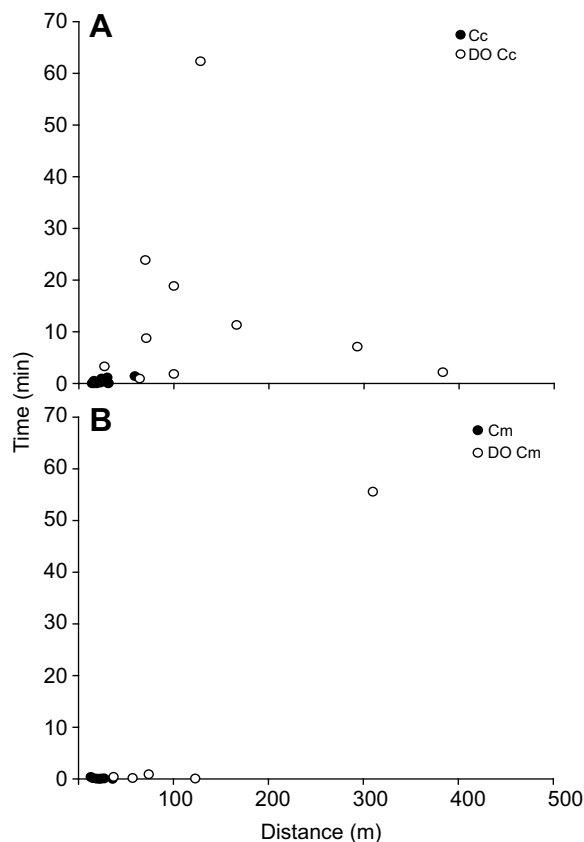
## DISCUSSION

Sea turtle hatchlings crawling on the beach are vulnerable to predation, thermal stress and dehydration. Disorientation and misorientation are likely to put hatchlings at greater risk because additional time on the beach increases exposure to predation, while metabolic effects such as glucose reduction and lactate build-up

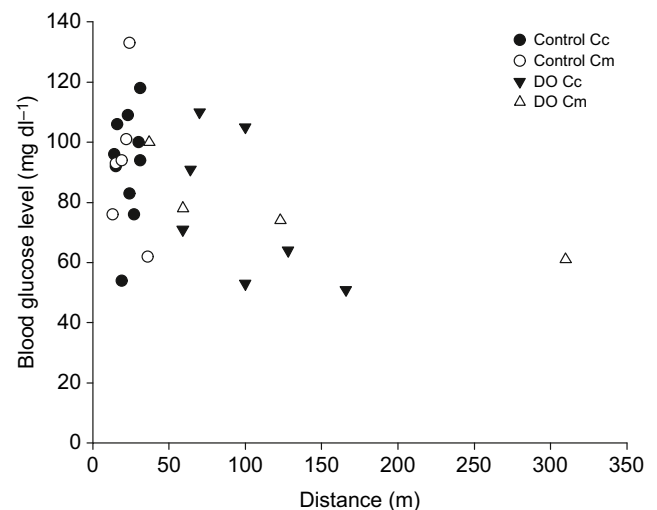
could affect crawling and swim performance. These experiments were designed to test the energetic effects of extended crawl distances on hatchlings and whether extended crawl distances have an impact on dispersal swim performance.

Hatchlings use both aerobic and anaerobic metabolism while digging, crawling and swimming (Dial, 1987; Baldwin et al., 1989). Anaerobic metabolism is necessary during the frenzy period when the hatchling has surpassed its aerobic scope during sustained vigorous exercise (Gatten, 1985). During undisrupted dispersal, anaerobic metabolism is utilized more during crawling than swimming (Pereira et al., 2012). Lactate build-up decreases blood pH and can thus decrease muscle performance. The build-up of lactate during extended crawls induces muscle fatigue, which requires rest periods for recovery (Gatten, 1985) and thus prolongs crawl time. A study by Hamann et al. (2007) suggested that carbohydrate metabolism is probably used during the digging, crawl frenzy and early stages of swimming dispersal, with a switch to fats and proteins as the swim continues. Hence, the digging phase consists of short bursts of intense activity interrupted by longer recovery periods that allow lactate levels to decline (Dial, 1987; Drake and Spotila, 2002), while the frenzy crawl results in higher lactate levels.

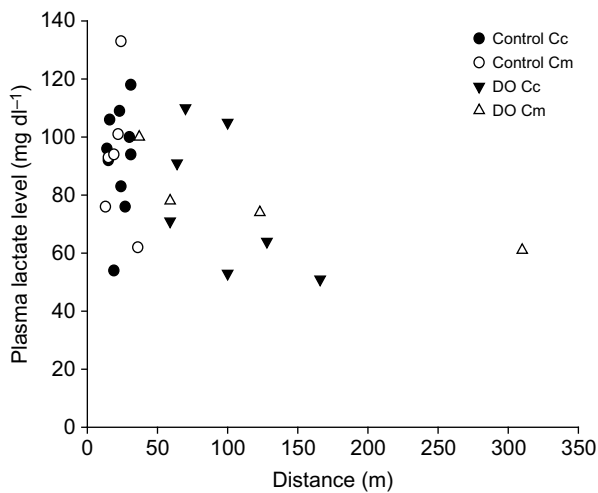
Mass-specific oxygen consumption in control (resting), crawling and frenzy swimming hatchlings of both species in this study were similar to those reported in previous studies (Wyneken and Salmon, 1992; Wyneken, 1997; Booth, 2009). Also, as has been reported (Prange and Ackerman, 1974), swim trial oxygen consumption rates were higher than resting but not as high as for crawling, as sea turtles



**Fig. 5. Time spent resting in relationship to total distance crawled on the beach for individual loggerhead and green sea turtle hatchlings. (A) Loggerhead: Cc, *Caretta caretta*. (B) Green: Cm, *Chelonia mydas* (green); DO, disoriented.**



**Fig. 6. Blood glucose levels showed no significant correlation with distance in either loggerhead or green sea turtle hatchlings crawling on the beach. Glucose linear regression: loggerhead power 0.109,  $P=0.462$ ; green power 0.325,  $P=0.128$ . Cc, *Caretta caretta* (loggerhead); Cm, *Chelonia mydas* (green); DO, disoriented.**



**Fig. 7. Plasma lactate levels showed no significant correlation with distance in either loggerhead or green sea turtle hatchlings crawling on the beach.** Lactate linear regression: loggerhead power 0.038,  $P=0.854$ ; green power 0.102,  $P=0.477$ . Cc, *Caretta caretta* (loggerhead); Cm, *Chelonia mydas* (green); DO, disoriented.

are adapted primarily for swimming (Wyneken, 1997). However, somewhat surprisingly, there were no differences in  $\dot{V}_{O_2}$  between the 200 and 500 m crawl distances or between species. As green turtles in general are known to have high aerobic scopes (Lutcavage and Lutz, 1997; Wyneken, 1997), we expected  $\dot{V}_{O_2}$  to be higher in these hatchlings than in loggerheads when crawling. However, as the belt speed of the treadmill was set in preliminary trials to loggerhead crawl speeds, and crawl speeds were thus the same for both species, this may explain the lack of difference in  $\dot{V}_{O_2}$ . The treadmill speed was apparently lower than the pace at which green hatchlings naturally crawl; the field data indicate that natural crawl speeds are approximately twice as fast as the belt speed ( $\sim 5.4$  versus  $2.4 \text{ m min}^{-1}$ ), and greens were observed to overtake the treadmill more often than loggerhead hatchlings. However, green hatchlings rested for significantly less time than loggerheads, such that the similar  $\dot{V}_{O_2}$  and lactate levels but more intense crawling still probably reflects the higher aerobic capacity of green turtle hatchlings. It would be of interest, though, to repeat the work using different belt speeds for each species.

There were also no differences in oxygen consumption with distance crawled. It was possible that hatchlings crawling the shorter 200 m distance would have higher  $\dot{V}_{O_2}$  than those crawling 500 m, if those crawling for 500 m became increasingly fatigued and slowed down (with lactate production either increasing to higher levels than in 200 m crawls, or levelling off), or, conversely, that longer crawl distances would result in higher  $\dot{V}_{O_2}$  overall. The high frequency and length of rests during the crawl trials, however, resulted in decreased mean oxygen consumption per unit time, and also prevented lactate levels from increasing as high as has been reported in previous field studies (Dial, 1987; Hamann et al., 2007) or as high as we found in the field portion of this study. Oxygen consumption rates during the crawl trials also varied greatly between individuals.

The energy for these activities of emergence and dispersal is drawn from the residual yolk sac (Kraemer and Bennett, 1981; Hewavisenthi and Parmenter, 2002). The yolk is composed mostly of fat with some carbohydrates (Kraemer and Bennett, 1981), but hatchling terrestrial dispersal seems to rely primarily on carbohydrates (Hamann et al., 2007) and so blood glucose concentrations can provide information about fuel use. As fuel

depletes as activity duration increases, and hatchlings appeared to rely on carbohydrates not only for the digging and crawling phases of dispersal but also for the initial hours of the frenzy swim (Hamann et al., 2007), hatchlings crawling longer distances as a result of disorientation should use up greater amounts of fuel than when crawling directly to the water. This carbohydrate depletion could reduce the amount of fuel available for the following frenzy swim and thus threaten the survival of disoriented hatchlings.

In this study, there were no significant differences in blood glucose concentrations between resting and the endpoint of experimental trials, either crawling or swimming, and levels were similar to previous reports in hatchlings following 2 h swim trials (Hamann et al., 2007; Pereira et al., 2013). It is a limitation of this study, however, that we were permitted to take only one blood sample per hatchling, at the end of each trial. Without repeated glucose assays over the course of the experiment we do not know whether glucose is decreasing or increasing over time in any animal, although when we standardized both glucose and lactate for the actual amount of time each individual animal was crawling, both glucose and lactate were lower in hatchlings that crawled 500 m versus those that only crawled 200 m. For the endurance activity of an extended crawl distance, lipid and catabolized protein from muscles mass may be utilized as during the migratory swim (Hamann et al., 2007), but the small amount of blood we could sample allowed for only glucose and lactate measurements in these experiments. While the relatively constant glucose levels across the various laboratory trials, which were similar in the field component, suggest that in these hatchlings glucose supply was sufficient to meet demand, we cannot know what percentage of available fuel was utilized. The lower standardized glucose values suggest a decline that could continue during the frenzy swim and leave hatchlings without enough fuel to maintain speed for the full 24 h, or require a shift to fat and protein fuel sources earlier than otherwise would occur. Pereira et al. (2013) report significant declines in loggerhead hatchling glucose over the first 2 h of frenzy swimming versus the end of frenzy crawling, with some additional decline in the following 2 h. The decline in lactate levels when standardized to per minute of crawl time also may suggest a change to fuels other than carbohydrates, or simply that the hatchlings metabolize some lactate as they crawl in an aerobic environment.

In this study we show that the additional crawl distances did not significantly affect swim performance, at least for the initial 2 h that we observed. Oxygen consumption was not different between swim controls and those that had crawled either 200 or 500 m, and glucose and lactate levels were also similar. There was no significant decline in the rates of powerstroking (other than for *C. mydas* crawling 200 m), nor an increase in respiratory frequency. Had glucose levels declined significantly or lactate levels increased enough to induce muscle fatigue, then impacts on swim performance would have been expected. However, the frequent rests that both green and loggerhead hatchlings exhibited while on the treadmill probably allowed them to maintain frenzy swimming. Although it should be noted that while the data were too variable to be significant, a decline in mean power stroke rates is observed in hatchlings that had crawled 500 versus 200 m; if the swim portion of the study had continued for longer, these declines may have been significant, and even the 5–10% decline noted here would affect hatchling dispersal over 24 h by reducing the distance covered.

However, in contrast to previous studies on lactate build-up during frenzy period activity, the laboratory results from these extended crawl distances (approximately 8–20 times longer than non-disoriented hatchlings crawled on the Boca Raton beach)

showed that plasma lactate levels do not increase significantly for either species crawling 200 or 500 m (or crawling plus swimming) and per minute concentrations decrease with distance. This is probably due to the frequent and extended periods of rest demonstrated by the hatchlings in the laboratory, as by contrast lactate levels in the field were significantly higher. While both loggerhead and green hatchlings rested a similar percentage of the time in both the laboratory and during disorientation events on the beach, beach lactate measurements were taken from hatchlings frenzied crawling immediately upon nest emergence, whereas the animals used in the laboratory were transported and kept quiescent for varying periods of time between emergence and testing. Thus it is likely that baseline levels of lactate in the laboratory ( $\sim 20\text{--}30\text{ mg dl}^{-1}$ ) were already lower than in the field prior to the beginning of the treadmill experiments, and so maximum levels during exercise would also be lower overall than on the beach. In addition, of course, the hatchlings in the field were crawling over rougher ground, with loose sand and rutted terrain, compared with the relatively smooth, flat surface of the treadmill. Other than plasma lactate levels, many of the field results were similar to the laboratory treatments and indicate that the laboratory experiments were a reasonable proxy for extended crawls on the beach.

Hatchling dispersal from the nest directly to the water has been studied previously (Dial, 1987; Ischer et al., 2009; Pereira et al., 2013); however, this is the first study that has measured distances and quantified both behavioral and metabolic markers for disoriented hatchlings in the field. Disorientation distances travelled on the beach in this study ranged from a low of 37 m to a high of 383 m, with reports of hatchlings crawling as much as 1 km on South Florida beaches (P. Sposato, personal communication), and thus were similar to distances selected for the laboratory extended crawl treatments. Resting behavior of hatchlings during crawls in the field was also similar to laboratory results, with loggerheads resting for more than twice as long on average as greens, and thus extending their time on the beach.

Interestingly, disoriented green hatchlings spent less time disoriented than the loggerhead turtles: approximately 32 min for greens versus 82 min for loggerheads, with a shorter median distance and a faster average crawl speed. Green turtles in general tend to nest further away from the waterline and closer to the dune toe (Wood, 2004; Turkozan et al., 2011), so they normally travel longer distances to the water than loggerheads, and may have adaptations to orient more effectively. In this study, however, the mean distance travelled by non-disoriented greens was shorter than the mean distance that non-disoriented loggerheads crawled. As most of the non-disoriented hatchlings were observed at park beaches that were not renourished, the narrow beaches restricted the distance that both nesting green and loggerhead females could travel inland. The fact that the non-disoriented hatchlings were observed at park beaches results from their higher dune silhouette and less beachfront lighting. Green hatchlings in general disorient less frequently (K.P., personal observation); the dune vegetation where greens prefer to nest (Turkozan et al., 2011) could provide a darker silhouette from which hatchlings would begin their navigation to the water. In individual loggerhead hatchlings we noted that some animals would crawl away from the water and towards various light sources, but once in the shadow of the dune would orient correctly and turn back towards the water – until they were away from the dune toe and misoriented again; this could go on for numerous cycles of orientation/misorientation. We also observed that green hatchlings were able to orient sooner than loggerhead hatchlings, thus reducing their disorientation crawl distances in this study by a

mean of 32.6 m. Besides travelling greater distances, disoriented loggerheads also spent much more time on the beach because they rested for a greater percentage of their crawl time than greens ( $\sim 22$  versus  $\sim 12\%$ ). Crawl speeds between the species also differed, with green hatchlings crawling at a much faster rate, perhaps as a result of a larger aerobic scope (Wyneken, 1997) and larger flipper to body ratio (Dougherty et al., 2010), although there was no significant correlation between body mass and physiological or performance measures, including crawling or swimming mass specific oxygen consumption, lactate or glucose levels, and swimming stroke rate (data not shown). Together these factors suggest that loggerheads will be more affected than greens by the negative impacts of disorientation.

Crawl speeds on the beach were different from crawl speeds in the laboratory. Loggerheads in the laboratory crawled at a set rate of  $2.4\text{ m min}^{-1}$  determined by average crawl speeds of an initial set of animals. Loggerheads in the field crawled at a mean speed of  $1.8\text{ m min}^{-1}$ , although this was a faster average speed than previously reported for loggerheads on a Mediterranean beach (Triessnig et al., 2012). The slightly faster speed of the treadmill did not cause hatchlings to fall behind the treadmill and seemed appropriate for the experiment. Animals in the laboratory were also crawling on a flat, firm surface rather than loose sand substrate and terrain that can slow down travel speeds; in one study by Triessnig et al. (2012), loggerhead hatchling crawl speed was affected most by substrate grain size, surface temperature, and orientation motivation. Green hatchlings in the field, however, crawling at  $4.8\text{--}5.4\text{ m min}^{-1}$  were twice as fast as the set treadmill speed. It was observed that the greens in the laboratory were sometimes outrunning the treadmill but were kept contained by the walls of the treadmill. If laboratory green hatchlings were run at a speed near to field measures of  $4.8\text{ m min}^{-1}$ , there may have been larger differences in  $\dot{V}_{O_2}$  and lactate levels than we observed. Despite the differences in crawl speed and distances travelled by each species, there were no significant differences in glucose or lactate levels between those that disoriented and those that did not, nor were there differences between species, and very little correlation between distance each individual crawled on the beach and either blood glucose or plasma lactate, suggesting that, as in the laboratory portion of the study, the extended crawling did not have an impact on hatchlings as much as might be expected by extrapolating measures taken only from properly oriented hatchlings (Dial, 1987; Booth, 2009; Pereira et al., 2013).

Thus while we hypothesized that the longer distances of disorientation crawling would decrease glucose levels and increase lactate, and thus affect swim performance, neither the laboratory nor field portions of this study support that hypothesis. However, this does not mean that disorientation does not negatively impact sea turtle hatchlings. One potential impact of extended crawling may be a reduction of limited energy stores; it has been calculated that loggerhead hatchlings may only have enough yolk reserve energy to swim continuously for  $\sim 70\text{ h}$  offshore (34 kJ; Kraemer and Bennett, 1981), although this may be an underestimate (Jones et al., 2007). The fact that lactate levels in the field were as high in disoriented hatchlings that had crawled for 300 m or more as those that were not disoriented, and in those that crawled for as long as 2 h as those that crawled directly to the sea within minutes, suggests an elevated mobilization of glucose for anaerobic metabolism that could still deplete total energy available for the initial days of life at sea. Newly emerged hatchlings may not eat for up to 1 week post-emergence when being raised in a laboratory setting, relying entirely on stored yolk energy (J. Wyneken, personal



communication). The elevated energy demand of extended crawling, as shown in this study by significantly higher mass-specific oxygen consumption in the laboratory compared with resting or swimming animals, would similarly suggest that overall energy stores will be depleted more rapidly in disoriented turtles than in non-disoriented animals.

Although further work would be required to examine fully the question of energy stores, it is clear that the numerous rest periods noted both in the laboratory and in the field could significantly have an impact on hatchling survival. These rest periods averaged almost a quarter of the total crawl time in disoriented loggerhead hatchlings both in the laboratory and in the field. The amount of time spent not crawling was in fact considerably higher in disoriented hatchlings of both species, compared with animals that crawled directly seaward. While these rest periods probably allowed for intermittent lactate recovery and prevented the negative effects of lactate accumulation and pH changes, they also significantly decreased mean crawl speeds and extended the time it took for disoriented animals to find the water (for those that managed to find the water at all: four out of nine observed disoriented loggerheads got lost in the dunes or in the street, although all the disoriented greens eventually found their way to the water). Hatchlings still on the shore at daylight risk death from dehydration and thermal stress as well as increased predation. As the hatchlings can be heavily preyed while crossing the beach even in groups (Tomillo et al., 2010; Peterson et al., 2013; Marco et al., 2015), and larger groups reduce predation risk (Santos et al., 2016), the longer a hatchling is on the beach, especially separated from the *en masse* emergence, the greater the likelihood of predation both on land and upon reaching the sea.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: S.L.M.; Methodology: K.P., S.L.M.; Formal analysis: K.P.; Investigation: K.P.; Resources: S.L.M.; Data curation: K.P.; Writing - original draft: K.P.; Writing - review & editing: K.P., S.L.M.; Supervision: S.L.M.; Project administration: S.L.M.; Funding acquisition: S.L.M.

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