Seasonal metabolism of juvenile green turtles (*Chelonia mydas*) at Heron Island, Australia

A.L. Southwood, R.D. Reina, V.S. Jones, J.R. Speakman, and D.R. Jones

Abstract: We investigated seasonal changes in the energetics of juvenile green turtles (*Chelonia mydas* (L., 1758)) (9.8–23.8 kg) from a resident population on the southern Great Barrier Reef. Doubly labeled water was used to determine water flux and field metabolic rate (FMR) of green turtles during summer (mean water temperature (T_W) = 25.8 °C) and winter (mean T_W = 21.4 °C), and muscle tissue was collected for analysis of thermal sensitivity of metabolic enzyme activity. There was no significant seasonal difference in total body water (TBW) content (73.7% and 75.1% of body mass in summer and winter, respectively) or water flux (7.5% and 6.1% of TBW per day in summer and winter, respectively). The mean FMR during winter (81 ± 15 $kJ \cdot kg^{-1} \cdot d^{-1}$) was 43% lower than that during summer (142 ± 33 $kJ \cdot kg^{-1} \cdot d^{-1}$), but the seasonal difference in FMR marginally failed to reach statistical significance (P = 0.17). Thermal dependence of citrate synthase, lactate dehydrogenase, and pyruvate kinase activity was relatively low (Q_{10} = 1.20–1.68) over the range of temperatures experienced by turtles during summer and winter, and there were no significant seasonal differences in enzyme activity. In summary, juvenile green turtles experience only moderate changes in metabolism within the temperature range and environmental conditions experienced seasonally at this subtropical site.

Résumé : Nous avons étudié les changements saisonniers des relations énergétiques de jeunes tortues vertes (*Chelonia mydas* L., 1758) (9,8–23,8 kg) d'une population résidente du sud du récif de la Grande Barrière. De l'eau doublement marquée a servi à déterminer le flux hydrique et le taux de métabolisme en nature (FMR) des tortues vertes pendant l'été (température moyenne de l'eau (T_W moyenne) = 25,8 °C) et l'hiver (T_W moyenne = 21,4 °C); nous avons aussi prélevé du tissu musculaire pour l'analyse de la sensibilité thermique de l'activité des enzymes métaboliques. Il n'y a pas de différence significative saisonnière dans le contenu hydrique du corps (TBW; 73,7 % de la masse corporelle en été et 75,1 % en hiver), ni dans le flux hydrique (7,5 % TBW par jour en été et 6,1 % TBW par jour en hiver). Le FMR moyen en hiver (81 ± 15 $kJ \cdot kg^{-1} \cdot j^{-1}$) est de 43 % inférieur à celui d'été (142 ± 33 $kJ \cdot kg^{-1} \cdot j^{-1}$), mais la différence saisonnière des FMR n'est pas statistiquement significative (P = 0,17), bien que de justesse. La dépendance thermique de l'activité de la citrate synthase, de la lactate déshydrogénase et de la pyruvate kinase est relativement faible (Q_{10} = 1,20–1,68) dans la gamme des températures rencontrées par les tortues en été et en hiver et il n'y a pas de différence saisonnière significative dans l'activité de ces enzymes. En résumé, les jeunes tortues vertes ne subissent que de faibles changements de métabolisme dans la gamme de températures et de conditions environnementales qu'elles connaissent au cours des saisons dans ce site subtropical.

[Traduit par la Rédaction]

Introduction

Juvenile green turtles (*Chelonia mydas* (L., 1758)) reside in coastal seagrass or reef habitats at tropical and subtropical latitudes. Although juvenile green turtles from certain populations undertake seasonal migrations (Morreale et al. 1992; Epperly et al. 1995; Musick and Limpus 1997), many remain in the same location year-round and experience fluctuations in local environmental conditions on a seasonal basis. Seasonal changes in biotic and abiotic factors can have profound effects on the energetics of reptiles (Bennett and Dawson 1976; Bennett 1982). Fluctuations in ambient temperature directly affect biochemical reaction rates and physiological processes (Davenport et al. 1982; Lutz et al. 1989; Penick et al. 1996; Southwood et al. 2003*a*; Hochscheid et al. 2004), and changes in activity patterns related to reproductive cycles and in ecological factors such as food availability may also contribute to seasonal differences in metabolism (Bennett and Dawson 1976; van Marken Lichtenbelt et al. 1993; Christian et al. 1995, 1996).

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The majority of field observations and remote monitoring studies indicate that juvenile green turtles remain active during the winter months when water temperature (T_W) is 15 °C or higher, although significant seasonal shifts in behavior may occur (Mendonca 1983; Read et al. 1996; Seminoff 2000; Southwood et al. 2003*b*). Seasonal behavioral adjustments can vary greatly depending on geographic location and local environmental factors. For example, juvenile green turtles in Mosquito Lagoon (28°44′N, 80°44′W), Florida, show an increase in daily movements, favor relatively deep troughs, and spend less time in shallow (<1.2 m) seagrass foraging pastures during winter when mean T_W is less than 18 °C (Mendonca 1983). In contrast, juvenile green turtles in coral reef habitats at Heron Island (23°26′S, 151°55′E), Australia, spend a significantly greater amount of time at shallow depths (<1 m) and show a trend towards longer dive times during the winter (mean T_W = 21.3 °C) compared with the summer (mean T_W = 26.2 °C) (Southwood et al. 2003*b*). Lower ambient temperatures and alterations in activity patterns during the winter may lead to decreased metabolic rates, which could account for the observed increase in dive times for turtles in the Heron Island population. Slower rates of oxygen utilization during a dive would ensure that oxygen stores last longer and breath-holds could be extended (Lutcavage and Lutz 1997; Hays et al. 2000).

The effect of temperature on oxygen consumption of sea turtles has been the subject of several laboratory investigations. Both loggerhead turtles (*Caretta caretta* (L., 1758)) and green turtles decrease oxygen consumption (Q_{10} = 2.4–2.7) with acute decreases in temperature over the range of 30 to 10 °C (Davenport et al. 1982; Lutz et al. 1989); however, the metabolic response during long-term cold acclimation is quite different for these two species. Hochscheid et al. (2004) observed that loggerhead turtles experience an 80% reduction in oxygen consumption when subjected to a seasonal decline in T_W of 25.4 to 15.7 °C. Green turtles, on the other hand, experience a moderate 24%–27% decrease in oxygen consumption when exposed to a long-term temperature regime reflective of seasonal temperature changes at subtropical locations (26 to 17 °C) (Southwood et al. 2003*a*). Furthermore, tissue metabolic rates and metabolic enzyme activities of green turtles have low thermal dependence within the temperature range typically experienced year-round (Penick et al. 1996; Southwood et al. 2003*a*). These observations suggest that green turtles, like many other species of reptiles, have a metabolism with relatively low thermal dependence over the range of temperatures in which they are normally active (Bennett and Dawson 1976; Waldschmidt et al. 1987; Angilletta 2001).

Although laboratory studies have shown only moderate decreases in metabolic rate with exposure to cold (Southwood et al. 2003*a*), alterations in diving patterns of green turtles in nearshore foraging habitats suggest that significant seasonal changes in metabolic rate may occur (Southwood et al. 2003*b*). Seasonal adjustments in metabolism and behavior of green turtles are likely due to a combination of thermal effects and ecological factors that cannot be fully accounted for in laboratory studies. In recent years, the use of doubly labeled water (DLW) to estimate CO_2 production and field metabolic rate (FMR) of free-living organisms has provided valuable information about the energetics of reptiles in their natural environment. We measured FMR of juvenile green turtles during the summer and the winter at a subtropical foraging site in the southern Great Barrier Reef to investigate seasonal energetics. Thermal effects on metabolic enzyme activity were also examined to assess the role of temperature in determining seasonal metabolism. This study was part of a larger project to evaluate seasonal changes in the physiology and behavior of green turtles, and a detailed analysis of diving behavior and the relationship between turtle body temperature and ambient T_W is presented in a separate publication (Southwood et al. 2003*b*).

Materials and methods

Study site

Studies were conducted at Heron Island, Queensland, Australia. Heron Island is a coral cay located in the northwestern corner of an elongate lagoonal reef that is approximately 11 km long and 5 km wide. The island is surrounded by an extensive and relatively shallow reef flat that supports coral and algal growth, numerous species of reef fishes, and a year-round population of green sea turtles (Forbes 1996). Mean T_W along the Heron Island reef varies from 26–27 °C in the summer to 20–21 °C in the winter. All turtles for this study were captured at night by hand (free diving) in the boat channel and harbor on the south side of Heron Island during the months of December 2000 and August 2001. Turtles were taken to the Heron Island Research Station (The University of Queensland, Brisbane) immediately after capture for blood sampling, morphological measurements, doubly labeled water injection, and instrument attachment. Procedures for turtle capture, handling, and data collection described in this manuscript were approved by the University of British Columbia Committee on Animal Care in accordance with the Canadian Council on Animal Care, the Queensland Parks and Wildlife Service, and the Great Barrier Reef Marine Park Authority (permit No. G00 568).

Field metabolic rate and water flux

Field metabolic rates for juvenile green turtles at Heron Island were determined using the doubly labeled water (DLW) technique (Lifson and McClintock 1966; Speakman 1997). Simply stated, the DLW technique involves injecting turtles with stable isotopes of hydrogen (^2H) and oxygen (^{18}O) and measuring the decline in activity of these isotopes in blood samples taken over time. Both ^2H and ^{18}O are excreted in the form of metabolically produced water, but ^{18}O is also excreted as metabolically produced CO_2. The difference in the washout slopes of ^2H and ^{18}O gives an estimate of CO_2 production, which is a measure of metabolic rate.

Turtles were weighed on a digital scale with a resolution of 0.05 kg over the range of 0–20 kg. A 4 mL blood sample was collected from the venous cervical sinus with a 21-gauge × 1.5-inch needle and a Vacutainer® tube (Becton, Dickinson and Co.) to determine baseline levels of ^2H and ^{18}O in the turtle's body water, and then a dose of DLW was injected into the turtle's intraperitoneal cavity anterior to the right rear flipper. Doses of DLW were drawn from a premixed solution of high-enrichment ^{18}O (40 g, 95 at. %) and ^2H (20 g, 99 at. %). The dose of DLW administered to each turtle was calculated based on body mass and using equations from Speakman (1997):

$$\text{DLW dose (mL)} = [(0.64 \times \text{body mass in grams})\text{DIE}]/\text{IE}$$

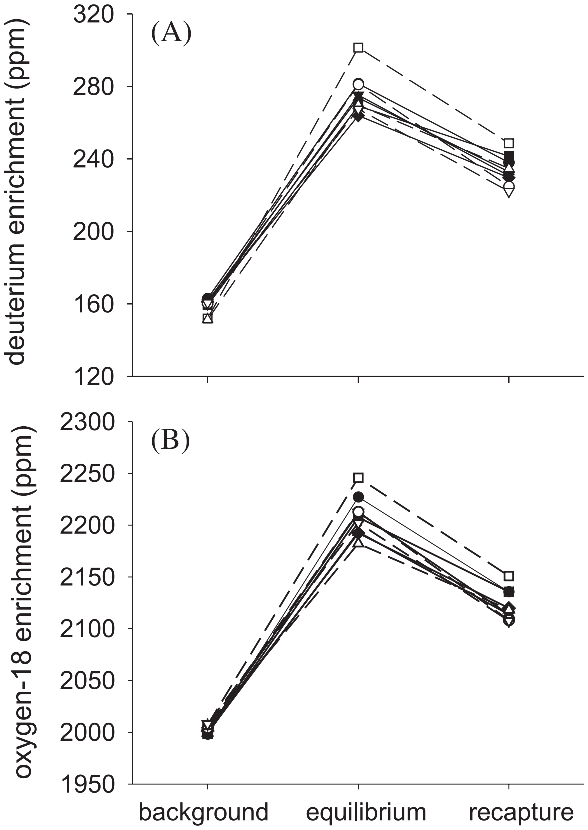
where 0.64 is the estimated proportion of a green turtle's body mass that is water (R.D. Andrews, unpublished observations; Jones et al. 2006), DIE is the desired initial enrichment in parts per million (ppm) (DIE = 618.923 × (body mass in kilograms)$^{-0.305}$), and IE is the injectate enrichment in ppm. The exact dose of DLW injected into each turtle was determined by measuring the mass of the injectate syringe and needle before and after injection using a digital scale with accuracy to four decimal places (Sartorius BP2105, Germany). Seven turtles were injected with DLW during the summer field season and six turtles were injected during the winter field season.

Turtles were kept out of water at an ambient temperature of 20–25 °C for 12 h following DLW injection so that isotopes could fully equilibrate with turtle body water (Jones et al. 2006). A blood sample was taken at the end of the 12 h equilibration period and the turtle was released within 500 m of the capture site. Sonic and very high frequency (VHF) radiotelemetry were used to periodically track turtles (see Diving behavior, below), and turtles were located and recaptured by hand after 4.8–5.4 days at sea in summer and 7.3–12.6 days at sea in winter. Turtles were weighed and a blood sample was obtained from the cervical sinus within 30 min of recapture.

The baseline, equilibration, and recapture blood samples were centrifuged in Vacutainer® tubes at 3000 r/min (Beckman GP centrifuge, Beckman, Palo Alto, California) for 5 min immediately following collection. Serum was transferred to cryo-safe plastic tubes (Sarstedt Inc., Montréal, Quebec) and stored for a period of up to 2 months at –70 °C. Samples were then analyzed for isotope enrichment using mass spectrometry following procedures described elsewhere (Speakman and Krol 2005). The turnover rates for ^{2}H (k_D) and ^{18}O (k_O) were calculated by analyzing the differences in isotope activity between the equilibration and recapture blood samples (Fig. 1). Turtles that had a k_D/k_O ratio greater than 0.9 were excluded from further analysis. High k_D/k_O ratios indicate that a large proportion of ^{18}O turnover is linked to ^{2}H turnover (Speakman 1997), and therefore the calculations to determine CO_2 production from the difference in turnover rates are less reliable.

Calculations of CO_2 production were made using the DLW.exe software developed by Speakman and Lemen (1999) with eq. r1 from Speakman et al. (1993). The plateau method was used for determination of isotopic dilution spaces, and the ^{18}O dilution space (N_O) was used as an estimate of total body water (TBW). We assumed that body water pools were stable during the course of our study. Water flux was determined by multiplying N_O by k_D. Juvenile green turtles from the Heron Island population forage almost exclusively on algae (Forbes 1996). Based on this herbivorous diet, an energy equivalent of 22.1 J·(mL CO_2)$^{-1}$ (respiratory quotient = 0.91) was used to convert CO_2 production to energy expenditure (Nagy and Shoemaker 1984). We used linear regression to assess the effect of mass on TBW, water flux, and FMR during winter and summer. Seasonal means for TBW, water flux, and FMR were compared using Student's *t* test (S-PLUS 6, Insightful Corp., Seattle, Washington). Differences were considered statistically significant when $P < 0.05$. Values are presented as the mean ± standard error of the mean (SEM).

Fig. 1. Isotope enrichment values for ^{2}H (A) and ^{18}O (B) for green sea turtles (*Chelonia mydas*) injected with doubly labeled water (DLW) during the summer ($N = 5$, closed symbols, solid lines) and the winter ($N = 4$, open symbols, dashed lines) at Heron Island, Australia. Equilibrium values were determined from blood samples collected 12 h after injection of DLW, and recapture values were determined from blood samples collected after turtles had been at sea for a period of 4.8–5.4 days in the summer and 7.3–12.6 days in the winter. Differences in the rate of washout for ^{2}H and ^{18}O were used to calculate field metabolic rates.



Metabolic enzyme activity

Tissue samples were obtained from iliotibialis muscle, a hip abductor, at initial capture so that the effects of temperature on muscle enzyme activity could be assessed. Before excising muscle tissue, the incision area was cleaned thoroughly with 95% ethanol and Betadine® topical antiseptic. A local anaesthetic (2% Lidocaine, Vetoquinol Inc., Lavaltrie, Quebec) was injected into the area from which the 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 $mg \cdot kg^{-1}$, Amiglyde-V®, Ayerst Veterinarian Laboratories, Guelph, Ontario) to reduce the risk of infection. The tissue samples were immediately frozen on dry ice and rapidly transferred to a –70 °C freezer for storage. Samples were stored for a maximum of 5 months before assays to determine activity of citrate synthase (CS), lactate dehydrogenase (LDH), and pyruvate kinase (PK) were performed.

Tissue samples were partially thawed, minced, and diluted to 1/10 volume in ice-cold 75 $mmol \cdot L^{-1}$ Tris-HCl homogenization buffer adjusted for pH 7.5 at room temperature. The dilution was homogenized using a Polytron® tissue homogenizer (model PT10, Brinkmann Instruments, Rexdale, Ontario) and sonicated using a Kontes MicroUltrasonic Cell Disrupter (model KT5, Kontes, Vineland, New Jersey). Whole homogenate was used for all assays, and each assay was run in duplicate. Enzyme activities were measured with a PerkinElmer spectrophotometer (model Lambda 2, PerkinElmer (Canada) Ltd., Rexdale, Ontario). Temperature in the spectrophotometer cells was controlled with a circulating water bath (MGW Lauda). Enzyme activity was measured at 15, 20, 25, and 30 °C. Assay temperatures encompassed the range of temperatures experienced by turtles during winter and summer. 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 the wavelength at which the reaction was monitored (λ) are indicated below for each assay.

Enzyme assay protocols were as follows: LDH, λ = 340 nm, ϵ = 6.22, pH 7.5, 50 $mmol \cdot L^{-1}$ imidazole-HCl, 0.15 $mmol \cdot L^{-1}$ β-nicotinamide adenine dinucleotide (reduced), 4 $mmol \cdot L^{-1}$ pyruvate, 1/1000 tissue dilution; PK, λ = 340 nm, ϵ = 6.22, pH 7.0, 50 $mmol \cdot L^{-1}$ imidazole-HCl, 10 $mmol \cdot L^{-1}$ magnesium chloride, 100 $mmol \cdot L^{-1}$ potassium chloride, 0.15 $mmol \cdot L^{-1}$ β-nicotinamide adenine dinucleotide (reduced), 7 $mmol \cdot L^{-1}$ phosphoenolpyruvate, 5 $mmol \cdot L^{-1}$ adenine diphosphate, excess LDH, 1/500 tissue dilution; CS, λ = 412 nm, ϵ = 13.6, pH 8.0, 100 $mmol \cdot L^{-1}$ Tris-HCl, 0.3 $mmol \cdot L^{-1}$ acetyl CoA, 0.5 $mmol \cdot L^{-1}$ oxaloacetate (omitted for control), 0.1 $mmol \cdot L^{-1}$ 5,5′-dithiobis-(2-nitrobenzoic acid), 1/10 tissue dilution. All chemicals were obtained from Sigma-Aldrich Canada Ltd. (Oakville, Ontario).

Thermal coefficients (Q_{10} values) for enzyme activity over the range of assay temperatures were calculated using the following equation: $Q_{10} = (k_2/k_1)^{10/(T_2-T_1)}$, where T_1 = 15 °C, T_2 = 30 °C, k_1 = enzyme activity at 15 °C, and k_2 = enzyme activity at 30 °C. Values for enzyme activity were log-transformed so that data met the assumptions for parametric statistical analysis. The effects of season and assay temperature on enzyme activity were analyzed using a two-factor ANCOVA with mass as a covariate (S-PLUS 6, Insightful Corp.).

Diving behavior

We used custom-built time–temperature–depth recorders (TTDRs; 180 g, 12.0 cm × 6.0 cm × 1.4 cm) to monitor at-sea diving behavior and ambient T_W during the FMR measurement period. Detailed descriptions of TTDR construction and specifications are given in Andrews (1998) and Southwood et al. (2003*b*). The TTDRs were programmed to record depth and T_W every 5 s. Depth resolution of the TTDRs varied from 0.2 to 0.5 m, depending on the pressure transducer components and the analog-to-digital mode of sampling. Pressure transducers were calibrated against a National Institute of Standards and Technology (NIST)-traceable precision pressure gauge. Thermistor components used to monitor T_W were calibrated against a NIST-traceable thermometer over 15–30 °C, and the TTDRs recorded T_W with a resolution of 0.4 °C.

Epoxy glue (SuperMend, Titan Corp., Lynnwood, Washington) was used to attach a TTDR to the turtle's carapace between the 2nd and 4th vertebral scutes during the 12 h period when isotopes were equilibrating with the turtle's body water. We also used epoxy to attach a sonic transmitter (model V16-5HR-01, Vemco Ltd., Shad Bay, Nova Scotia; model CHP87L, Sonotronics, Tucson, Arizona) and a VHF radio beacon (model SI-2sp, Holohil Systems, Carp, Ontario) to the rear marginal scutes of the turtle's carapace so that we could relocate turtles to retrieve the TTDR and obtain a recapture blood sample for FMR measurements. Sonic tags (1.5 cm diameter, 9–11 cm long) transmitted at a frequency of 38–57 kHz and could be detected within a range of 1.5 km. VHF radio beacons (1.5 cm diameter, 4 cm long) transmitted a 149 MHz signal at a frequency of 1 Hz and had a range of approximately 3 km in air.

A submergence to a depth of greater than 1 m was considered a dive and was analyzed for duration and maximum depth. A dive cycle was defined as an individual dive and the subsequent post-dive surface interval. High-resolution dive records showed that turtles could be submerged at depths of less than 1 m and not be solely at the surface, so a set of criteria was established to distinguish typical post-dive surface intervals from prolonged periods at shallow depths (Southwood et al. 1999, 2003*b*). The mean surface interval for all dive cycles was calculated, and dive cycles with surface intervals that were 3 times greater than the mean surface interval were eliminated from analysis of dive duration and dive depth. Surface intervals that were 3 times greater than the mean surface interval were counted as shallow-depth periods. Descriptive statistics were calculated for time spent at shallow depths, dive duration, surface interval, and depth of dives that met the dive criteria (>1 m depth).

Results

Field metabolic rate and water flux

Results from isotopic analysis are summarized in Table 1. One turtle could not be recaptured before the end of the summer field season, and three turtles were excluded from the analysis owing to high k_D/k_O ratios (>0.9; Speakman 1997). This reduced the number of turtles for analysis to five for summer and four for winter. One turtle (No. 38309) was sampled during both the summer and the winter field season. The seasonal differences in values of isotopic measurements for turtle No. 38309 were larger than seasonal differences for other turtles of similar mass; therefore, it is unlikely that inclusion of this turtle in our parametric statistical analysis would result in an underestimation of the standard error.

Table 1. Water flux and field metabolic rates for juvenile green sea turtles (*Chelonia mydas*) at Heron Island, Australia, during December 2000 (summer) and August 2001 (winter).

Turtle No.	Mass (kg)*	Recapture interval (d)	Mean T_W (°C)†	TBW (% of mass)	Water flux ($mL \cdot d^{-1}$)	Water flux (% TBW per day)	k_D/k_O	FMR ($kJ \cdot d^{-1}$)	FMR ($kJ \cdot kg^{-1} \cdot d^{-1}$)
Summer									
35982	11.1	5.3	26.6±1.6	72.8	689	8.2	0.89	617	55
38305	11.6	5.3	25.6±1.3	74.3	438	5.0	0.70	2517	217
38306	15.7	5.3	25.3±0.8	73.2	903	7.9	0.84	1615	103
38309	21.7	5.4	—	71.4	1383	8.9	0.77	4799	221
94946	16.3	4.8	25.8±1.0	76.8	999	7.7	0.86	1824	112
$\bar{x}$ ± SEM	15.3±1.91	5.2±0.1	25.8±0.3	73.7±0.9	882±158	7.5±0.7	0.81±0.03	2274±700	142±33
Winter									
5276	15.9	7.4	21.2±0.1	72.5	966	8.4	0.89	795	50
35133	9.8	8.1	20.9±0.1	73.2	385	5.3	0.84	612	62
38309	23.8	7.3	21.9±1.4	77.5	879	4.8	0.80	2744	115
38311	17.2	9.3	21.7±1.4	77.0	758	5.7	0.82	1688	98
$\bar{x}$ ± SEM	16.7±2.87	8.0±0.5	21.4±0.2	75.1±1.3	747±128	6.0±0.8	0.84±0.02	1460±488	81±15

*Mass at time of initial capture.

†Mean of all T_W measurements recorded by a data logger during the period when the turtle was at liberty. Values are means ± SEM.

There was no significant effect of mass on TBW during either winter ($r^2 = 0.56$, $P = 0.25$) or summer ($r^2 = 0.09$, $P = 0.62$), and there was no significant difference in TBW between seasons ($t = -0.89$, df = 7, $P = 0.40$). Likewise, mass had no significant effect on water flux in winter ($r^2 = 0.04$, $P = 0.80$) or summer ($r^2 = 0.38$, $P = 0.26$), and there was no significant difference in mean water flux between seasons ($t = 1.44$, df = 7, $P = 0.19$). Regressions of absolute FMR ($kJ \cdot d^{-1}$) against mass had relatively high r^2 values (0.85 in winter, 0.64 in summer) and only marginally failed to reach significance ($P = 0.07$ in winter, $P = 0.10$ in summer), likely owing to the low sample sizes for individual seasons. We used mass-specific FMR ($kJ \cdot kg^{-1} \cdot d^{-1}$) in our comparison between seasons to account for mass effects and to facilitate comparisons with results of other studies. The mean FMR of juvenile green turtles during winter was 43% lower than that during summer (Table 1), but this difference was not statistically significant ($t = 1.52$, df = 7, $P = 0.17$).

Metabolic enzyme activity

Turtle body mass did not have a significant effect on CS ($F_{[1,44]} = 0.16$, $P = 0.69$), LDH ($F_{[1,48]} = 0.58$, $P = 0.45$), or PK activity ($F_{[1,48]} = 2.66$, $P = 0.11$), so this covariate was removed from the analysis of thermal and seasonal effects on metabolic enzyme activity. Activity of CS showed low thermal dependence (i.e., $Q_{10} < 2$) over the range of 15–30 °C, with a Q_{10} of 1.44 for tissue collected during the summer and a Q_{10} of 1.20 for tissue collected during the winter (Fig. 2). There was no significant difference in CS activity between muscle tissue collected during summer and that collected during winter ($F_{[1,43]} = 2.36$, $P = 0.13$), nor was there a significant effect of assay temperature on CS activity ($F_{[3,43]} = 1.03$, $P = 0.39$) (Fig. 2).

Assay temperature did have a significant effect on both LDH ($F_{[3,47]} = 16.46$, $P < 0.01$) and PK activity ($F_{[3,47]} = 19.24$, $P < 0.01$) (Fig. 2). Fisher's LSD method was used to test for significant differences in enzyme activity at different assay temperatures. LDH activity was significantly different between all assay temperatures except 20 and 25 °C, and PK activity was significantly different between all assay temperatures except 25 and 30 °C. The Q_{10} values for LDH over the range of 15–30 °C were 1.60 for tissue collected during the summer and 1.47 for tissue collected during the winter, and Q_{10} values for PK were 1.66 during summer and 1.68 during winter. There was no significant seasonal difference in LDH ($F_{[1,47]} = 0.38$, $P = 0.54$) or PK activity ($F_{[1,47]} = 0.92$, $P = 0.34$) (Fig. 2).

Diving behavior

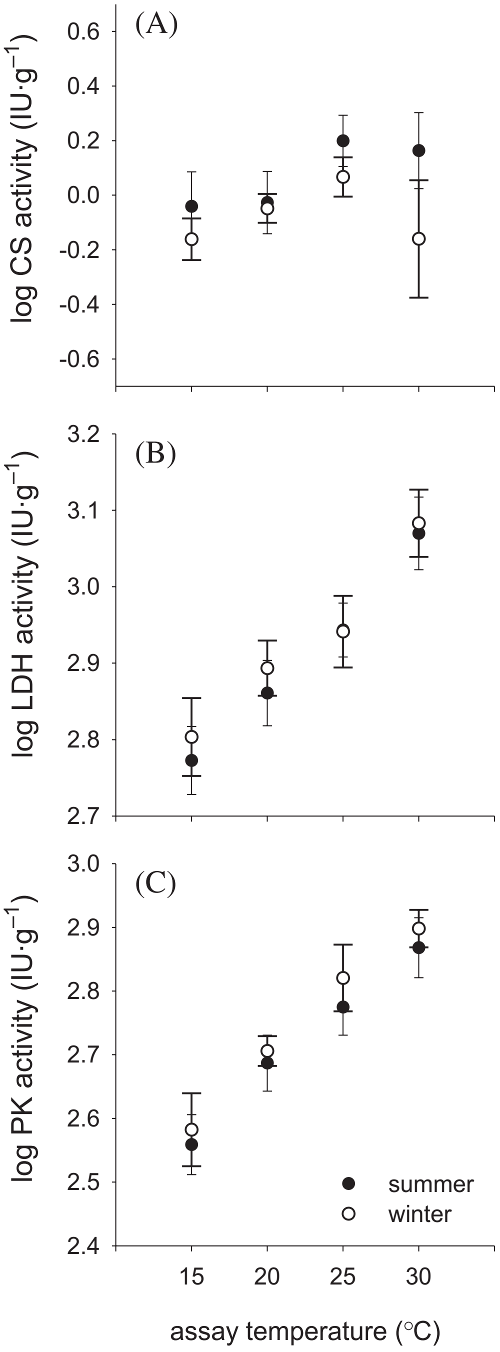
In the current study, dive data and T_W data were obtained for five turtles injected with DLW during the summer and two turtles injected during the winter. The mean T_W experienced during winter (21.4 °C) was significantly lower than the mean T_W experienced during summer (25.8 °C) ($t = 12.22$, df = 6, $P < 0.01$). Table 2 provides a summary of dive variables for turtles and Fig. 3 shows representative dive traces for summer and winter. Green turtles spent almost twice as much time at shallow depths (<1 m) during winter compared with summer. For dives that exceeded 1 m depth, mean dive duration was twice as long during winter compared with summer. Likewise, post-dive surface intervals for dives exceeding 1 m depth were 3 times longer during winter compared with summer. The amount of time spent submerged during dive cycles was similar between seasons (95.4% ± 0.2% in summer, 93.1% ± 0.1% in winter).

Discussion

Field metabolic rate and water flux

The use of DLW to determine FMR and water flux of reptiles was pioneered by Nagy and colleagues in the 1970s (Nagy 1975; Bennett and Nagy 1977). Since that time, the DLW technique has become a common tool used to investigate the ecological energetics of terrestrial and semi-aquatic reptiles in temperate, subtropical, and tropical climates. The validity of using the DLW technique to estimate metabolic rates of sea turtles was recently confirmed by using open-flow respirometry to record oxygen consumption of juvenile

Fig. 2. Activities (IU·(g wet mass)$^{-1}$, mean ± SEM) of (A) citrate synthase (CS), (B) lactate dehydrogenase (LDH), and (C) pyruvate kinase (PK) in iliotibialis muscle tissue collected from juvenile green turtles at Heron Island, Australia, during December 2000 ($N = 7$, ●) and August 2001 ($N = 6$, ○). There was no significant seasonal difference in activity for CS ($P = 0.13$), LDH ($P = 0.54$), or PK ($P = 0.34$). Thermal dependence of enzyme activity was relatively low over the range of 15–30 °C (Q_{10} = 1.2–1.68).



green turtles ($N = 4$, 16.8 ± 1.5 kg) that had been injected with DLW (Jones et al. 2006). At a T_W of 24 °C, estimates of metabolic rate obtained using the DLW technique were 9% lower than respirometry measurements of metabolic rate for fed green turtles, and there was no statistically significant difference between values obtained using the two methods.

Estimates of FMR for juvenile green turtles obtained in the current study are 2.7–4.2 times higher than DLW-determined estimates of metabolic rate for captive juvenile green turtles (Jones et al. 2006). There is a strong correlation between activity and metabolic rate in green turtles (Davenport et al. 1982; Butler et al. 1984; Southwood et al. 2003*a*) and activity patterns are obviously much more limited under captive conditions, so it is not surprising that wild green turtles have comparatively higher mean metabolic rates. Studies of other species of reptiles have also shown that FMRs are generally higher than metabolic rates recorded in the laboratory (Bennett and Nagy 1977; Christian et al. 1995, 1996; Thompson et al. 1997). Small insectivorous lizards (*Sceloporus occidentalis* Baird and Girard, 1852) have FMRs that are 2.0–2.5 times higher than resting metabolic rates recorded using respirometry (Bennett and Nagy 1977), and varanid lizards have FMRs that are 1.5–4.5 times higher than resting metabolic rates in the laboratory (Christian et al. 1995, 1996; Thompson et al. 1997).

Prange and Jackson (1976) investigated the effect of body mass on metabolic rate of green turtles over the range of 0.03 to 142 kg and found scaling exponents of 0.826 for resting turtles and 0.944 for active turtles. A log–log regression of absolute metabolic rate (kJ·d^{-1}) against mass (kg) that incorporates our data for summer FMR of juvenile green turtles and literature values for moderately active hatchling (0.03 kg) (Prange and Ackerman 1974; Wyneken 1997) and small juvenile (<1 kg) green turtles (Prange 1976; Butler et al. 1984) at ambient temperatures of 25–28 °C results in a scaling exponent of 0.889 (Fig. 4). We excluded data for adult green turtles from this regression, as published values are for either supine turtles or vigorously exercising turtles (Prange and Jackson 1976). If data for active adult green turtles (Prange and Jackson 1976) are included in the regression, the scaling exponent increases to 0.910. The scaling exponent for metabolic rate of green turtles derived using the data in Fig. 4 falls within the broad range of values that have been reported for reptiles (0.62–1.09; Schmidt-Nielsen 1984) and matches the scaling exponent reported by Nagy (2005) for FMR in 55 species of reptiles.

The only other species of sea turtle for which FMR has been measured is the leatherback turtle, *Dermochelys coriacea* (Vandelli, 1761) (Wallace et al. 2005). Mass-specific FMRs for juvenile green turtles in foraging habitat are greater than the mean mass-specific FMR reported for adult female leatherback turtles (268 ± 44 kg) during the inter-nesting interval (35 ± 17 kJ·kg^{-1}·d^{-1}) (Wallace et al. 2005); mass effects may account for a large proportion of the difference in FMR between these two species. Among reptiles, sea turtles and varanid lizards are recognized for their impressive capacity for prolonged activity and wide aerobic scope (Bartholomew and Tucker 1964; Prange and Jackson 1976; Wood et al. 1978; Butler et al. 1984), and mass-specific FMRs for juvenile green turtles are similar to mass-specific FMRs reported for tropical varanid lizards (7.7–16.6 kg, 77–121 kJ·kg^{-1}·d^{-1}) (Dryden et al. 1992; Thompson et al. 1997). Mass-specific FMRs for juvenile green turtles are higher than mass-specific FMRs of marine iguanas (*Amblyrhynchus cristatus* Bell, 1825) (1–2 kg, 56–

Table 2. Summary of dive variables for juvenile green sea turtles injected with doubly labeled water during the summer and the winter at Heron Island, Australia.

Turtle	Dive depth (m)*	Dive duration (min)*	Surface interval (min)*	% time in shallows (<1 m)
Summer				
35982	2.1±0.1	12.8±0.6	0.7±0.03	50.2
38305	2.3±0.1	12.6±0.6	0.5±0.02	20.4
38306	4.4±0.2	15.2±0.9	0.7±0.03	37.7
38309	2.9±0.1	14.3±0.6	0.7±0.03	40.2
94946	3.7±0.2	11.9±0.5	0.6±0.02	24.2
$\bar{x}$ ± SEM	3.1±0.4	13.4±0.6	0.6±0.04	34.5±5.5
Winter				
5276	—	—	—	—
35133	—	—	—	—
38309	4.9±0.4	22.7±1.4	1.7±0.2	60.7
38311	3.8±0.2	25.9±2.2	1.9±0.5	67.9
$\bar{x}$ ± SEM	4.4±0.6	24.3±1.6	1.8±0.1	64.3±3.6

*For dives that met the dive criteria of >1 m depth. Values are means ± SEM.

60 $kJ \cdot kg^{-1} \cdot d^{-1}$) (Nagy and Shoemaker 1984), possibly due to the higher cost of transport (swimming) for green turtles (Bennett 1982).

Seasonal shifts in FMR are common for reptiles, even in the tropics where ambient temperatures are stable year-round (van Marken Lichtenbelt et al. 1993; Christian et al. 1995, 1996). For example, Gould's goanna (*Varanus gouldii* (Gray, 1838)) and the Argus monitor (*Varanus panoptes* Storr, 1980) experience a 71%–76% decrease in FMR and a 60%–71% decrease in water flux during the dry season compared with the wet season (Christian et al. 1995); lack of water and prey in the dry season is thought to initiate a period of inactivity, resulting in lower energy expenditure (Christian et al. 1995). In our study, the lack of a significant seasonal difference in water flux suggests that food intake of juvenile green turtles was similar during the winter and the summer; however, more detailed information on diet composition is necessary to properly assess seasonal differences in foraging success. Green turtles at Heron Island forage primarily on a heterogeneous assemblage of numerous species of Rhodophyta, Chlorophyta, and Phaeophyta algae. The species composition of the algal assemblage, as well as the nutrient, water, and energy content of individual algal species, varies temporally (Forbes 1996). We did not directly document the diet composition of green turtles in this study, so estimates of food intake based on our water flux data would be highly speculative. The water flux values we observed for free-ranging juvenile green turtles were within the range of values for captive juvenile green turtles (6%–11% of TBW per day; Jones et al. 2006) and well below values reported for adult leatherback turtles (24% of TBW per day; Wallace et al. 2005) and adult Kemp's ridley turtles, *Lepidochelys kempii* (Garman, 1880) (16% of TBW per day; Ortiz et al. 2000).

For green turtles at Heron Island, the trend towards lower FMR during the winter compared with the summer may be the result of changes in diving patterns and the direct effects of temperature on metabolic processes. Previous studies have shown that juvenile green turtles do not maintain a thermal gradient between body temperature and T_W (Southwood et al. 2003*b*), so metabolic processes are not buffered from environmental temperature fluctuations. Over the course of our study, the mean T_W experienced by green turtles at Heron Island during winter was only 4.4 °C lower than mean T_W during summer. The lack of a significant difference between winter and summer FMR may be due in part to the low thermal dependence of metabolic enzyme activity over this narrow range of temperatures.

Metabolic enzyme activity

Thermal coefficients for CS activity in muscle tissue collected from green turtles at Heron Island were similar to values reported for captive green turtles (Q_{10} = 1.44, 15–30 °C) (Southwood et al. 2003*a*) and other aquatic reptiles such as the American alligator, *Alligator mississippiensis* (Daudin, 1801), (Q_{10} = 1.45–1.95, 15–30 °C) (Seebacher et al. 2003) and the Australian snake-necked turtle, *Chelodina longicollis* (Shaw, 1794) (Q_{10} = 1.12–1.81, 10–25 °C) (Seebacher et al. 2004). Likewise, thermal coefficients for LDH and PK of green turtles at Heron Island were comparable to values observed for captive green turtles (Q_{10} = 1.48–1.69, 15–30 °C) (Southwood et al. 2003*a*), the American alligator (Q_{10} = 1.4–1.6, 15–30 °C) (Seebacher et al. 2003), and the Australian snake-necked turtle (Q_{10} = 1.12–2.10, 10–25 °C) (Seebacher et al. 2004). Bennett (1982) noted that the anaerobic scope and the anaerobic capacity of terrestrial reptiles have low thermal sensitivity, with Q_{10} values of 0.76–1.39 over the range of temperatures in which activity is maintained. The low Q_{10} values for glycolytic enzymes observed in our study and several others (Seebacher et al. 2003, 2004) suggest that this may be the case for aquatic reptiles as well.

The lack of a significant seasonal difference in glycolytic enzyme activity for green turtles at Heron Island is in contrast to results from studies of captive green turtles that showed compensation of LDH and PK activity with prolonged cold exposure. Enzyme activity in muscle tissue collected from captive green turtles exposed to 17 °C for several weeks was significantly higher than activity in muscle tissue collected at 26 °C before cold exposure (Southwood et al. 2003*a*). Acclimatization of glycolytic en-

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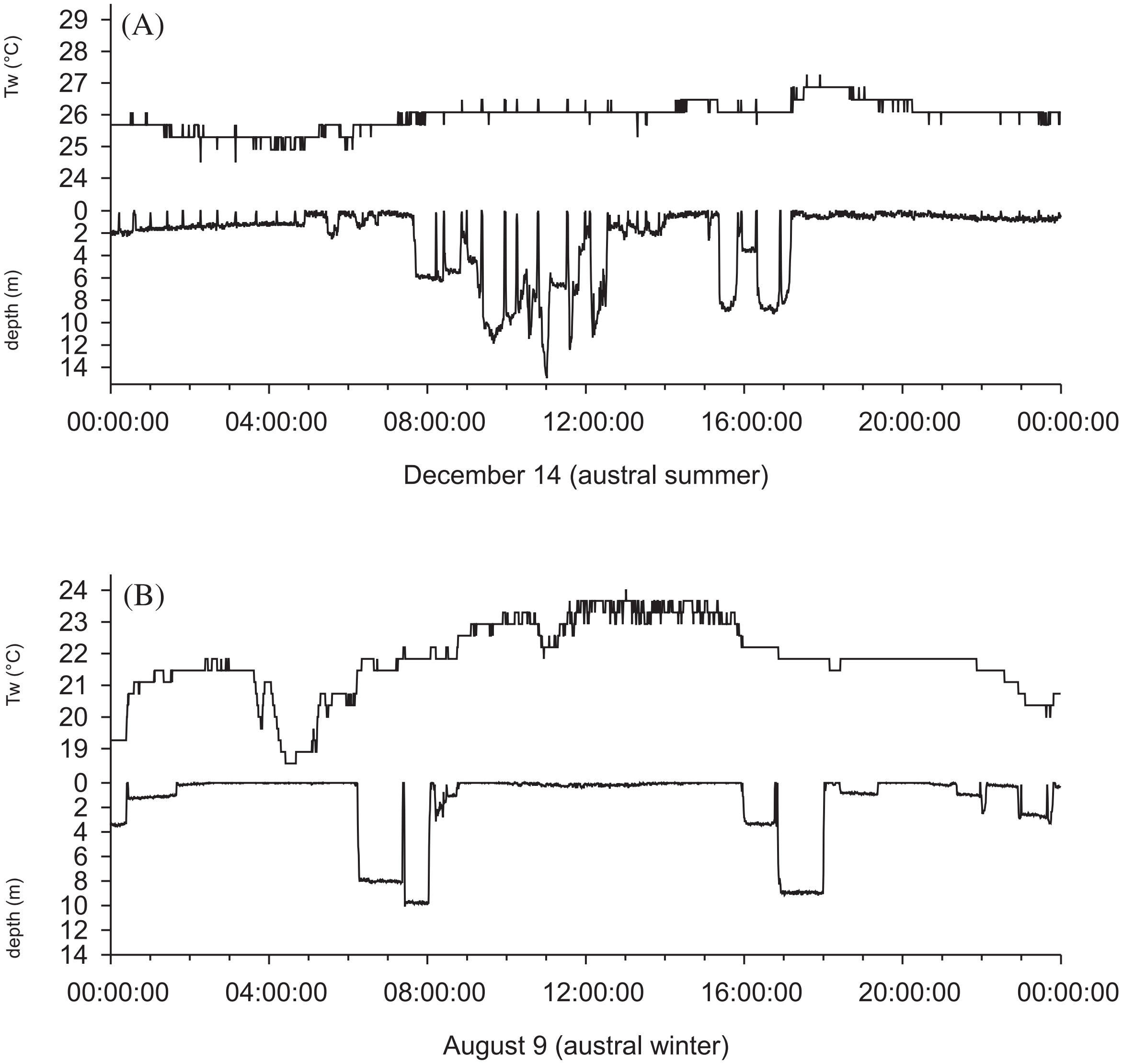
Fig. 3. Representative dive traces for juvenile green turtle No. 38309 during the summer (A) and turtle No. 38311 during the winter (B) at Heron Island, Australia. There was a seasonal shift towards spending a greater amount of time at shallow depths (<1 m) during the winter (60.7%–67.9% of recording period) compared with the summer (20.4%–50.2% of recording period). For dives that exceeded 1 m in depth, winter dives tended to be of longer duration, with longer post-dive surface intervals, compared with summer dives.

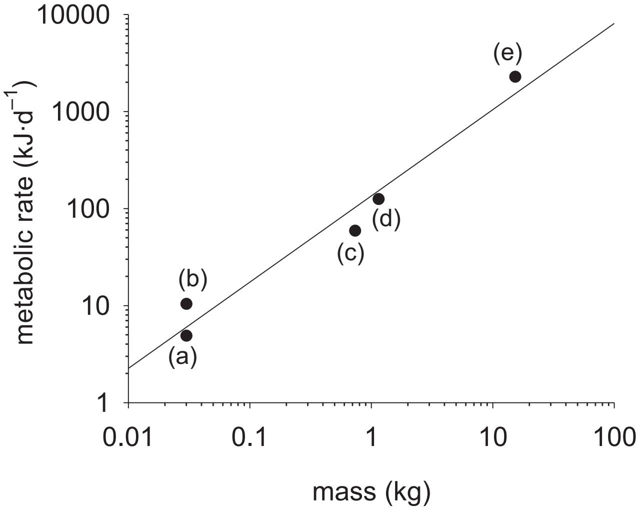


zyme activity during seasonal cold exposure has also been observed in other aquatic reptiles (Seebacher et al. 2003, 2004) and is well documented in fish (Hochachka and Somero 2002). Compensatory adjustments in glycolytic enzyme function during winter months may ensure that the capacity for anaerobic means of energy production is preserved over the range of temperatures experienced annually. Green turtles rely heavily on anaerobic metabolism during high-intensity burst activity (Jackson and Prange 1979; Dial 1987; Baldwin et al. 1989), and preservation of anaerobic capacity by means of low temperature sensitivity of glycolytic pathways or acclimatization of glycolytic enzymes may be a critical component of predator avoidance and survival. The lack of evidence for thermal acclimatization of glycolytic enzyme activity in the current study may be explained by the fact that green turtles at Heron Island experienced a relatively small change in mean T_W from summer to winter. Our results suggest that low thermal dependence of glycolytic enzymes is sufficient for maintenance of anaerobic function with relatively small shifts in temperature; however, larger shifts in temperature may induce a compensatory response to ensure that anaerobic capacity is preserved (Southwood et al. 2003*a*).

Diving behavior

As with many species of air-breathing, diving vertebrates, sea turtles are assumed to rely primarily on aerobic metabolic pathways while submerged (Lutcavage and Lutz 1997). This strategy allows turtles to avoid a buildup of anaerobic by-products during submergence that would necessitate a lengthy amount of time at the surface to repay the oxygen debt incurred while diving. Reduction of aerobic metabolism during a dive results in a slower rate of oxygen utilization, so oxygen stores in the lungs, blood, and tissues may last longer and dive duration may be extended. The trend towards longer dive durations in winter compared with sum-

Fig. 4. Metabolic rate (kJ·d^{-1}) plotted against body mass (kg) on logarithmic coordinates for green turtles ranging in size from 0.03 to 15.3 kg. All measurements of metabolic rate were made at ambient temperature (25–28 °C). Data points represent mean values for metabolic rate of (*a*) crawling hatchlings (Prange and Ackerman 1974), (*b*) post-frenzy swimming hatchlings (Wyneken 1997), (*c*) small juvenile turtles swimming at 0.25 m·s^{-1} (Prange 1976), (*d*) small juvenile turtles swimming at 0.4 m·s^{-1} (Butler et al. 1984), and (*e*) juvenile turtles in the field during the summer at Heron Island, Australia (Table 1). The allometric equation describing the relationship between metabolic rate (kJ·d^{-1}) and body mass (kg) for moderately active green turtles is as follows: metabolic rate = 134.83(body mass)$^{0.889}$ ($F_{[1,3]} = 78.18$, $P < 0.01$, $r^2 = 0.96$).



mer for green turtles at Heron Island suggests that metabolic rates during extended winter dives were lower than metabolic rates during summer dives.

A major complicating factor in our examination of the possible links between diving behavior and FMR is the large amount of time that turtles spent at very shallow depths of 1 m or less (Table 2). Green turtles spent an average of 34.5% of the recording period at depths of less than 1 m during the summer and almost double that amount of time during the winter. Given the resolution of our recording instruments, it is difficult to say with any certainty what proportion of time at shallow depths was spent submerged or at the surface. The seasonal shift in time spent at shallow depths may reflect an increase in surface basking behavior during the winter, or turtles may be spending a greater amount of time in the shallow reef flat habitat during winter for foraging purposes, in response to predator behavior or abundance, or as a means of behavioral thermoregulation (Southwood et al. 2003*b*). Daytime T_W on the shallow reef flats and at the surface is likely to be warmer than T_W in deeper waters (Fig. 3), and the shift towards shallow daytime diving in winter may be motivated by thermal preference. The lack of a significant seasonal difference in FMR could be partially explained by thermal effects associated with the increase in daytime shallow-water utilization during the winter. This effect may be offset, however, by the low T_W experienced by turtles during periods of nighttime shallow diving in winter (Fig. 3).

Another possibility is that the increase in time spent at shallow depths during winter reflects a shift in biochemical pathways used for energy metabolism while diving. If turtles depleted oxygen stores and resorted to anaerobic metabolic pathways during extended winter dives, then the increase in time spent at shallow depths may reflect prolonged recovery periods at the surface. It is difficult to assess this possibility without knowing the actual percentages of time spent submerged and at the surface during shallow-water periods. A shift towards increased reliance on anaerobic metabolism during routine activity at colder temperatures has been demonstrated for lizards (Bennett 1982) and is assumed to be mainly the result of lower thermal dependence of anaerobic metabolic pathways compared with aerobic metabolic pathways. Results from our analysis of the effects of temperature on metabolic enzyme activity do not support the idea of an aerobic-to-anaerobic shift in diving metabolism during the winter, since the thermal dependence of LDH and PK was actually greater than that of CS; however, thermal acclimatization of glycolytic enzymes with larger seasonal decreases in temperature may foster a shift towards anaerobiosis.

Conclusions

In summary, juvenile green turtles at Heron Island showed a moderate decrease in FMR during winter compared with summer. This finding is in agreement with results of laboratory studies of the seasonal changes in oxygen consumption and the effects of temperature on metabolic biochemistry of this species. Green turtles from most populations remain active at temperatures above 15 °C, but temperatures below this threshold may induce metabolic downregulation and seasonal torpor (Felger et al. 1976; Seminoff 2000; Godley et al. 2002). This aspect of the green turtle's biology has not been well investigated but certainly warrants further study. It is likely that thermal effects act in tandem with other site-specific environmental and ecological factors to determine seasonal FMR and behavior of green turtles.

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