Research article

Physiological changes in post-hatchling green turtles (*Chelonia mydas*) following short-term fasting: implications for release protocols

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Relocation of sea turtle nests and the retention of post-hatchlings for head-starting programs are both commonly used to improve conservation outcomes and facilitate eco-tourism ventures. Currently, there is little literature surrounding the husbandry protocols required during these programs to optimize post-release outcomes. To assess the impact of varied feeding regimes on exercise performance, (which will hereafter be referred to as 'fitness'), 40 10-month-old captive post-hatchling green turtles (*Chelonia mydas*) were divided into four groups of 10 and fasted for either 3, 9, 10 or 15 h. The animals were then subjected to a fitness test via repetitive use of the 'righting reflex' on land. Health assessments were conducted prior to the fitness test, including; heart rate, haematocrit (Hct), heterophil to lymphocyte ratio and the measurement of 11 biochemical analytes, including pH, partial pressures of carbon dioxide (P_vCO_2) and oxygen (P_vO_2), lactate, bicarbonate (HCO_3^-), sodium (Na^+), potassium (K^+), chloride (Cl^-), ionized calcium (iCa^{2+}), glucose and urea. Results were corrected for multiple comparisons and significant differences among groups were demonstrated for temperature, pH, HCO_3^-, iCa^{2+}, urea and lactate. To investigate physiological relationships between analytes, correlation coefficients were calculated between fitness and glucose, fitness and lactate, glucose and lactate, pH and iCa^{2+}, pH and K^+, pH and P_vCO_2, pH and HCO_3^- and Hct and K^+. Following correction for multiple comparisons, significant relationships were seen between pH and iCa^{2+} and pH and HCO_3^-. Post-hatchling turtles appear to enter a catabolic state when exposed to short-term fasting. While this did not have a direct impact on fitness, the production of an intense energetic output from a catabolic state may induce a physiological debt. This study suggests that handling that induces a physical response should be minimized and animals should be fed within 10 h of release.

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Introduction

The management of post-hatchling sea turtles in captive environments is becoming increasingly common. This is due to both conservation strategies, where relocation is used as an effective strategy to decrease hatchling mortality and assist with population management in areas of high tidal inundation (Dutton *et al.*, 2005; Wyneken *et al.*, 1988) and, more recently, to facilitate eco-tourist–based ventures (Tisdell and Wilson, 2005). Extensive research has determined the factors that are required to optimize the success of nest relocation including not moving the eggs between 12 h and day 21 of gestation (Harry and Limpus, 1989; Limpus *et al.*, 1979; Parmenter, 1980), minimizing movement during transport (Limpus *et al.*, 1979), maintaining optimal incubation temperatures (Burgess *et al.*, 2006; Fisher *et al.*, 2014) and controlling moisture content (McGehee, 1990), and new research is examining the efficacy of delaying embryonic development prior to movement via the creation of hypoxicx conditions (Williamson *et al.*, 2017). The process of hatching and emergence can take several days (Fisher *et al.*, 2014; Godfrey and Mrosovsky, 1997), and following this, the hatchlings can be successfully released from the beach adjacent to the nest location. The 'swim frenzy' is then utilized to propel them through predator-rich near shore environments to access the required, deeper offshore currents (Wyneken and Salmon, 1992).

However, in sea turtle hatcheries such as those located in Sri Lanka (Tisdell and Wilson, 2005), Indonesia (Haryati *et al.*, 2016) and Brazil (de Vasconcellos Pegas and Stronza, 2010), the release dates for animals can be planned to coincide with peak tourist numbers, resulting in hatchlings that can remain in care for months prior to release (Tisdell and Wilson, 2005). Prolonged retention of hatchlings may compromise a turtle's chance of survival post-release (Pilcher and Enderby, 2001), and therefore, maintenance of appropriate husbandry regimes and the employment of optimal pre-release processes are required for successful post-release outcomes. Digestion and fasting can both alter physiological processes and feeding regimes and should be considered in the release plan. Adult sea turtles have unique physiological adaptions to deal with the extended periods of fasting that occur during the nesting period (Perrault *et al.*, 2016; Perrault *et al.*, 2014), including changes in hormone levels associated with appetite control (Goldberg *et al.*, 2013) and the mobilization of fat stores to provide the energy required to complete the nesting process (Hamann *et al.*, 2002; Perrault and Stacy, 2018; Plot *et al.*, 2013). Physiological tolerance to short-term fasting has also been documented in sub-adult animals, where a decrease in blood glucose was not detected until animals were fasted for 1 week (Moon *et al.*, 1997). Digestion has been shown to induce a postprandial metabolic alkalosis in other reptiles (Hicks and Bennett, 2004) and an increase in metabolic rate called the 'specific dynamic action' (SDA) (Secor, 2009). The SDA of captive post-hatchlings is relatively small compared to large, carnivorous reptiles, with the upregulation of the metabolic rate to facilitate digestion lasting up to 4 h post-feeding (Kowalski, 2005). The impact of digestion and fasting on post-hatchling exercise output is unknown, and hence it is difficult to predict how each process may affect post-release outcomes.

Metabolic studies in wildlife have become increasingly feasible with the advent of hand-held point of care blood analysers, such as the VetScan i-STAT®. (Alberghina *et al.*, 2015; Hunt *et al.*, 2016; Perrault *et al.*, 2018; Stoot *et al.*, 2014; Wolf *et al.*, 2008). These machines are capable of assessing blood gases and plasma analytes and have the potential to provide near real-time insights into the metabolic state of an animal. Here, we assess the impacts of short-term fasting on exercise performance, which will be referred to in this study as 'fitness' and use a hand-held analyser to detect the associated physiological changes in blood analytes, in post-hatchling green turtles.

Materials and methods

Housed at James Cook University following collection from Heron Island in Southern Queensland, Australia (government permit WITK15765815; Animals Ethics Committee Permit Number A2309) were 40 green turtle hatchlings, 10 months prior to the following experimental protocols. The animals were collected during an afternoon emergence, held overnight and then transported to the research facility the following day, arriving ~30 h after collection. Hatchlings were housed in individual sea water tanks and were fasted for 9 days to allow the yolk sac to be utilized. Following this, food was introduced slowly over a 2-week period until the animals were eating 5% of their body mass per day with a diet that composed of one-third fish/prawn meat, one-third commercial fish pellets and one-third mixed green vegetables. All of the ingredients were finely diced and bonded with gelatin and a multivitamin supplement (SeaTabs B.T.F.S, Pacific Research Labs, Ramona, CA) and were frozen prior to feeding. Adjustments were made fortnightly based on weight to maintain feeding at 5% body mass per day. At the time of the experiment, the mean (± standard deviation) body mass of the post-hatchlings was 129.6 ± 21.9 g. Each day, the tanks housing the animals were placed in direct sunlight for 1 h to provide ultraviolet (UV) exposure. To avoid the introduction of movement stress associated with transferring the animals outside, on the 2 days of the experiment, the animals were not placed outside for routine UV supplementation. The tank temperatures were allowed to equilibrate to ambient temperatures, and the tank temperature for each animal was recorded immediately prior to the health assessment.

The turtles were divided into 4 groups of 10 and were subjected to either a 3-, 9-, 10- or 15-h fasting period; a health assessment (described below) was conducted prior to the fitness test. The time intervals were selected to include both the presence and absence of the SDA but were also limited due to logistical constraints. Given the pelagic nature

of these animals at this life stage, swim tests were attempted to measure fitness; however, in response to current, the animals would passively drift. Hence, to assess fitness, all animals were placed in dorsal recumbency to encourage the 'righting reflex', which is a commonly used measure of hatchling fitness (Booth *et al.*, 2013; Maulany *et al.*, 2012; Wood *et al.*, 2014) and provided a consistent fitness challenge among groups for this experiment. To measure fitness, the number of times the animal successfully turned over, within a maximum period of 3 min or until the animals remained in dorsal recumbency for a period in excess of 20 s, was recorded.

Data collected during health assessments included heart rate and analysis of blood health analytes. Heart rate was measured using an ultrasound machine (GE Logiq Book) in B-mode using a curve-linear probe positioned on the centre of the plastron at a frequency of 5 MHz, while the animal was held in an upright position. Heart beats were counted for 30 s and multiplied by 2 to calculate the beats per minute. Collected from the dorsal occipital sinus was 150 μL of venous blood using a 25-gauge, 1.6 cm needle and a 1-mL syringe following aseptic preparation of the skin based on previously described techniques (Owens and Ruiz, 1980), and the sample was carefully observed during collection to monitor for lymphatic contamination. A blood smear was prepared immediately with fresh blood and stained with Diff Quik (Point of Care Diagnostics Healthcare, Artarmon, NSW) to enable the heterophil to lymphocyte ratio to be calculated using standardized methods (Fudge, 2000). The remaining sample was transferred to a 1-mL BD Microtainer® tube (Beckton Dickson, Franklin Lakes, NJ) containing lithium heparin. Biochemical analysis of the heparinized blood was conducted immediately using a VetScan i-STAT®1 System (Analyzer Model 300A; Abaxis, Union City, CA, USA). CG4+ (ABBT-03P85–25) cartridges were used to analyse pH, partial pressures of carbon dioxide (P_vCO_2,) and oxygen (P_vO_2), lactate and bicarbonate (HCO_3^-). Chem8+ (ABBT-09P31–25) cartridges were used to measure sodium (Na^+), potassium (K^+), chloride (Cl^-), ionized calcium (iCa^{2+}), glucose, urea and haematocrit (Hct). The CG4+ analysis was conducted before the Chem8+ and the time between analyses was minimized to avoid temporal changes. The total volume of blood used in the sample collection did not exceed the recommended maximum amount for chelonian species of 0.8% of body weight (Mans, 2008).

The i-STAT® machine performs analysis of samples at 37°C, and hence the following parameters were temperature corrected $_{(TC)}$ as per previous studies in sea turtles (Harms *et al.*, 2003; Keller *et al.*, 2012), where $\Delta T = 37°C$—tank temperature. Data were corrected to the temperature of the tank, as opposed to the body temperature of the animal. While a degree of endothermy has been documented in sub-adult and adult green turtles, with deep body temperatures ranging from $2.2 \pm 0.2°C$ to $1.7 \pm 0.2°C$ above ambient temperatures at 20°C and 30°C, respectively (Heath and McGinnis, 1980),

tank temperature was selected given the potential trauma involved in obtaining a deep body temperature in animals with small cloaca's.

$$pH_{TC} = pH_{37°C} + (0.014/°C) \times \Delta T \text{ (Kraus and Jackson, 1980).}$$

$$P_vCO_2{}_{TC} = P_vCO_2{}_{37°C} \times (10^{-0.019 \Delta T}) \text{ (Ashwood } et al., 1983).$$

$$P_vO_2{}_{TC} = P_vO_2{}_{37°C} \times (10^{-0.0058 \Delta T}) \text{ (Ashwood } et al., 1983).$$

HCO_3^- was calculated using the Henderson–Hasselbalch equation $HCO_3^- = \alpha CO_2 \times P_vCO_2 \times 10^{(pH-pka)}$, where αCO_2 and pKa were calculated using the equations described by Stabenau and Heming (1993) and $P_vCO_2{}_{TC}$ and pH_{TC}.

$$iCa^{2+}{}_{TC} = iCa^{2+} (1 + 0.53(pH-pH_{TC}))$$
(Fogh-Andersen, 1981).

The mean $\pm$ standard error was calculated for each analyte, including the mass of the animals in each group and the data were assessed for homogeneity of variance and normality of distribution using Levene's test and the Shapiro–Wilk test, respectively. To determine differences among groups of varied fasting intervals, where data met these assumptions, a one-way analysis of variance (ANOVA) for each analyte was performed. Temperature, heart rate, fitness outcomes, P_vO_2, lactate, glucose and K^+ did not meet these assumptions, and hence a Kruskal–Wallis analysis was used. Given the potential for inflated type 1 error due to multiple comparisons, the Holm–Bonferroni sequential correction method (Abdi, 2010; Holm, 1979) was also used. To assess for pairwise differences between groups where significant differences were detected, Bonferroni and Dunn's *post hoc* analyses were used for ANOVA and the Kruskal–Wallis results, respectively. Pearson correlation coefficients (r) were calculated for hypotheses about the relationships among analytes and fitness; however, given multiple correlations increased the chance of type 1 error, only select variables were assessed, and correction for multiple comparisons was conducted via the Bonferroni method (Curtin and Schulz, 1998). All analyses were conducted on IBM SPSS Statistics Version 24 Licence 9.6.0.0.

Results

The median and range of the analytes for each group, along with an indication of the analytes that failed Levene's test for homogeneity of variance and the groups that failed the Shapiro–Wilk test for normality, are presented in Table 1. Also presented in Table 1 is the fact that for 12 data points, K^+ levels were below the detectable limit. Where this occurred, a nominal value equal to the lowest detection point, 2 mmol/L, was assigned. Analytes that were calculated to be

Table 1: Median and *range* of blood analytes, temperature and heart rate for groups fasted for 3, 9 10 and 15 h

	3 h		9 h		10 h		15 h	
Weight (g)	129.5	*(98.0–164.0)*	128.0	*(104.0–164.0)*	124.0	*(96.0–156.0)*	134.6	*(83.0–169.0)*
Fitness[¥]	8.5	*(5.0–12.0)*	6.0	*(4.0–15.0)*	5.0	*(2.0–7.0)*	8.0	*(3.0–9.0)*
Temperature[¥] (°C)	24.2	*(23.5–25.3)*	26.1	*(25.9–26.3)*	23.8	*(23.6–26.4)*	30.5	*(30.3–30.9)*
Hct (%)	20.0	*(17.0–23.0)*	21.0	*(19.0–27.0)*	19.0	*(14.0–23.0)*	18.0	*(13.0–22.0)*
Heart Rate[¥]	23.5	*(10.0–47.0)*	45.0	*(12.0–50.0)*	39.0	*(20.0–54.0)*	41.0	*(16.0–58.0)*
H:L	0.52	*(0.12–1.1)*	0.48	*(0.29–0.89)*	0.73	*(0.42–1.5)*	0.48[†]	*(0.37–1.2)*
Glucose[¥] (mg/dL)	111.6	*(102.6–117.0)*	120.6	*(95.4–136.8)*	124.2	*(102.6–172.8)*	122.4[†]	*(113.4–145.8)*
Lactate (mmol/L)	2.1	*(1.1–4.8)*	2.7	*(1.0–5.0)*	5.8	*(5.1–10.5)*	5.9	*(4.3–8.1)*
pH	7.64	*(7.59–7.85)*	7.55	*(7.44–7.68)*	7.59	*(7.45–7.66)*	7.43	*(7.34–7.55)*
HCO_3^- (mmol/L)	41.6	*(34.5–56.0)*	33.9	*(21.9–47.0)*	27.9	*(22.0–43.0)*	25	*(22.0–34.6)*
Urea (mmol/L)	14.6	*(11.3–18.2)*	14.9	*(10.1–18.8)*	19.7	*(13.4–23.5)*	20.4	*(15.8–23.9)*
iCa^{2+} (mmol/L)	0.78	*(0.64–0.92)*	0.91	*(0.78–1.11)*	1.03	*(0.86–1.18)*	1.10	*(0.95–1.29)*
P_vCO_2 (mmHg)	31.0	*(18.9–37.4)*	34.9	*(28.4–41.4)*	26.3	*(21.4–32.6)*	37.7	*(21.8 42.0)*
P_vO_2 (mmHg)	55.4	*(47.7–94.6)*	51.4	*(43.2–60.6)*	59.2	*(47.3–73.7)*	56.3	*(42.1–59.5)*
Na^+ (mmol/L)	144.0	*(139.0–147.0)*	141.0	*(139.0–145.0)*	145.0	*(141.0–148)*	144.5	*(139–148.0)*
Cl^- (mmol/L)	100.0	*(88.0–105.0)*	101.0	*(93.0–109.0)*	102.0	*(91.0–106.0)*	103.0	*(96.0–107.0)*
K^+ (mmol/L)	2.2	*(2.0–2.6)*	2.6	*(2.0–2.9)*	2.1	*(2.0–2.4)*	2.4[†]	*(2.2–3.0)*

Hct = haematocrit, *K^+ = 12 values were below detectable limit, P_vCO_2 = partial pressure carbon dioxide, Na^+ = sodium, iCa^{2+} = ionized calcium, HCO_3^- = bicarbonate.

[¥] Analyte failed Levene's test for homogeneity of variance between groups

[†] Analyte failed the Shapiro–Wilk test for normality of distribution within the group.

significantly different among groups following correction are shown in Fig. 1. Significant differences were calculated for temperature ($P < 0.01$), lactate ($P < 0.01$), pH ($P < 0.01$), HCO_3^- ($P < 0.01$), urea ($P < 0.01$), iCa^{2+} ($P < 0.01$) and P_vCO_2 ($P < 0.01$).

Pairwise analysis showed there was no significant difference in the temperature of the holding tanks between animals fasted for 3 h compared to 9 h ($P = 0.08$) or 10 h ($P > 0.99$) or between animals fasted for 9 h compared to 10 h ($P = 0.29$) or 15 h ($P = 0.11$). Significant differences in holding tank temperatures were calculated between groups fasted for 3 h compared to 15 h ($P < 0.01$) and between animals fasted for 10 h compared to 15 h ($P < 0.01$). There was no significant difference in lactate between animals fasted for 3 h and 9 h ($P > 0.99$) or animals fasted for 10 h compared to 15 h ($P > 0.99$). There was a significant difference between animals fasted for 3 h compared to animals fasted for 10 h ($P < 0.01$) and 15 h ($P < 0.01$) and between animals fasted for 9 h compared to animals fasted for 10 h ($P < 0.01$) and 15 h ($P < 0.01$). The pH declined significantly during the postprandial period, and significant differences were calculated among all groups with the exception of animals fasted for 9 h compared to animals fasted for 10 h ($P > 0.99$). A similar postprandial decline was observed with HCO_3^- with

significant differences measured when animals were fasted for 3 h were compared to animals that were fasted for 10 h ($P < 0.01$) and 15 h ($P < 0.01$). There was no significant difference in urea between animals fasted for 3 h and 9 h ($P > 0.99$) or animals fasted for 10 h compared to 15 h ($P > 0.99$). There was a significant difference between animals fasted for 3 h compared to animals fasted for 10 hs ($P < 0.01$) and 15 h ($P < 0.01$) and between animals fasted for 9 h compared to animals fasted for 10 h ($P < 0.01$) and 15 h ($P < 0.01$). iCa^{2+} increased during the postprandial interval with significant differences calculated between animals fasted for 3 h compared to animals fasted for 9 h ($P < 0.01$), 10 h ($P < 0.01$) and 15 h ($P < 0.01$) and between animals fasted for 9 h compared to animals fasted for 15 h ($P < 0.01$). However, there was no significant difference between animals fasted for 9 h compared to animals fasted for 10 h ($P > 0.05$) or animals fasted for 10 h compared to 15 h ($P > 0.99$). Significant differences in P_vCO_2 levels were calculated for animals that were fasted for 10 h compared to 9 h ($P < 0.01$) and 15 h ($P < 0.01$).

The results of the Pearson's correlation assessment can be seen in Table 2. Following adjustment of the alpha value via the Bonferroni correction technique, there was a significant negative correlation between pH and iCa^{2+} ($r = -0.58$,
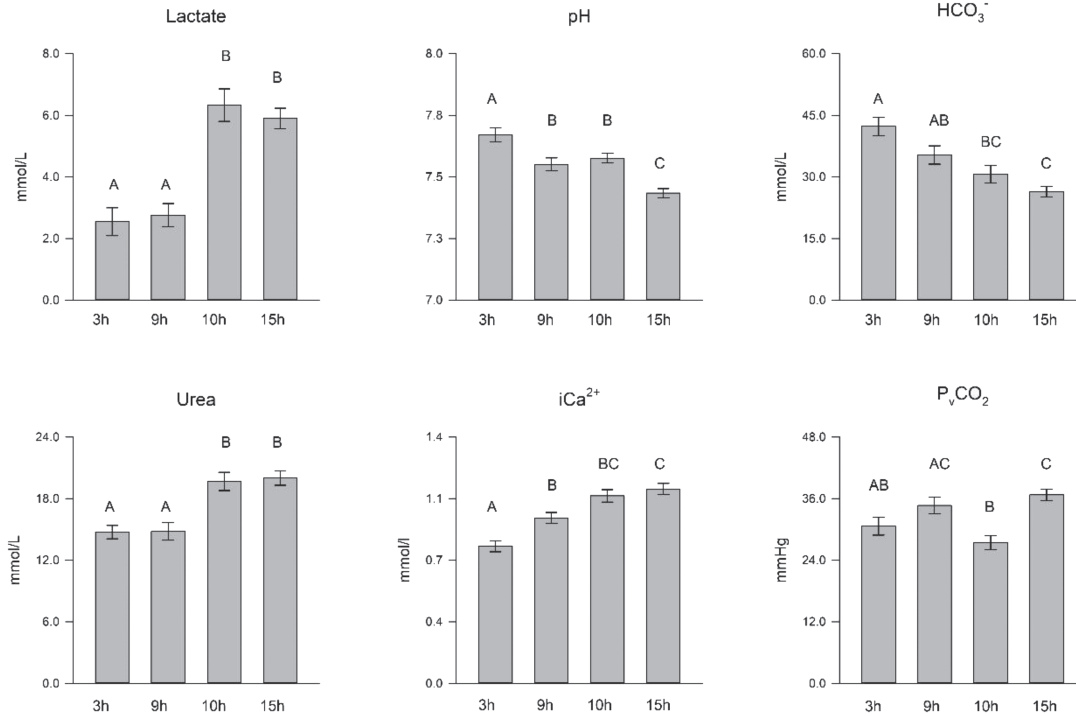
Figure 1: Mean and standard error for groups at 3, 9, 10 and 15 h after feeding for analytes where significant differences were detected with letters denoting pairwise differences.

$P < 0.01$) and a significant positive correlation between pH and HCO_3^- ($r = 0.77$, $P < 0.01$); however, no significant correlations were calculated within groups.

Discussion

The mean values for glucose, Cl^- and, Na^+ fall into the local reference intervals for green turtles (Flint *et al.*, 2010). K^+ levels were lower than these reference intervals and levels recorded in other studies on different species of sub-adult sea turtles (Hunt *et al.*, 2016; Innis *et al.*, 2014). While hypokalemia can be associated with metabolic alkalosis (Campbell, 2006), no significant correlation with pH was calculated, and hence the low readings may reflect decreased dietary intake. The P_vCO_2 is similar to previous studies using point-of-care analysers in sea turtles; however, the P_vO_2 was lower (Hunt *et al.*, 2016). Tissue hypoxia can be related to a functional deficiency of mature red blood cells (Hodges *et al.*, 2007), and while the Hct was within the normal reference intervals for sub-adult and adult green sea turtles in Australia, it would be considered low compared to studies conducted in sub-adult animals in the northern hemisphere (Bolten and Bjorndal, 1992; Page-Karjian *et al.*, 2014; Stacy *et al.*, 2018) and is lower than previously recorded values in leatherback hatchlings (Reina *et al.*, 2002). Hct levels can be impacted by iatrogenic error, such as lymphatic contamination or

sample hemolysis. If the former were to occur, based on the comparatively low levels of K^+ in lymphatic fluid compared to plasma (Thrall *et al.*, 2012), a positive correlation would be seen between the two analytes. This is in contrast to hemolysis, where the destruction of the cell would liberate the intracellular K^+ and elevate K^+ levels (Yücel and Dalva, 1992) in the analysed sample, while the cellular destruction would decrease the Hct. This process would generate a negative correlation between the two analytes. However, there was no significant correlation calculated between Hct and K^+, and hence neither hypothesis adequately explains the results. Previous studies utilizing the i-STAT machine in fish have shown reduced Hct readings relative to microcapillary controls (Harter *et al.*, 2014). The proposed explanation for this, which may be relevant to sea turtles, is that the algorithm used to calculate Hct is based on a non-nucleated, spherical red blood cell, as opposed to the elliptical, nucleated red blood cells that fish and reptiles have and hence is not as accurate when used in these species (Harrenstien *et al.*, 2005).

The pH declined postprandially for green turtles in two distinct phases. The initial decline between 3 and 9 h may be reflective of a waning metabolic alkalosis that accompanies digestion (Hicks and Bennett, 2004) and is supported by decreasing HCO_3^- levels during this period. This timeline is consistent with the SDA calculated for post-hatchling green turtles fed 2% of their bodyweight, where a peak increase occurred 1 h post-ingestion, with VO_2 returning to near

Table 2: Pearson's correlation coefficient (r) and the associated P value for selected analytes within groups

	Overall		3 h		9 h		10 h		15 h	
	r	P	r	P	r	P	r	P	r	P
Fitness: glucose	−0.17	0.29	−0.15	0.70	0.12	0.75	0.07	0.85	−0.08	0.82
Fitness: lactate	−0.16	0.34	0.25	0.52	0.42	0.23	−0.13	0.72	0.72	0.02
Glucose: lactate	0.34	0.03	−0.71	0.03	0.36	0.30	−0.03	0.93	−0.10	0.79
pH: iCa^{2+}	−0.58	<0.01*	−0.24	0.53	−0.24	0.53	0.17	0.66	−0.47	0.20
pH: K$^+$	−0.35	0.04	0.51	0.17	−0.21	0.58	0.67	0.04	−0.28	0.47
pH: P$_v$CO$_2$	−0.37	0.03	−0.22	0.56	−0.23	0.56	−0.60	0.10	−0.03	0.95
pH: HCO$_3^-$	0.77	<0.01*	0.42	0.26	0.71	0.03	0.38	0.28	0.79	0.01
Hct: K$^+$	0.23	0.18	0.31	0.41	0.50	0.17	0.19	0.63	−0.33	0.39

Hct = haematocrit, K$^+$ = potassium, P$_v$CO$_2$ = partial pressure carbon dioxide, iCa^{2+} = ionized calcium, HCO$_3^-$ = bicarbonate.

*Denotes significant correlation following Holm–Bonferroni correction.

normal levels by 4 h (Kowalski, 2005). The decline in pH that occurs between animals fasted for 10 h compared to animals fasted for 15 h may be the result of an accumulation of acidic by-products produced via catabolic metabolism, as increases in the ketone body β-hydroxybutyrate have been documented previously in sub-adult green turtles following fasting (Price et al., 2013). The steady rise in iCa^{2+} postprandially and the significant negative correlation with pH are reflective of this acidifying state, as H$^+$ can displace iCa^{2+} from their binding sites, increasing serum levels (Wang et al., 2002).

The lactate levels observed in the early postprandial stages are typical of hatchlings at rest, while the later stages of fasting are more consistent with levels following anaerobic exercise (Hamann et al., 2007), indicating a shift in these animals towards a state of catabolic metabolism. The increased lactate levels likely occurred secondary to the liberation of pyruvate from skeletal muscle following the onset of glycogenolysis (Rui, 2014) that allows lactate to be used directly by skeletal muscles as a gluconeogenic substrate (Gleeson, 1996). The hypothesis of metabolic transition to catabolism is also supported by the elevation in urea in fasted animals, as elevated levels of urea have been recorded during fasting in nesting hawksbill (Goldberg et al., 2013) and leatherback turtles (Plot et al., 2013), as amino acids are catabolized from muscle protein. The decrease in P$_v$CO$_2$ calculated at 10 h may be a compensatory mechanism associated with this metabolic transition, as hyperventilation can be used to compensate for metabolic acidosis (Hartzler et al., 2006).

The temperatures of the holding tanks were measured immediately prior to blood collection to provide the information needed to temperature correct the i-STAT data. Significant variations in pre-assessment temperature among groups represent a potential confounding factor in this study. To address this, analysis of covariance (ANCOVA) analysis was the preferred method of analysis, with temperature as the covariate; however, many analytes displayed heterogeneous regression slopes, which precluded the use of this analysis technique. There was no significant difference between the mean temperature of animals fasted for 3 h compared to animals fasted for 10 h or 9 h compared to 15 h, and yet significant differences were observed between these groups for a number of analytes, supporting the hypothesis that physiological changes were induced via varied postprandial periods independent of temperature variations.

Despite the elevated VO$_2$ that is seen during the SDA to facilitate digestion in reptiles that have consumed large, high protein meals, these animals are also able to mobilize metabolic resources to facilitate exercise during this period (Bennett and Hicks, 2001; Secor et al., 2000). Post-hatchling green turtles generally employ anaerobic metabolic pathways to produce the energy required to perform intense exercise (Hamann et al., 2007), and hence repeat analysis after the fitness test would have been of interest to assess the physiological changes induced by animals that were already engaged in a state of catabolic metabolism. The ability of turtles fasted for 15 h to perform an equal number of turnovers as the turtles fasted for 3 h suggests that this is possible. Unfortunately, repeat analysis was not conducted due to the small size of the animals, limiting the volume of blood available for analysis.

Short-term fasting appears to induce a catabolic state of metabolism in post-hatchling green turtles; however this does not appear to directly impact upon the ability of the animal to produce physical activity. While these animals appear to be able to physiologically compensate for this process, they may incur a metabolic debt that the animal will need to repay once caloric input and pathways of aerobic metabolism are reinitiated. As more intensive management options are employed to improve the conservation of sea turtles, both the

immediate and medium-term fitness of these animals post-release need to be considered. Further work investigating energetics in post-hatchlings, including the time to physiologically recover from intense exercise in both fasted and fed animals, is required before best practice regimes can be employed in release protocols.

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References

Abdi H (2010) Holm's sequential bonferroni procedure. *Encyclopedia of Research Design* 1: 1–8.

Alberghina D, Marafioti S, Spadola F, Panzera M, Piccione G (2015) Influence of short-term storage conditions on the stability of total protein concentrations and electrophoretic fractions in plasma samples from loggerhead sea turtles, caretta caretta. *Comp Clin Path* 24: 1091–1095.

Ashwood E, Kost G, Kenny M (1983) Temperature correction of blood-gas and pH measurements. *Clin Chem* 29: 1877–1885.

Bennett AF, Hicks JW (2001) Postprandial exercise: prioritization or additivity of the metabolic responses? *J Exp Biol* 204: 2127–2132.

Bolten AB, Bjorndal KA (1992) Blood profiles for a wild population of green turtles (Chelonia mydas) in the southern Bahamas: size-specific and sex-specific relationships. *J Wildl Dis* 28: 407–413.

Booth DT, Feeney R, Shibata Y (2013) Nest and maternal origin can influence morphology and locomotor performance of hatchling green turtles (chelonia mydas) incubated in field nests. *Mar Biol* 160: 127–137.

Burgess EA, Booth DT, Lanyon JM (2006) Swimming performance of hatchling green turtles is affected by incubation temperature. *Coral Reefs* 25: 341–349.

Campbell T (2006) Clinical pathology in reptiles. In Mader D ed. *Reptile Medicine and Surgery*, Elsevier Health Sciences, Philadelphia, pp. 453–470.

Curtin F, Schulz P (1998) Multiple correlations and Bonferroni's correction. *Biol Psychiatry* 44: 775–777.

de Vasconcellos Pegas F, Stronza A (2010) Ecotourism and sea turtle harvesting in a fishing village of Bahia, Brazil. *Conservation and Society* 8: 15.

Dutton DL, Dutton PH, Chaloupka M, Boulon RH (2005) Increase of a Caribbean leatherback turtle dermochelys coriacea nesting population linked to long-term nest protection. *Biol Conserv* 126: 186–194.

Fisher LR, Godfrey MH, Owens DW (2014) Incubation temperature effects on hatchling performance in the loggerhead sea turtle (caretta caretta). *PLoS One* 9: e114880.

Flint M, Morton JM, Limpus CJ, Patterson-Kane JC, Murray PJ, Mills PC (2010) Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (Chelonia mydas). *Vet J* 185: 299–304.

Fogh-Andersen N (1981) Ionized calcium analyzer with a built-in pH correction. *Clin Chem* 27: 1264–1267.

Fudge AM (2000) *Laboratory Medicine: Avian and Exotic Pets*, Elsevier Health Sciences, Philadelphia.

Gleeson TT (1996) Post-exercise lactate metabolism: a comparative review of sites, pathways, and regulation. *Annu Rev Physiol* 58: 565–581.

Godfrey M, Mrosovsky N (1997) Estimating the time between hatching of sea turtles and their emergence from the nest. *Chelonian Conserv Biol* 2: 581–584.

Goldberg DW, Leitão SAT, Godfrey MH, Lopez GG, Santos AJB, Neves FA, de Souza ÉPG, Moura AS, Bastos JC, Bastos VLFC (2013) Ghrelin and leptin modulate the feeding behaviour of the hawksbill turtle eretmochelys imbricata during nesting season. *Conserv Physiol* 1: doi: 10.1093/conphys/cot016.

Hamann M, Jessop TS, Schäuble CS (2007) Fuel use and corticosterone dynamics in hatchling green sea turtles (chelonia mydas) during natal dispersal. *J Exp Mar Bio Ecol* 353: 13–21.

Hamann M, Limpus C, Whittier J (2002) Patterns of lipid storage and mobilisation in the female green sea turtle (Chelonia mydas). *J Comp Physiol B* 172: 485–493.

Harms CA, Mallo KM, Ross PM, Segars A (2003) Venous blood gases and lactates of wild loggerhead sea turtles (caretta caretta) following two capture techniques. *J Wildl Dis* 39: 366–374.

Harrenstien LA, Tornquist SJ, Miller-Morgan TJ, Fodness BG, Clifford KE (2005) Evaluation of a point-of-care blood analyzer and determination of reference ranges for blood parameters in rockfish. *J Am Vet Med Assoc* 226: 255–265.

Harry J, Limpus C (1989) Low-temperature protection of marine turtle eggs during long-distance relocation. *Wildl Res* 16: 317–320.

Harter T, Shartau R, Brauner C, Farrell A (2014) Validation of the i-STAT system for the analysis of blood parameters in fish. *Conserv Physiol* 2: doi: 10.1093/conphys/cou037.

Hartzler LK, Munns SL, Bennett AF, Hicks JW (2006) Recovery from an activity-induced metabolic acidosis in the American alligator, Alligator mississippiensis. *Comp Biochem Physiol A Mol Integr Physiol* 143: 368–374.

Haryati JR, Putri JF, Chairiyah N, Harris A, Putri HA, Pamungkas RN (2016) Action plan in developing sea turtle conservation as ecotourism

attraction in Sukamade, Meru Betiri National Park. *Journal of Indonesian Tourism and Development Studies* 4: 67–74.

Heath ME, McGinnis SM (1980) Body temperature and heat transfer in the green sea turtle, chelonia mydas. *Copeia* 767–773.

Hicks JW, Bennett AF (2004) Eat and run: prioritization of oxygen delivery during elevated metabolic states. *Respir Physiol Neurobiol* 144: 215–224.

Hodges VM, Rainey S, Lappin TR, Maxwell AP (2007) Pathophysiology of anemia and erythrocytosis. *Crit Rev Oncol Hematol* 64: 139–158.

Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 65–70.

Hunt KE, Innis CJ, Kennedy AE, McNally KL, Davis DG, Burgess EA, Merigo C (2016) Assessment of ground transportation stress in juvenile Kemp's ridley sea turtles (Lepidochelys kempii). *Conserv Physiol* 4: doi: 10.1093/conphys/cov071.

Innis CJ, Merigo C, Cavin JM, Hunt K, Dodge KL, Lutcavage M (2014) Serial assessment of the physiological status of leatherback turtles (Dermochelys coriacea) during direct capture events in the northwestern Atlantic Ocean: comparison of post-capture and pre-release data. *Conserv Physiol* 2: doi: 10.1093/conphys/cou048.

Keller KA, Innis CJ, Tlusty MF, Kennedy AE, Bean SB, Cavin JM, Merigo C (2012) Metabolic and respiratory derangements associated with death in cold-stunned Kemp's ridley turtles (Lepidochelys kempii): 32 cases (2005–2009). *J Am Vet Med Assoc* 240: 317–323.

Kowalski A (2005) *Specific Dynamic Action in Hatchling and Post-hatchling Green (Chelonia mydas) and Loggerhead (Caretta caretta) Sea Turtles*, Florida Altantic University, Boca Raton.

Kraus DR, Jackson DC (1980) Temperature effects on ventilation and acid-base balance of the green turtle. *Am J Physiol* 239: R254–R258.

Limpus CJ, Baker V, Miller JD (1979) Movement induced mortality of loggerhead eggs. *Herpetologica* 35: 335–338.

Mans C (2008) Venipuncture techniques in chelonian species. *Lab Anim* 37: 303.

Maulany R, Booth D, Baxter G (2012) The effect of incubation temperature on hatchling quality in the olive ridley turtle, Lepidochelys olivacea, from Alas Purwo National Park, East Java, Indonesia: implications for hatchery management. *Mar Biol* 159: 2651–2661.

McGehee MA (1990) Effects of moisture on eggs and hatchlings of loggerhead sea turtles (caretta caretta). *Herpetologica* 46: 251–258.

Moon D-Y, MacKenzie DS, Owens DW (1997) Simulated hibernation of sea turtles in the laboratory: I. Feeding, breathing frequency, blood ph, and blood gases. *J Exp Zool* 278: 372–380.

Owens DW, Ruiz GJ (1980) New methods of obtaining blood and cerebrospinal fluid from marine turtles. *Herpetologica* 36: 17–20.

Page-Karjian A, Rivera S, Torres F, Diez C, Moore D, Dam R, Brown C (2014) Baseline blood values for healthy free-ranging green sea turtles (chelonia mydas) in Puerto Rico. *Comp Clin Path* 24: 567–573.

Parmenter C (1980) Incubation of the eggs of the green sea turtle, chelonia mydas, in Torres Strait, Australia: the effect of movement on hatchability. *Wildl Res* 7: 487–491.

Perrault JR, Bresette MJ, Mott CR, Stacy NI (2018) Comparison of whole blood and plasma glucose concentrations in green turtles (chelonia mydas) determined using a glucometer and a dry chemistry analyzer. *J Wildl Dis* 54: 196–199.

Perrault JR, Page-Karjian A, Miller DL (2016) Nesting leatherback sea turtle (Dermochelys coriacea) packed cell volumes indicate decreased foraging during reproduction. *Mar Biol* 163: 232.

Perrault JR, Stacy NI (2018) Note on the unique physiologic state of loggerhead sea turtles (caretta caretta) during nesting season as evidenced by a suite of health variables. *Mar Biol* 165: 71.

Perrault JR, Wyneken J, Page-Karjian A, Merrill A, Miller DL (2014) Seasonal trends in nesting leatherback turtle (Dermochelys coriacea) serum proteins further verify capital breeding hypothesis. *Conserv Physiol* 2: doi: 10.1093/conphys/cou002.

Pilcher NJ, Enderby S (2001) Effects of prolonged retention in hatcheries on green turtle (chelonia mydas) hatchling swimming speed and survival. *J Herpetol* 633–638.

Plot V, Jenkins T, Robin J-P, Fossette S, Georges J-Y (2013) Leatherback turtles are capital breeders: morphometric and physiological evidence from longitudinal monitoring. *Physiol Biochem Zool* 86: 385–397.

Price ER, Jones TT, Wallace BP, Guglielmo CG (2013) Serum triglycerides and ß-hydroxybutyrate predict feeding status in green turtles (chelonia mydas): evaluating a single blood sample method for assessing feeding/fasting in reptiles. *J Exp Mar Biol Ecol* 439: 176–180.

Reina RD, Jones TT, Spotila JR (2002) Salt and water regulation by the leatherback sea turtle Dermochelys coriacea. *J Exp Biol* 205: 1853–1860.

Rui L (2014) Energy metabolism in the liver. *Compr Physiol* 4: 177–197.

Secor SM (2009) Specific dynamic action: a review of the postprandial metabolic response. *J Comp Physiol B* 179: 1–56.

Secor SM, Hicks JW, Bennett AF (2000) Ventilatory and cardiovascular responses of a python (Python molurus) to exercise and digestion. *J Exp Biol* 203: 2447–2454.

Stabenau EK, Heming TA (1993) Determination of the constants of the Henderson-Hasselbalch equation, (alpha) co2 and pka, in sea turtle plasma. *J Exp Biol* 180: 311–314.

Stacy NI, Bjorndal KA, Perrault JR, Martins HR, Bolten AB (2018) Blood analytes of oceanic-juvenile loggerhead sea turtles (Caretta caretta) from azorean waters: reference intervals, size-relevant correlations and comparisons to neritic loggerheads from western Atlantic coastal waters. *Conserv Physiol* 6: doi:10.1093/conphys/coy006.

Stoot LJ, Cairns NA, Cull F, Taylor JJ, Jeffrey JD, Morin F, Mandelman JW, Clark TD, Cooke SJ (2014) Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conserv Physiol* 2: doi: 10.1093/conphys/cou011.

Thrall MA, Weiser G, Allison R, Campbell T (2012) *Veterinary Hematology and Clinical Chemistry*, John Wiley & Sons, New Jersey.

Tisdell C, Wilson C (2005) Do open-cycle hatcheries relying on tourism conserve sea turtles? Sri lankan developments and economic–ecological considerations. *Environ Manag* 35: 441–452.

Wang S, McDonnell EH, Sedor FA, Toffaletti JG (2002) pH effects on measurements of ionized calcium and ionized magnesium in blood. *Arch Pathol Lab Med* 126: 947–950.

Williamson SA, Evans RG, Robinson NJ, Reina RD (2017) Hypoxia as a novel method for preventing movement-induced mortality during translocation of turtle eggs. *Biol Conserv* 216: 86–92.

Wolf KN, Harms CA, Beasley JF (2008) Evaluation of five clinical chemistry analyzers for use in health assessment in sea turtles. *J Am Vet Med Assoc* 233: 470–475.

Wood A, Booth DT, Limpus CJ (2014) Sun exposure, nest temperature and loggerhead turtle hatchlings: implications for beach shading management strategies at sea turtle rookeries. *J Exp Mar Biol Ecol* 451: 105–114.

Wyneken J, Burke T, Salmon M, Pedersen D (1988) Egg failure in natural and relocated sea turtle nests. *J Herpetol* 88–96.

Wyneken J, Salmon M (1992) Frenzy and postfrenzy swimming activity in loggerhead, green, and leatherback hatchling sea turtles. *Copeia* 478–484.

Yücel D, Dalva K (1992) Effect of in vitro hemolysis on 25 common biochemical tests. *Clin Chem* 38: 575–577.