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Metabolic and cardiovascular adjustments of juvenile green turtles to seasonal changes in temperature and photoperiod

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Summary

We measured activity levels, oxygen consumption, metabolic enzyme activity, breathing frequency, heart rate and blood chemistry variables of juvenile green turtles exposed to a laboratory simulation of subtropical winter and summer temperatures (17–26°C) and photoperiod (10.25 h:13.75 h to 14 h:10 h light:dark). The activity level of turtles had a significant effect on oxygen consumption and breathing frequency but there was no significant change in activity level between the summer and winter simulations. There was a moderate 24–27% decrease in oxygen consumption during exposure to winter conditions compared with summer conditions, but this difference was not statistically significant. Likewise, there was no statistically significant difference in breathing frequency between summer and winter simulations. Exposure to winter conditions did result in a significant decrease in activity of the aerobic enzyme citrate synthase. By contrast, activities of the glycolytic enzymes pyruvate kinase and lactate dehydrogenase were significantly higher in tissue collected during exposure to winter conditions compared with summer conditions. Citrate synthase, pyruvate kinase and lactate dehydrogenase had relatively low thermal dependence over the range of assay temperatures (15–30°C; Q_{10}=1.44–1.69). Heart rate was 46–48% lower during the winter simulation compared with the summer simulation, and this difference was statistically significant. Exposure to winter conditions resulted in a significant decrease in plasma thyroxine and plasma proteins and a significant increase in plasma creatine phosphokinase and hematocrit. Overall, our results suggest that green turtles have a relatively low thermal dependence of metabolic rate over the range of temperatures commonly experienced at tropical to subtropical latitudes, a trait which allows them to maintain activity year-round.

Key words: green turtle, *Chelonia mydas*, metabolism, heart rate, enzyme activity, temperature, season.

Introduction

Green turtles (*Chelonia mydas*) are distributed circumglobally in coastal habitats such as reefs, lagoons and estuaries. Although green turtles primarily inhabit tropical to subtropical latitudes, they experience significant shifts in environmental conditions on a seasonal basis. Seasonal changes in water temperature (T_W), photoperiod and food availability may have profound effects on the metabolism and physiology of ectotherms, but metabolic adjustments that occur in response to seasonal variations in environmental conditions remain largely unexplored in sea turtles.

There is evidence that green turtles from some populations may enter a state of torpor or dormancy during the winter, when environmental conditions are sub-optimal. Felger et al. (1976) observed Eastern Pacific green turtles (*Chelonia mydas agassizii*) in the Gulf of California partially buried in the muddy substrate on the seafloor during winter when T_W was less than 15°C; these turtles were lethargic and unresponsive when handled. Although cold temperatures or changes in food availability may induce metabolic torpor in green turtles, this does not seem to be the common response to typical winter conditions. Sonic tracking studies conducted by Seminoff (2000) show that Eastern Pacific green turtles reside year-round at foraging pastures in the Gulf of California and can maintain activity during both summer and winter months. Based on sea surface temperature data and movement patterns of turtles, Seminoff (2000) suggested that the 'inactivity threshold' for turtles at this site was 15°C and that turtles may become torpid when temperatures drop below this threshold. Studies of the behavior and movements of green turtles from other populations also indicate that turtles can remain active over a wide range of environmental temperatures. Mendonca (1983) tracked the movements of juvenile green turtles in a subtropical lagoon in Florida and found that turtles were active and continued to forage at a T_W as high as 34°C in the summer and as low as 18°C in the winter. Likewise, Read et al. (1996) observed that green turtles in subtropical Moreton Bay, Queensland, Australia were active year-round. Turtles at this study site rapidly fled approaching research boats during the

winter when T_W was 15.0–22.7°C and had food in their buccal cavities upon capture.

Remote monitoring studies and direct observations allow us to monitor the behavior of green turtles in their natural environment during summer and winter, but technological and logistical limitations make at-sea measurements of metabolic and physiological variables quite difficult to accomplish. Laboratory studies with captive turtles provide an attractive alternative to field studies because factors such as temperature and photoperiod may be controlled, and the metabolic and physiological responses to changes in environmental conditions may be closely monitored. Previous experiments with captive green turtles have shown that acute decreases in temperature result in a decrease in metabolic rate over the range of 30–10°C (Davenport et al., 1982), a decrease in heart rate over the range of 35–15°C (Smith et al., 1986) and greatly reduced peripheral blood flow over the range of 32–17°C (Hochscheid et al., 2002). However, the metabolic and cardiovascular responses of green turtles to gradual and prolonged shifts in temperature and photoperiod, such as are experienced on a seasonal basis, have not previously been investigated.

Green turtles from many populations appear to be capable of maintaining activity year-round in tropical and subtropical habitats, despite fluctuations in environmental conditions. The purpose of our study was to investigate the effects of seasonal changes in temperature and photoperiod on the metabolism and physiology of green turtles and to assess the ability of green turtles to acclimate to changing environmental conditions. Maintenance of activity over the range of temperatures experienced seasonally may be accomplished by either a low thermal dependence of metabolism and supporting physiological functions or thermal acclimation of metabolic rate. We exposed captive turtles to an environmental simulation based on seasonal changes in T_W and photoperiod commonly experienced by green turtles at subtropical latitudes. Changes in temperature and photoperiod were specifically modeled after environmental conditions in Moreton Bay, Queensland, Australia (27°30′ S, 153°18′ E), as this site harbors a resident population of green turtles that are known to remain active year-round. Oxygen consumption ($\dot{V}_{O_2}$), breathing frequency (f_B) and heart rate (f_H) of turtles were measured during exposure to simulated summer and winter conditions. We also collected muscle tissue samples from turtles during the summer and winter simulations so that we could determine the effects of temperature on the function of important enzymes in aerobic and anaerobic metabolic pathways. In particular, our goal was to assess the thermal dependence of these metabolic enzymes and whether metabolic compensation of reaction rates occurred with prolonged exposure to low T_W during the winter simulation. Blood samples were collected from turtles during exposure to summer and winter conditions so that hematocrit and plasma levels of glucose, proteins, ions, creatine phosphokinase and thyroxine (T4) could be determined.

Materials and methods

Animals

Five juvenile green turtles (*Chelonia mydas* L.) were transported from the Cayman Island Turtle Farm (Grand Cayman, British West Indies) to the Zoology Animal Care Facility at the University of British Columbia (Vancouver, Canada). The turtles were kept in a 10 m×2 m×1.5 m oval fiberglass tank with 13 250 l of re-circulating seawater filtered through a series of mechanical and biological filters. The tank was located in a covered enclosure, and a series of lighting fixtures with UV bulbs (Vita-Lite; Houston, TX, USA) were placed over the tank to ensure proper lighting conditions. Seawater was obtained from the Vancouver Aquarium and Marine Science Center each month to replace the water in the tanks. Water temperature was maintained at 26°C and salinity was 26–32 p.p.m. The turtles were fed a diet of vitamin D- and calcium-supplemented trout chow (0.5–1.0% of body mass daily). Turtles were weighed on a monthly basis. Turtle body masses ranged from 18.9 kg to 43.1 kg during the course of this study. The mean mass of turtles was 24.1±1.9 kg (mean ± S.E.M.) at the beginning of the study and had increased to 32.5±2.8 kg by the end of the study.

All procedures were approved by the University of British Columbia's Animal Care Committee in accordance with guidelines set by the Canadian Committee on Animal Care.

Seasonal simulation

Turtles were exposed to changes in temperature and photoperiod that simulated seasonal changes in these variables at Moreton Bay, Australia. Mean T_W during summer at Moreton Bay is 26°C, and mean T_W during the winter is 17°C (Read et al., 1996). In our study, turtles were initially held at a T_W of 26°C for a period of several months. T_W was then decreased by 3°C every 2 weeks until it reached 17°C. Turtles were maintained in 17°C water for 16 weeks and then T_W was increased by 3°C every 2 weeks until it was once again 26°C. Photoperiod was modified based on seasonal sunrise and sunset times at Moreton Bay: 14 h:10 h light:dark at 26°C; 12.75 h:11.25 h light:dark at 23°C; 11 h:13 h light:dark at 20°C; and 10.25 h:13.75 h light:dark at 17°C. We defined 'summer conditions' as a T_W of 26°C with a 14 h:10 h light:dark photoperiod. 'Winter conditions' were defined as a T_W of 17°C with a 10.25 h:13.75 h light:dark photoperiod.

Turtle body temperature (T_B), specifically the subcarapace temperature, was measured periodically using temperature-sensitive passive induced transponder (PIT) tags (BioMedic Data Systems, Maywood, NJ, USA) implanted beneath left marginal scute #2, just above the left front flipper. The tags had a resolution of ±0.1°C and were calibrated over the range of 20–35°C using a National Institute of Standards and Technology traceable thermometer (0.05°C gradations) before implantation.

Oxygen consumption and breathing frequency

Open-flow respirometry was used to measure oxygen consumption ($\dot{V}_{O_2}$) of turtles. Two days before a respirometry

trial, the turtle was moved into a smaller circular fiberglass tank (2 m diameter). The surface of the tank was covered except for a 30 cm^2 area. Turtles were trained to surface in this area and to breathe under a Plexiglas funnel placed over the water surface. A one-way respiratory valve (Hans Rudolph, Kansas City, MO, USA) was placed on the inflow port of the funnel, and a T-junction with one arm leading to a latex gas collection bag to receive overflow expired gas was placed on the outflow port of the funnel. Air was pulled through the funnel at a rate of 4.0 l min^{-1} by an air pump (Shop-vac Canada Ltd., Burlington, ON, Canada), and air flow was monitored with a mass flowmeter (Omega Engineering Inc., Stamford, CT, USA). Exhaled air that entered the overflow bag was eventually pulled back through the T-junction and into the main airstream. A sub-sample of funnel gas was drawn through Drierite and soda-lime before passing through an SAE 300 oxygen analyzer at a rate of 150 ml min^{-1}. The percentage of oxygen (%O_2) in the sub-sample from the funnel was recorded on a computer at a frequency of 1 Hz using LabTech software. Downward deflections from 20.94% O_2 (concentration of O_2 in dry air) occurred when the turtle breathed into the funnel. $\dot{V}_{O_2}$ was determined by measuring the area of downward deflections in a data analysis program (Acqknowledge, BIOPAC Systems, Inc., Santa Barbara, CA, USA). The respirometry system was calibrated by injecting known quantities of nitrogen into the funnel. Values for $\dot{V}_{O_2}$ were corrected for STPD.

Respirometry trials lasted 3–5 h and were conducted between 10:00 h and 15:00 h. A video camera was set up above the experiment tank and all trials were videotaped so that behavior and breathing frequency could be monitored continuously. The amount of time spent resting or active during the trial was calculated. The turtle was considered to be resting if motionless on the tank bottom. Active periods consisted of turtles slowly paddling around the side of the tank or shuffling along the tank bottom.

$\dot{V}_{O_2}$ and f_B were recorded during exposure to summer conditions before and 20 weeks after the winter simulation (26-pre and 26-post, respectively), and at 8 weeks and 16 weeks exposure to winter conditions (17-8w and 17-16w, respectively). Turtles were fasted for 5–7 days before respirometry trials in summer conditions and for 10–12 days before trials in winter conditions to ensure that they were in a post-absorptive state (Davenport et al., 1982; Hadjichristophorou and Grove, 1983; Brand et al., 1999). $\dot{V}_{O_2}$ was corrected for differences in body mass using an exponent of 0.83. This exponent was derived for green turtles weighing between 0.3 kg and 141.5 kg (ambient temperature T°=23–27°C; Prange and Jackson, 1976) and is similar to exponents derived for the Aldabra giant tortoise (*Geochelone gigantea*; exponent=0.82, mass=0.1–35.5 kg, T°=21–26°C; Hughes et al., 1971), 10 species of aquatic turtles (exponent=0.86, mass=0.3–132 kg, T°=20°C; Bennett and Dawson, 1976) and 26 species of lizards (exponent=0.83, mass=0.001–4.4 kg, T°=30°C; Bennett and Dawson, 1976). The effect of the seasonal simulation on $\dot{V}_{O_2}$ and f_B was analyzed using repeated measures analysis of covariance (ANCOVA) with activity level as the co-variate. Activity levels of turtles during summer and winter trials were compared using repeated measures analysis of variance (ANOVA). Mean values for $\dot{V}_{O_2}$ and f_B in summer conditions (26-pre and 26-post combined) and winter conditions (17-8w and 17-16w combined) were calculated for each turtle, and Pearson correlation was used to investigate the relationship between mean $\dot{V}_{O_2}$ and mean f_B during simulated summer and winter conditions.

Metabolic enzyme activity

Muscle tissue samples were obtained so that the effects of temperature on muscle enzyme activity could be assessed. Muscle tissue samples were obtained from the flexor tibialis muscle of the rear flipper during exposure to summer conditions before the winter simulation and after 4 weeks exposure to winter conditions. The flexor tibialis flexes and retracts the rear flipper and controls the shape of the flipper for steering (Wyneken, 2001). Before excising muscle tissue, the incision area was cleaned thoroughly with 95% EtOH and Betadine topical antiseptic. A local anaesthetic (2% Lidocaine; Vetoquinol Inc., Lavaltrie, QC, Canada) was injected into the area from which the biopsy sample was to be taken. A 1.5 cm incision was made in the skin and approximately 100–200 mg of muscle tissue was excised using surgical scissors. Dissolvable sutures were used to sew muscle tissue together and close the incision wound. The area was treated with topical antibiotic cream (Furacin; Vetoquinol Inc.) and the turtle was given an intramuscular injection of antibiotics (5 mg kg^{-1} Amiglyde-V; Ayerst Veterinarian Laboratories, Guelph, ON, Canada) to reduce the risk of infection. The tissue samples were immediately frozen in liquid nitrogen and rapidly transferred to a –70°C freezer for storage. Tissue samples were stored for 20–22 months before assays were performed. Activity of citrate synthase (CS), lactate dehydrogenase (LDH) and pyruvate kinase (PK) were determined for all tissue samples.

Tissue samples were partially thawed, minced and diluted to 1/10 volume in ice-cold 75 mmol l^{-1} Tris-HCl homogenization buffer adjusted for pH 7.5 at room temperature. The dilution was homogenized using a Polytron tissue homogenizer (model PT10; Brinkman Instruments, Rexdale, ON, Canada) and sonicated using a MicroUltrasonic Cell Disrupter (model KT5; Kontes, Vineland, NJ, USA). Whole homogenate was used for all assays and each assay was run in duplicate. Enzyme activities were measured with a Perkin-Elmer spectrophotometer [model Lambda 2; Perkin-Elmer (Canada) Ltd, Rexdale, ON, Canada]. Temperature in the spectrophotometer cells was controlled with a circulating water bath (MGW Lauda, Lauda, Germany). Enzyme activity was measured at assay temperatures of 15°C, 20°C, 25°C and 30°C. The order in which activity was measured at different assay temperatures was randomized. Stock solutions for assays were prepared using buffers adjusted for pH at each temperature. All reactions were initiated by the addition of substrate. The millimolar extinction coefficient (ϵ) and

wavelength at which the reaction was monitored (λ) are indicated for each assay below.

Enzyme protocols were as follows.

LDH: λ=340 nm, ϵ=6.22, pH 7.5, 50 mmol l^{-1} imidazole-HCl, 0.15 mmol l^{-1} β-nicotinamide adenine dinucleotide (reduced), 4 mmol l^{-1} pyruvate, 1/1000 tissue dilution.

PK: λ=340 nm, ϵ=6.22, pH 7.0, 50 mmol l^{-1} imidazole-HCl, 10 mmol l^{-1} magnesium chloride, 100 mmol l^{-1} potassium chloride, 0.15 mmol l^{-1} β-nicotinamide adenine dinucleotide (reduced), 7 mmol l^{-1} phosphoenol pyruvate, 5 mmol l^{-1} adenine diphosphate, excess LDH, 1/500 tissue dilution.

CS: λ=412 nm, ϵ=13.6, pH 8.0, 100 mmol l^{-1} Tris-HCl, 0.3 mmol l^{-1} acetyl-CoA, 0.5 mmol l^{-1} oxaloacetate (omitted for control), 0.1 mmol l^{-1} 5,5′-dithiobis-(2-nitrobenzoic acid), 1/10 tissue dilution.

All chemicals were obtained from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada).

The effects of the seasonal simulation and assay temperature on enzyme activity were analyzed using repeated measures two-way ANCOVA with turtle body mass as co-variate. Thermal coefficients (Q_{10} values) were calculated for enzyme activity over the assay temperature range of 15–30°C.

Heart rate

Electrocardiograms (ECGs) were recorded from turtles using custom-built data loggers (Andrews, 1998). Electrode assemblies were constructed of 34G polyethylene-coated medical stainless steel wire (5 cm length; Cooner Wire, Chatsworth, CA, USA) soldered to waterproof connectors (model MS1F-1; Underwater Systems Inc., Stanton, CA, USA). The distal tip of the medical wire was stripped of 0.5 cm of the polyethylene coat. The medical wire portion of the assembly served as the internal electrode, and the waterproof connectors were externalized so that electrodes could be interfaced with the data logger. Medical wire was inserted into the body cavity of the turtle through 2 mm holes drilled through left marginal scute #9 (LM9) and right marginal scute #5 (RM5) of the carapace 1–2 days before ECG trials. A blunted stainless steel shaft inserted through the drill hole in scute LM9 was used to guide an electrode to a depth of 5 cm into the body cavity. The same procedure was used to insert another electrode to a depth of 5 cm through scute RM5. The positioning and polarity of electrodes corresponded with ECG Lead II of Einthoven's triangle. The holes in the carapace were treated with local anaesthetic (2% Lidocaine) as soon as they were drilled and coated with topical antibacterial gel (Furacin). Electrodes were secured in the holes with tissue glue (3M; Vet-Bond, St Paul, MN, USA) and the hole was sealed with fast-curing epoxy (5-Cure; Industrial Formulators, Burnaby, BC, Canada). The externalized waterproof connector was glued flat to the surface of the carapace at the site of electrode insertion. Electrodes remained implanted for a maximum of 5 days, during which time the turtle was isolated in a 2 m diameter tank (1 m depth) so that other turtles would not disturb the externalized portion of the electrode assembly.

ECG was recorded during exposure to summer conditions and after 16 weeks exposure to winter conditions. ECG trials were conducted between 10:00 h and 17:00 h in the 2 m-diameter isolation tank, and trials were videotaped so that activity could be monitored. Behavior was categorized as 'active' or 'resting' in the same manner as for turtles during respirometry trials. When the ECG recordings were completed, the electrodes were removed and the drill holes were treated with topical antibiotics and re-sealed with epoxy. Antibiotics (2.5 mg kg^{-1} Amiglyde-V) were administered to the turtle intramuscularly every 3 days for 2 weeks following electrode removal to lower the risk of infection. Drill holes healed completely within 4 weeks of electrode removal.

Heart rate was determined from the ECG trace using the Acknowledge data analysis program (BIOPAC systems, Inc). Cardiac intervals (CI) were derived from the ECG trace by measuring the time between consecutive R-R peaks. Values for CI were converted to instantaneous heart rate in beats min^{-1}. Sections of the ECG trace in which the QRS complex could not be distinguished were excluded from data analysis. Mean resting f_H was determined for all five turtles but, because of increased background noise due to electrical signals from muscle contraction during swimming, active heart rates could only be distinguished on ECG traces from three turtles. Mean active f_H was calculated for these three turtles. Increased EMG activity was associated with breathing episodes for all turtles, so breathing f_H could not be determined.

The effects of the seasonal simulation and activity state on f_H were analyzed using two-way repeated measures ANOVA.

Blood chemistry

Blood samples (5 ml) were collected from turtles during exposure to summer conditions and after 16 weeks exposure to winter conditions. All blood samples were drawn from the venous cervical sinus using heparinized Vacutainer™ tubes with 21G×1½ needles. A subsample of blood was immediately transferred to duplicate microcapillary tubes and centrifuged so that hematocrit could be determined. The remainder of the blood was centrifuged at 3000 r.p.m. for 5 min. Plasma was transferred to cryo-safe plastic tubes (Sarstedt Inc., Montreal, QC, Canada) and stored in a –70°C freezer. Samples were sent to a pathology laboratory (Central Laboratory for Veterinarians Ltd, Langley, BC, Canada) for analysis of glucose, proteins, ion and mineral content and thyroxine (T4). Creatine phosphokinase (CPK) activity at 37°C was determined using an automated clinical chemistry analyzer (Dade-Behring Canada, Mississauga, ON, Canada). Paired *t*-tests were used to test for significant differences in blood chemistry variables for turtles during exposure to summer and winter conditions.

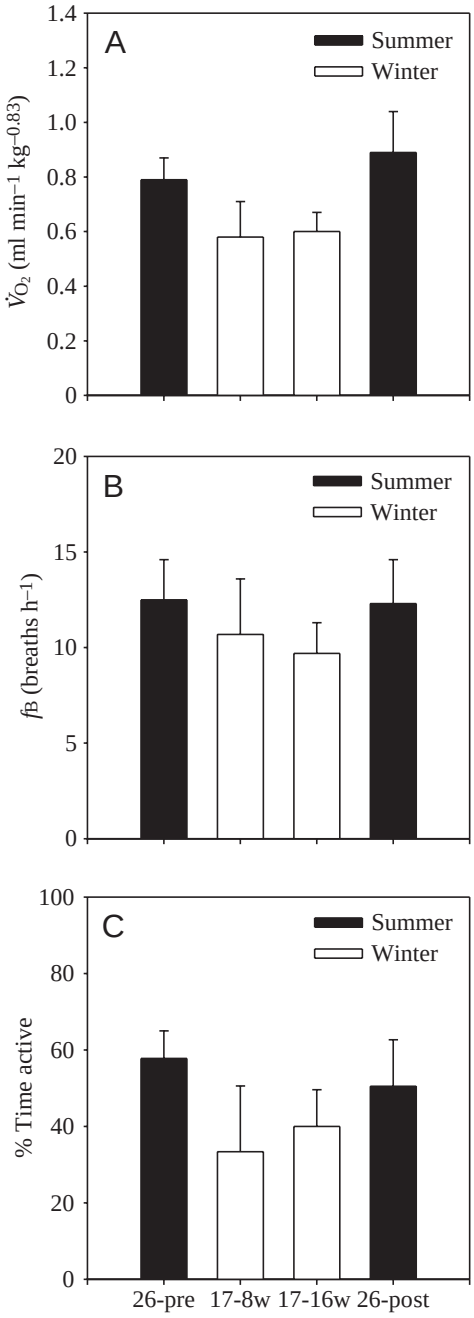
Statistics

Statistical analyses described in the previous sections were performed using JMPIN software (SAS Institute, Inc., Cary, NC, USA). Differences were considered to be significant at $P<0.05$. Values are presented as means ± S.E.M.

Results

Oxygen consumption and breathing frequency

Fig. 1 shows the effects of the seasonal simulation on $\dot{V}_{O_2}$, f_B and activity. Mean $\dot{V}_{O_2}$ during exposure to summer conditions was 0.79±0.08 to 0.89±0.15 ml min^{-1} kg$^{-0.83}$ (26-pre and 26-post, respectively). During exposure to winter conditions, mean $\dot{V}_{O_2}$ decreased to 0.58±0.13 to 0.60±0.07 ml min^{-1} kg$^{-0.83}$ (17-8w and 17-16w, respectively) but there was no statistically significant difference in $\dot{V}_{O_2}$ between summer and winter conditions (P=0.059, F=3.469, d.f.=3; Fig. 1A). Activity level of turtles had a significant effect on $\dot{V}_{O_2}$ (P<0.001, F=25.747, d.f.=1), with higher activity levels resulting in higher $\dot{V}_{O_2}$ (Fig. 2).



There was no significant decrease in f_B during exposure to winter conditions (P=0.288, F=1.444, d.f.=3; Fig. 1B). Turtles had a mean f_B of 10.8±1.5 breaths h^{-1} at 26-pre and 12.3±2.3 breaths h^{-1} at 26-post. Mean f_B decreased to 9.6±3.4 breaths h^{-1} at 17-8w and 9.1±2.0 breaths h^{-1} at 17-16w. There was a significant correlation between mean f_B and mean $\dot{V}_{O_2}$ during exposure to summer conditions (r=0.982, P=0.018, t=7.430, d.f.=3; Fig. 3A), but the correlation was not significant in winter conditions (r=0.514, P=0.375, t=1.040, d.f.=3; Fig. 3B). Activity level of turtles had a significant effect on f_B (P<0.001, F=24.638, d.f.=1).

Turtles were active for an average of 61.0±16.8% of the time during trials at 26-pre and 50.5±12.8% of the time during trials at 26-post (Fig. 1C). The mean time spent active was 38.3±21.3% during trials at 17-8w and 47.8±7.4% during trials at 17-16w. Mean percent time spent active during exposure to winter conditions was not significantly lower than mean percent time spent active in summer conditions (P=0.400, F=1.073, d.f.=1).

Turtle T_B was not significantly higher than T_W during respirometry trials conducted during exposure to either summer or winter conditions (Student's t-test, P=0.563–0.834, t=0.378–0.612). Growth rates during exposure to winter conditions (0.78±0.12 g day^{-1} kg^{-1}) were significantly lower compared with summer conditions (1.59±0.10 g day^{-1} kg^{-1}) (P=0.010, t=4.608).

Metabolic enzyme activity

Activity of the aerobic enzyme citrate synthase at any given assay temperature was significantly higher in muscle tissue collected during summer conditions compared with tissue collected during winter conditions (P<0.001, F=17.313, d.f.=1; Fig. 4A). CS activity was significantly affected by mass of the turtle (P=0.002, F=11.711, d.f.=1), with larger turtles having lower mass-specific CS activity. The Q_{10} value for CS activity over the range of 15–30°C was 1.44 for both summer- and winter-acclimated tissue. CS activity at an assay temperature of 30°C was significantly higher than activity at all other assay temperatures (P=0.022, F=3.734, d.f.=3). There was no significant difference in CS activity at assay temperatures of 15°C, 20°C and 25°C.

The enzymes LDH and PK showed similar responses to

Fig. 1. Effects of a laboratory simulation of summer and winter conditions on (A) oxygen consumption ($\dot{V}_{O_2}$), (B) breathing frequency (f_B), and (C) percent time spent active of captive juvenile green turtles. Filled bars represent simulated summer conditions (T_W=26°C, photoperiod 14 h:10 h light:dark) and open bars represent simulated winter conditions (T_W=17°C, photoperiod 10.25 h:13.75 h light:dark). Variables were measured during exposure to summer conditions before and after the winter simulation (26-pre and 26-post, respectively) and after 8 weeks and 16 weeks exposure to winter conditions (17-8w and 17-16w, respectively). There was no statistically significant difference in $\dot{V}_{O_2}$ (P=0.059), f_B (P=0.288) or percent time spent active (P=0.400) between treatment groups. N=5 for all treatment groups except 26-post, in which N=4.

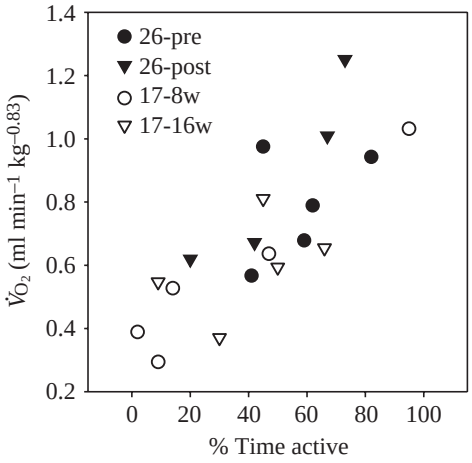


Fig. 2. There was a significant positive relationship between activity and oxygen consumption ($\dot{V}_{O_2}$; r^2=0.63, P<0.001) for juvenile green turtles. Data points for individual turtles are shown for the summer simulation (filled symbols) and winter simulation (open symbols).

changes in temperature. For both enzymes, activity at any given assay temperature was significantly higher in tissue collected during exposure to winter conditions than in tissue collected during exposure to summer conditions (LDH: P<0.001, F=59.014, d.f.=1; PK: P<0.001, F=25.255, d.f.=1; Fig. 4B,C). Mass did not significantly affect activity of LDH (P=0.230, F=1.499, d.f.=1) or PK (P=0.224, F=1.539, d.f.=1). The Q_{10} value for LDH was 1.61 for summer-acclimated tissue and 1.48 for winter-acclimated tissue. The Q_{10} of PK activity was 1.67 for summer-acclimated tissue and 1.69 for winter-acclimated tissue. There was no significant difference in activity at assay temperatures of 20–25°C for either LDH or PK. Activity of LDH and PK at 15°C was significantly lower than at all other assay temperatures, and activity at 30°C was significantly higher than at all other assay temperatures (LDH: P<0.001, F=46.283, d.f.=3; PK: P<0.001, F=33.627, d.f.=3).

Heart rate

Heart rate was significantly lower during exposure to winter conditions compared with summer conditions (P<0.001, F=89.030, d.f.=1). Active f_H in winter conditions (12.9±0.6 beats min^{-1}) was 46% lower than active f_H in summer conditions (24.0±2.4 beats min^{-1}), and resting f_H in winter conditions (10.2±0.9 beats min^{-1}) was 48% lower than resting f_H in summer conditions (19.6±1.5 beats min^{-1}) (Fig. 5). There was a significant difference between active and resting f_H at both temperatures (P=0.012, F=9.293, d.f.=1).

Blood chemistry

There was no significant difference in the plasma levels of glucose, sodium, potassium, calcium, phosphorus, chloride or globulin for turtles during exposure to summer and winter conditions (Table 1). Plasma levels of albumin (P<0.001, t=9.129), total protein (P<0.047, t=2.845) and T4 (P<0.001, t=11.918) were significantly lower in turtles during exposure

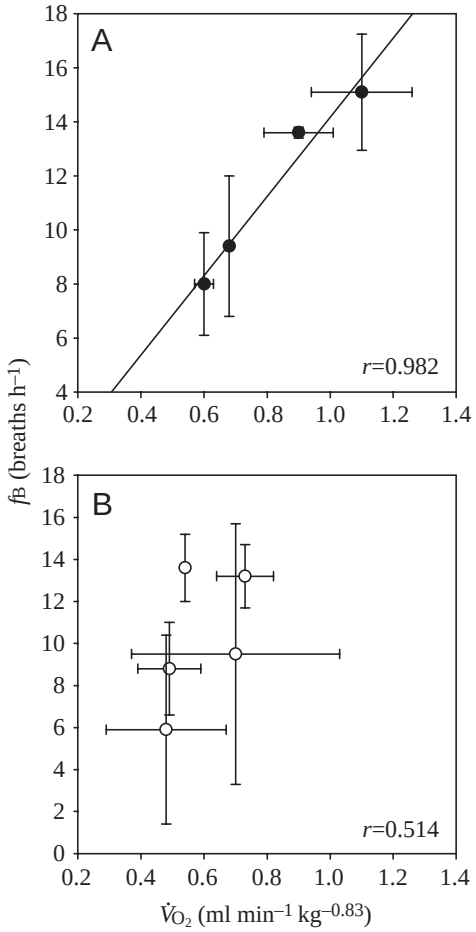


Fig. 3. (A) There was a significant correlation between breathing frequency (f_B) and oxygen consumption ($\dot{V}_{O_2}$) of green turtles during exposure to summer conditions (f_B=–0.496+14.660×$\dot{V}_{O_2}^{-0.83}$, r=0.982, P=0.018, N=4). Data points represent means ± S.E.M. for individual turtles during exposure to summer conditions before (26-pre) and after (26-post) the winter simulation. (B) The correlation between f_B and $\dot{V}_{O_2}$ was much weaker and not significant during exposure to winter conditions (r=0.514, P=0.375, N=5). Data points represent means ± S.E.M. for individual turtles after 8 weeks (17-8w) and 16 weeks (17-16w) exposure to winter conditions.

to winter conditions compared with levels during exposure to summer conditions. Blood variables that increased significantly during exposure to winter conditions were plasma CPK (P=0.024, t=3.545) and hematocrit (P=0.003, t=6.216).

Discussion

One of the primary goals of this study was to develop an integrative picture of the metabolic response of green turtles to simulated seasonal changes in temperature and photoperiod. To this end, indices of metabolism were measured at the molecular and organismal levels during exposure to summer and winter conditions. Activities of aerobic and anaerobic enzymes were measured to determine the effects of temperature variation and acclimation on the function of

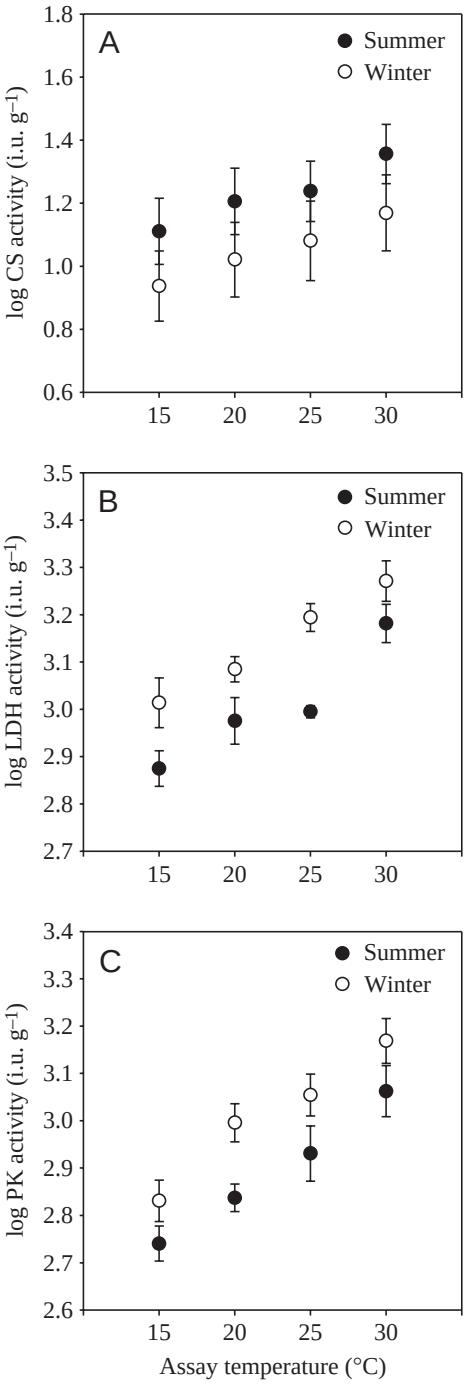


Fig. 4. Activity (i.u. g^{-1} wet mass) of (A) citrate synthase (CS), (B) lactate dehydrogenase (LDH) and (C) pyruvate kinase (PK) in flexor tibialis muscle of captive juvenile green turtles (N=5) exposed to summer conditions (filled circles) and after 4 weeks exposure to winter conditions (open circles). CS activity was significantly lower in tissue collected from turtles exposed to winter conditions compared with summer conditions (P<0.001). Conversely, LDH and PK activity were significantly higher during exposure to winter conditions compared with summer conditions (P<0.001), a pattern typical of thermal acclimation. CS, LDH and PK had relatively low thermal dependence over the 15–30°C range of assay temperatures (Q_{10}=1.44–1.69).

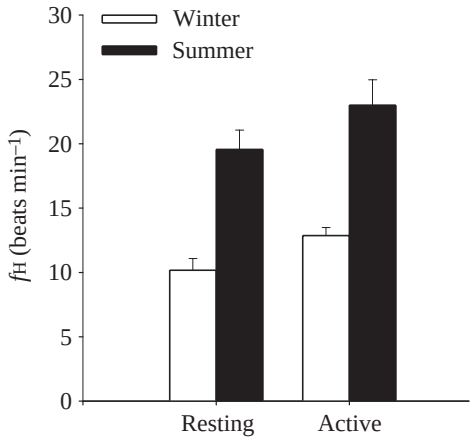


Fig. 5. A comparison of resting (N=5) and active (N=3) heart rates of juvenile green turtles during exposure to summer (filled bars) and winter (open bars) conditions. Heart rates during exposure to winter conditions were significantly lower than heart rates during the summer simulation (P<0.001). Active heart rates were significantly higher than resting heart rates during the summer and winter simulations (P=0.012).

molecular metabolic machinery, and $\dot{V}_{O_2}$ was measured so that the overall metabolic effect of seasonal changes in environmental conditions could be assessed. Our integrative analysis of metabolism provides insight not only into the metabolic response at the whole animal level but also into the potential mechanisms underlying this response.

Given the pervasive effects of temperature on biochemical reaction rates, structure and stability of proteins and membranes, and physiological processes, it was not surprising to see a trend of decreased $\dot{V}_{O_2}$ with exposure to a lower temperature (17°C) during the winter simulation (Fig. 1A). Although statistically there was no significant difference in $\dot{V}_{O_2}$ between simulated summer and winter conditions (P=0.059), the trend warrants comment. Previous studies with green turtles have provided values for $\dot{V}_{O_2}$ at various temperatures, but the thermal dependence of $\dot{V}_{O_2}$ (i.e. Q_{10} value) has not been explicitly stated (Kraus and Jackson, 1980; Davenport et al., 1982), perhaps because factors other than temperature, such as digestive state or activity level, were not controlled. Lutz et al. (1989) reported a Q_{10} of 2.4 for $\dot{V}_{O_2}$ of loggerhead sea turtles (4.3–22.7 kg) exposed to acute decreases in temperature over the range of 30–10°C. A Q_{10} of 2.4 applied to our green turtle data results in a predicted $\dot{V}_{O_2}$ of 0.36 ml min^{-1} $kg^{-0.83}$ at 17°C, a decrease of 54% from the mean $\dot{V}_{O_2}$ at 26°C before initiation of the winter simulation. In fact, mean $\dot{V}_{O_2}$ during exposure to 17°C (0.58–0.60 ml min^{-1} $kg^{-0.83}$) was 24–27% lower than mean $\dot{V}_{O_2}$ at 26°C (0.79–0.89 ml min^{-1} $kg^{-0.83}$); the magnitude of the decrease in $\dot{V}_{O_2}$ of green turtles between summer and winter conditions is much less than that predicted using a Q_{10} of 2.4.

One possible explanation for this discrepancy is that turtles undergo some degree of metabolic compensation during chronic exposure to colder temperatures. If thermal

Table 1. *Blood chemistry variables for captive juvenile green turtles during exposure to 26°C and 17°C*

	26°C	17°C	*t*	*P*
Glucose (mmol l^{-1})	5.0±0.4	5.2±0.2	0.590	0.587
Total protein (g l^{-1})	44.6±2.4	38.2±1.5	2.845	0.047*
Albumin (g l^{-1})	22.4±0.8	17.4±0.4	9.129	<0.001*
Globulin (g l^{-1})	22.2±1.9	20.8±1.4	0.787	0.475
Sodium (mmol l^{-1})	146.0±1.5	147.8±0.7	1.686	0.167
Potassium (mmol l^{-1})	4.1±0.5	3.1±0.2	1.739	0.157
Calcium (mmol l^{-1})	2.3±0.2	2.1±0.1	0.542	0.617
Phosphorus (mmol l^{-1})	2.2±0.1	1.8±0.1	2.525	0.065
Chloride (mmol l^{-1})	103.6±1.2	104.4±1.2	0.691	0.528
Creatine phosphokinase (i.u. l^{-1})	450.0±146.6	2091.2±525.2	3.545	0.024*
Thyroxine (nmol l^{-1})	17.8±1.5	3.4±0.7	11.918	<0.001*
Hematocrit	33.6±0.5	40.0±1.3	6.216	0.003*

Values are means ± S.E.M. (N=5).
*Values for this variable were significantly different at 26°C and 17°C (P<0.05).

acclimation of metabolic rate occurs, it should be reflected at the molecular level by adjustments in metabolic enzyme activity. One of the most common mechanisms to compensate for decreased kinetic energy of reactant molecules at low temperature is an increase in enzyme concentration (Hochachka and Somero, 2002). If a compensatory increase in enzyme concentration occurs with prolonged cold exposure, maximal enzyme activity in tissue obtained from cold-acclimated animals is higher than activity in tissue obtained from warm-acclimated animals when compared at a common temperature. In the current study, activity of the aerobic enzyme CS in tissue collected during exposure to 17°C was significantly lower than CS activity in tissue collected during exposure to 26°C (Fig. 4A), a pattern opposite to what we would expect if a compensatory adjustment in CS activity had been made during cold exposure (see discussion below). Therefore, there is no evidence of thermal acclimation of oxygen consumption at the molecular level.

An alternative explanation for the moderate decrease in $\dot{V}_{O_2}$ during exposure to 17°C compared with 26°C is that metabolic rate has a low thermal dependence over this temperature range. This speculation is supported by the fact that CS activity had a low thermal dependence (Q_{10}=1.44) over the 15–30°C range of assay temperatures, and there was no significant difference in CS activity at assay temperatures of 15°C, 20°C and 25°C. Several species of reptiles display a metabolic plateau over the temperature range in which they are normally active, i.e. metabolic rate remains stable and Q_{10} approaches 1 within a range of several °C (Bennett and Dawson, 1976; Waldschmidt et al., 1987; Angilletta, 2001). Penick et al. (1996) measured tissue metabolic rates ($\dot{V}_{O_2}$) of isolated skeletal muscle, heart, liver, intestine and kidneys from freshly killed juvenile green turtles and found that Q_{10} values for tissue $\dot{V}_{O_2}$ were relatively low within the temperature range of 12.5–27.5°C (Q_{10}=0.79–1.82). Green turtle tissues generally had a lower thermal dependence than tissues from cold-climate garter snakes (*Thamnopnis* spp.) and eurythermic lizards (*Leiolopisma zelandica* and *Eumeces obsoletus*; Penick et al., 1996).

In the current study, $\dot{V}_{O_2}$ and metabolic enzyme activity data indicate that green turtles have a low thermal dependence of aerobic metabolism over the range of temperatures experienced during the seasonal simulation (17–26°C). If this is the case, then populations of green turtles that experience seasonal fluctuations in temperature within this range should be capable of maintaining activity year-round. Field observations of green turtles at Moreton Bay, the study site that was used to model our laboratory seasonal simulation, support this speculation (Read et al., 1996). It should be noted, however, that Q_{10} values typically increase at the lower and upper limits of an animal's optimal thermal range (Bennett and Dawson, 1976; Hochachka and Somero, 2002). Increased thermal dependence of physiological and biochemical processes at lower temperatures may result in a drastic reduction in activity and metabolic rate. Laboratory and field studies have shown that green turtles become quiescent and cease feeding at a T_W of 15°C or lower (Felger et al., 1976; Moon et al., 1997; Seminoff, 2000), and green turtles subjected to rapid decreases in T_W within the range of 8–15°C commonly develop a pathological condition called 'cold-stunning', which results in a loss of respiratory function and buoyancy control (Sadove et al., 1998). Obviously, metabolic and physiological responses to decreases in environmental temperature depend on both the magnitude of the temperature change and how quickly the temperature changes.

Plasma T4 levels of vertebrates are commonly associated with metabolic state, and seasonal variation in plasma T4 has been observed in several reptile species. Changes in reproductive or developmental state, nutritional status, general activity levels and temperature may all result in changes in plasma T4 (Lynn, 1970). Given that the last of these three factors varied for green turtles exposed to our seasonal simulation, it is difficult to pinpoint the exact cause of the decrease in T4 during exposure to winter conditions. The fact

that growth rates, blood protein levels and presumably food consumption were lower during exposure to winter conditions suggests that nutritional status may play a key role. This assertion is supported by data from earlier studies, which show that at a constant temperature increases in food quantity and quality result in significantly higher plasma T4 levels in juvenile green turtles (Moon et al., 1999). Variation in temperature and photoperiod may also affect T4 production, but it is difficult to assess the direct effects of these variables in the current study.

The decrease in plasma T4 during exposure to winter conditions may provide an explanation for the pattern observed for CS activity during the seasonal simulation. CS activity during exposure to winter conditions was significantly lower than during exposure to summer conditions. Several studies have shown that CS activity of reptiles can be affected by increases or decreases in T4 levels. Lizards injected with T4 or implanted with subcutaneous T4 pellets had significantly higher CS activity in liver and muscle tissue than control lizards, whereas thyroidectomy of lizards resulted in significantly reduced plasma T4 and reduced CS activity (John-Alder, 1983, 1990a,b). Other studies have shown that CS activity, maximal $\dot{V}_{O_2}$ and endurance of field-active lizards undergo a pattern of seasonal variation that parallels changes in plasma T4 levels (Lynn, 1970; John-Alder, 1984), which suggests that thyroid hormones may be involved in regulating aerobic energetic capacities. If green turtles in the field experience a decrease in T4 levels during the winter months, a concurrent decrease in aerobic capacity may also occur. Interestingly, and in contrast to the current study, blood samples collected monthly from juvenile green turtles at a tropical field site showed no evidence of a seasonal shift in plasma T4 (Moon, 1992). As noted previously, there are numerous environmental and physiological factors that affect plasma T4 levels on a seasonal basis. These factors may be different between laboratory and field conditions, as well as between different age classes of turtles and different geographic populations of turtles. The concurrent decrease in plasma T4 and CS activity during exposure to winter conditions in our laboratory simulation supports the proposition that aerobic capacity may fluctuate seasonally, but exercise variables such as maximal $\dot{V}_{O_2}$ and endurance capacity would need to be measured to confirm this.

In contrast to the results obtained for CS, activity of enzymes associated with the glycolytic pathway of energy production (LDH and PK) was significantly higher during exposure to winter conditions compared with summer conditions (Fig. 4B,C). Glycolytic enzymes showed a pattern of thermal acclimation even though they had a low thermal dependence over the range of 15–30°C (Q_{10}=1.48–1.69). There was no significant difference in activity of LDH and PK at 20°C and 25°C, but activity at 15°C was significantly lower than at all other assay temperatures. One interpretation of these results is that low thermal dependence of glycolytic enzymes is sufficient for maintenance of anaerobic function with relatively small shifts in temperature, but larger shifts in temperature induce a compensatory response to ensure that enzyme function is preserved. Field observations of green turtles from sub-tropical populations show that these animals are capable of quick bursts of activity to escape pursuing predators during both winter and summer (Read et al., 1996; Southwood, 2002). Although green turtles are known to have a high aerobic capacity (Butler et al., 1984), they rely heavily on their large anaerobic reserves for high intensity activity (Prange and Jackson, 1976; Jackson and Prange, 1979; Dial, 1987; Baldwin et al., 1989; Wyneken, 1997). Preservation of anaerobic means of energy production, *via* low thermal dependence and/or compensation of biochemical reaction rates, may be critically important for predator avoidance and survival in a changing thermal environment.

Phosphocreatine (PCr) provides a large anaerobic energy buffer in muscle cells during burst activity *via* a reversible reaction catalyzed by CPK ($PCr + ADP + H^+ \leftrightarrow ATP + Cr$). This reaction takes place in the cytosol of cells, and the presence of large amounts of CPK in the bloodstream is generally used as an indicator of cellular injury or death. In green turtles, the mean level of plasma CPK during exposure to winter conditions was 4.6 times higher than during exposure to summer conditions (Table 1), and the significant increase in CPK could represent breakdown of muscle tissue during prolonged exposure to sub-optimal conditions. However, turtles continued to feed and gain weight and showed no overt signs of malaise during the winter simulation. Another possibility is that the occurrence of cell death may be the same in winter and summer, and the increase in plasma CPK is due to increased amounts of CPK being released from individual cells. A comparison of muscle tissue and plasma activity of CPK in green iguanas (*Iguana iguana*) and yellow rat snakes (*Elaphe obsoleta quadrivitatta*) showed that low plasma activity of CPK coincided with low tissue activity, and high plasma activity of CPK was associated with moderate to large amounts of tissue activity (Wagner and Wetzel, 1999; Ramsay and Dotson, 1995). It is possible that elevated plasma CPK during exposure to winter conditions reflects elevated concentrations of intracellular CPK and therefore compensation of CPK activity. CPK activity in muscle tissues should be measured directly to confirm whether or not a compensatory adjustment occurs.

In addition to the changes we observed in metabolic variables, we also found that prolonged exposure to winter conditions resulted in alterations in respiratory and cardiovascular variables. Interestingly, the changes in oxygen delivery variables that we measured did not match the changes in oxygen consumption. For example, the relationship between $\dot{V}_{O_2}$ and f_B was strong and highly significant during exposure to summer conditions (r^2=0.965, P=0.018), but the relationship between these variables broke down in winter conditions (r^2=0.265, P=0.375) (Fig. 3). This suggests that the relative contributions of f_B and tidal volume to ventilation may change with temperature. Kraus and Jackson (1980) found that for juvenile green turtles increases in ventilation between 15°C and 25°C were due exclusively to changes in tidal volume,

whereas increases in ventilation between 25°C and 35°C were due to changes in f_B. The weak relationship between f_B and $\dot{V}_{O_2}$ during exposure to winter conditions supports the idea that turtles alter tidal volume, rather than f_B, to match O_2 supply to O_2 demand at cooler temperatures.

As with f_B, seasonally induced changes in f_H did not correspond well with the changes in $\dot{V}_{O_2}$. Mean f_H during the winter simulation was 46–48% lower than mean f_H during the summer simulation – approximately double the decrease observed for $\dot{V}_{O_2}$ (24–27%). If cardiac output is primarily controlled by changes in f_H, then the greater decrease in f_H compared with $\dot{V}_{O_2}$ could result in an imbalance between O_2 delivery and O_2 demand at lower temperatures. Hudson and Bertram (1966) suggested that discrepancies in thermal dependence of f_H and $\dot{V}_{O_2}$ could be explained by alterations in stroke volume, with increased filling time between systolic contractions at lower temperatures resulting in larger stroke volumes. The increase in stroke volume could offset the decrease in f_H, so that cardiac output is stabilized and oxygen delivery is maintained during exposure to cool temperatures. Temperature effects on stroke volume of sea turtles have not been investigated, but stroke volume is highly variable in many other species of turtle and is affected by both activity state and temperature (White, 1976). Furthermore, simulation of winter conditions resulted in a significant increase in hematocrit. The range of hematocrit observed in our study (32–42%) was within the range observed for wild loggerhead sea turtles (*Caretta caretta*; 28–48%), although hematocrit for loggerhead sea turtles either did not change seasonally or decreased during the winter months (Lutz and Dunbar-Cooper, 1987). An increase in hematocrit during the winter, if mirrored by an increase in hemoglobin concentration, could enhance blood oxygen transport capabilities, thereby partially offsetting the seasonal bradycardia.

Conclusions

The magnitude of seasonal shifts in temperature and photoperiod, as well as other site-specific environmental factors such as food availability, may play a large role in determining seasonal metabolic response. Our study shows that metabolic enzyme activity and overall metabolic rate of juvenile green turtles have a relatively low thermal dependence over the range of temperatures commonly experienced at tropical and subtropical latitudes (17–26°C). Low thermal dependence of aerobic metabolism and metabolic compensation of anaerobic means of energy production may allow turtles to remain active year-round if other environmental conditions are favorable. Results from previous studies suggest that the pivotal temperature for metabolic downregulation and torpor in green turtles is close to 15°C (Moon et al., 1997; Seminoff, 2000). Whether or not other environmental cues contribute to the induction of torpor in this species remains to be determined.

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