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Bioenergetics and diving activity of internesting leatherback turtles *Dermochelys coriacea* at Parque Nacional Marino Las Baulas, Costa Rica

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Summary

Physiology, environment and life history demands interact to influence marine turtle bioenergetics and activity. However, metabolism and diving behavior of free-swimming marine turtles have not been measured simultaneously. Using doubly labeled water, we obtained the first field metabolic rates (FMRs; 0.20–0.74 W kg^{-1}) and water fluxes (16–30% TBW day^{-1}, where TBW=total body water) for free-ranging marine turtles and combined these data with dive information from electronic archival tags to investigate the bioenergetics and diving activity of reproductive adult female leatherback turtles *Dermochelys coriacea*. Mean dive durations (7.8±2.4 min (±1 S.D.), bottom times (2.7±0.8 min), and percentage of time spent in water temperatures (T_w) ≤24°C (9.5±5.7%) increased with increasing mean maximum dive depths (22.6±7.1 m; all P≤0.001). The FMRs increased with longer mean dive durations, bottom times and surface intervals and increased time spent in T_w≤24°C (all r^2≥0.99). This suggests that low FMRs and activity levels, combined with shuttling between different water temperatures, could allow leatherbacks to avoid overheating while in warm tropical waters. Additionally, internesting leatherback dive durations were consistently shorter than aerobic dive limits calculated from our FMRs (11.7–44.3 min). Our results indicate that internesting female leatherbacks maintained low FMRs and activity levels, thereby spending relatively little energy while active at sea. Future studies should incorporate data on metabolic rate, dive patterns, water temperatures, and body temperatures to develop further the relationship between physiological and life history demands and marine turtle bioenergetics and activity.

Key words: leatherback turtle, *Dermochelys coriacea*, bioenergetics, field metabolic rate, diving physiology.

Introduction

Physiology, environment and life history constrain animal energetics and behavior. Marine turtles are attractive subjects for investigation of trade-offs between physiological constraints, life history demands and activity levels because they play several important ecological roles (Bjorndal and Jackson, 2003), migrate long distances between distinct foraging and reproductive areas (Plotkin, 2003) and are long-lived and iteroparous (Miller, 1997). At-sea metabolic rates for marine turtles are the most critical components in calculating individual and population energy requirements, improving our understanding of physiological limitations on diving and thermoregulation, and for refinement of demographic parameters necessary to estimate population trends (Jones et al., 2004). However, concurrent measurements of metabolism and diving activity of free-swimming marine turtles have not yet been documented.

Leatherback turtles *Dermochelys coriacea* Vandelli 1761 are critically endangered (Spotila et al., 2000) and range circumglobally from sub-polar to tropical waters (Goff and Lien, 1988; Paladino et al., 1990). Their unique thermoregulatory adaptations (Frair et al., 1972; Greer et al., 1973; Paladino et al., 1990), pan-oceanic migrations (Morreale et al., 1996; Hays et al., 2004a,b; Ferraroli et al., 2004), prodigious growth rate (Zug and Parham, 1996), reproductive output (Reina et al., 2002b) and size (200–900 kg) make quantification and understanding of the energy–activity trade-offs of the species' distinctive physiology, movements, and life history crucial to their conservation.

Leatherbacks utilize gigantothermy – a suite of physiological adaptations including low metabolic rate, large thermal inertia, blood flow adjustments and peripheral insulation – to maintain elevated body temperatures in cold

water and avoid overheating in the tropics (Paladino et al., 1990). Such thermal tolerance probably allowed leatherbacks to exploit an ecological niche unavailable to other marine turtle species, similar to the thermal niche expansion theory proposed by Block et al. (1993) to explain the multiple and diverse origins of endothermy in the Family Scombroidei (tunas, billfish). Leatherback metabolic rate (MR) during nesting is intermediate between reptilian and mammalian resting metabolic rates (RMRs) scaled to leatherback size (Paladino et al., 1990, 1996). However, all metabolic measurements have been on adult leatherbacks during nesting, walking on the beach, or while restrained in nets (Lutcavage et al., 1990, 1992; Paladino et al., 1990, 1996), and not during in-water activities that constitute the vast majority of the lifespan of adult leatherbacks. Therefore, quantification of metabolic rates for free-swimming leatherbacks would provide ecologically relevant measures of energy expenditure during at-sea activity.

The energetic costs of activity and maintenance physiological processes during the internesting period are unknown. Internesting leatherbacks swim continuously, displaying distinct swim-speed patterns for diving and traveling (Eckert, 2002; Reina et al., 2005; Southwood et al., 2005), in contrast to hypotheses that turtles rest or bask for extended periods at or near the surface (Eckert et al., 1986, 1989; Southwood et al., 1999). Leatherbacks exhibit distinct dive patterns during different activities. For instance, U-shaped dives, during which turtles decrease activity on or near the ocean bottom, are thought to serve a resting or energy conservation purpose, in contrast to V-shaped dives, which appear to serve mainly a transit purpose (Reina et al., 2005). Southwood et al. (1999) hypothesized that leatherback metabolism at sea might be higher than during oviposition due to other costs (reproduction, swimming, foraging, etc.); however, swimming in other vertebrates is more energetically efficient than walking (Schmidt-Nielsen, 1972) and elevated water temperatures in the tropics might constrain leatherback activity due to the possibility of overheating, as reported for giant bluefin tuna (Blank et al., 2004). Furthermore, given the competing reproductive energy requirements of round-trip migration between foraging and nesting grounds, egg production and nesting, and internesting activity at sea, leatherbacks should conserve energy while at sea during the internesting period in order to enhance their seasonal reproductive success.

Aerobic dive limit (ADL) can provide estimates of physiological and energetic constraints on activity in air-breathing, diving animals (Costa et al., 2001). The ADL concept specifically refers to the dive duration beyond which blood lactate levels increase above resting levels (Kooyman et al., 1980). However, direct measurements of post-dive blood lactate concentrations are difficult to obtain from free-swimming animals, so many reports combine data on individual total oxygen stores and at-sea metabolic rates to obtain calculated aerobic dive limits (cADL; for a review, see Costa et al., 2001). Leatherback respiratory and cardiovascular physiology allows for deep and prolonged diving (Lutcavage et al., 1990; Paladino et al., 1996), with the deepest recorded dive to 1230 m (Hays et al., 2004b) and the longest dive duration in excess of 1 h (Southwood et al., 1999). Lutcavage et al. (1992) combined measurements of nesting leatherback metabolic rates, blood O_2-carrying capacity and tissue myoglobin concentration (Lutcavage et al., 1990) with data on blood and lung volumes to calculate total O_2 stores of 27 ml kg^{-1}, and estimated that leatherback cADL was between 5–70 min. Southwood et al. (1999) recorded the longest dive duration for a leatherback (67.3 min) and refined the cADL estimate to 33–67 min, based on heart rates and dive patterns of free-swimming adult female leatherbacks during the internesting period. In order to better estimate the cADL, however, measurements of metabolic rates of free-swimming leatherbacks are necessary.

Using conventional respirometry to measure metabolic rates of free-ranging marine animals is logistically infeasible in most cases. However, the doubly labeled water (DLW) method has proved a useful tool for studying field energetics and diving activity of marine animals (Costa, 1988; Arnould et al., 1996; Costa and Gales, 2000, 2003). The DLW method estimates CO_2 production (rCO_2) from the divergence between washout curves of hydrogen (deuterium, D or tritium, T) and oxygen (18-oxygen, ^{18}O) isotopes introduced into an animal's total body water (Lifson et al., 1955). Disadvantages of the method include the high cost of the isotopes and the reliance of the method on significant divergence of the isotope washout curves that is created by a relatively higher rCO_2 than water turnover rate (rH_2O). The accuracy of the DLW method decreases considerably as the ratio of rCO_2 to rH_2O decreases (Butler et al., 2004). Although the DLW method has been used to measure the field metabolic rate (FMR) and water turnover of many terrestrial reptilian species (for a review, see Speakman, 1997), Booth (2002) concluded that DLW would not work for aquatic turtles because their water turnover rates are too high (approximately 1.6–4.3×TBW day^{-1}, where TBW=total body water). Clusella Trullas et al. (in press) recently reported DLW-derived FMRs and water turnover rates during dispersal in hatchling olive ridley turtles *Lepidochelys olivacea*, but there are no published reports of DLW being used to quantify the FMRs of free-swimming adult marine turtles. However, since marine turtles face a different osmoregulatory challenge from freshwater turtles, and osmoregulate efficiently (Reina, 2000; Reina et al., 2002a), they should have a lower water turnover rate than their freshwater counterparts and sufficient divergence in the isotopes should occur to allow measurement of FMRs in this species.

Therefore, using highly enriched DLW, we measured for the first time the FMRs and water turnover rates for free-swimming adult marine turtles and used electronic archival tags to record diving activity of 18 adult female leatherbacks during the internesting period. Here we combine metabolic and diving data to examine relationships between physiology, environment and activity in leatherbacks.

Materials and methods

We conducted this study at Playa Grande, Parque Nacional

Marino Las Baulas (PNMB), Costa Rica. We performed the DLW experiment on five turtles *Dermochelys coriacea*, two in 2002 and three more in 2003–2004. First, we weighed the turtles using a tripod, winch, cargo net and hanging scale (Chatillon, Largo, FL, USA; 500±2 kg capacity). Next, we took initial blood samples (5–20 ml) from the dorsal cervical sinus (Owens and Ruiz, 1980) for determination of background D and ^{18}O levels. We then intravenously injected 15–30 ml $D_2{}^{18}O$ [99 APE (atom percent excess) D_2 and 75 APE ^{18}O solution; Isotec, Inc., Miamisburg, OH, USA] into the dorsal cervical sinus in order to ensure rapid equilibration of the isotopes in the turtles' body water (Speakman, 1997). We used equation 12.1 from Speakman (1997) to estimate the DLW dosages required for leatherbacks within the range of body sizes we studied. In 2002–2003, we only had approximately 30 g of the DLW available, so we took a conservative approach to the use of our expensive labeled water. Due to the highly exploratory nature of this study, we decided to divide the DLW we had into two doses and attempt the experiment on two turtles, rather than putting all of the DLW into one turtle. This way we avoided the risk that that turtle would not return to nest, in which case, we would have had no possibility of obtaining any results. In the 2003–2004 season we adhered closely to the Speakman (1997) equation.

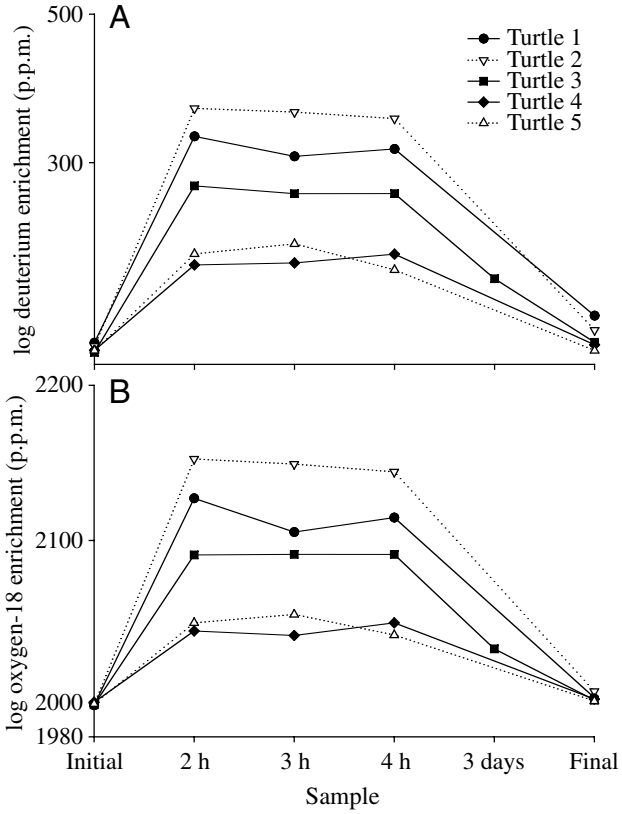

Fig. 1. Log(isotopic enrichment values) for (A) deuterium and (B) oxygen-18 for five leatherback turtles. The filled circle, square and diamond with solid lines represent the isotopic enrichments and washouts for the three turtles for which we were able to calculate FMRs. Open triangles and dotted lines represent the isotopic enrichments and washouts for the two turtles for which we were unable to calculate FMRs. Note the 3-day values for Female 3, which allowed for calculation of an FMR for the first 3 days of her internesting period and an FMR for her entire internesting period.

We sampled blood (≤5 ml) hourly from a rear flipper to establish equilibration of the isotopes with body water and released the turtles after approximately 4 h. Subsequent analyses confirmed that the injected isotopes had equilibrated with the animals' body water in this time period, as indicated by the stable plateau of isotopic enrichments between 2–4 h after injection of isotopes (Fig. 1). A recent DLW study on Atlantic walruses (body mass=1310 kg; ~5 times the body mass of the leatherbacks in our study) reported that the isotopes (intravenous injection) equilibrated in 2.5–3 h (Aquarone, 2004).

Because the DLW method requires recapture to measure final plasma isotope levels, and female leatherbacks at PNMB nest on average 6–8 times in a season (Reina et al., 2002b), we selected female turtles that were early in their nesting season (first to fourth nest), to ensure return and recapture upon subsequent nesting. We took a final blood sample (≤5 ml) from a rear flipper and albumen samples from shelled albumen gobs (SAGs; Wallace et al., 2004) when the turtles returned to nest in order to measure final isotope concentrations remaining in turtle body water at the end of the study period. While recent blood biochemistry analyses on reptiles indicate more variability in samples taken from hind limbs than from jugular veins (Jacobson et al., http://accstr.ufl.edu/blood_chem.htm), we found that isotopic concentrations in samples taken from the hind flippers were similar to those from the cervical sinus. We sampled mainly from a rear flipper because it was a less invasive procedure and we only needed small volumes of blood. All blood and albumen samples were later analyzed for D_2 and ^{18}O isotope concentrations by Metabolic Solutions, Inc. (Nashua, NH, USA), which ensures the accuracy of their analyses to 2% of 1 S.D. for deuterium and 0.4% of 1 S.D. for ^{18}O.

We calculated total body water (TBW) from oxygen dilution space and water turnover (rH_2O) using TBW derived from deuterium dilution space (Speakman, 1997). We calculated CO_2 production (rCO_2) assuming an RQ of 0.7 for nesting leatherbacks (Paladino et al., 1996) and using a 2-pool equation 7.43 from Speakman (1997), recommended for large animals.

We used LTD (light–temperature–depth) 2310 archival tags (Lotek Wireless, Inc., Newfoundland, Canada) attached to the anterior portion of the pygal process (Morreale, 1999) of 18 turtles, four of which were also subjects of the DLW experiments, to record their diving activity. The LTDs were programmed to record time, depth, water temperature and light level data at intervals of 4–60 s (depending on the tag) and had a maximum depth rating of 2000 m, with 1% accuracy to full scale. We analyzed dive data using Surface Adjust and Dive Analysis Programs from Lotek Wireless, Inc. To improve the reliability of classifying true surfacing events for the purposes of dive analysis, the automated Surface Adjust program was arbitrarily limited to search within areas of the data containing readings of <10 m when referenced to the daily minimum depth value. This assumes that the zero offset error on any

given day will be not fluctuating by more than 10 m. Regions of data that met this condition were processed and the median depth values determined as estimates of the zero offset error. The zero offset error for a given dive was then calculated by averaging the median value from the surface events that preceded and followed each dive.

Once the depth data were adjusted based on the zero offset, the entire data set was processed by Dive Analysis, which classified surfacing events as those regions of the data where the corrected depth records were exactly zero. We further filtered the adjusted data and accepted only dives >3 m to limit our analyses to true diving events. We calculated bottom time as the portion of a dive at or below 85% of maximum depth. A dive was counted as a U-dive if the turtle spent ⩾1 min on the 'bottom' (Reina et al., 2005). Based on video footage of breathing episodes at the surface (Reina et al., 2005), and because extended surface intervals correspond to traveling periods near the surface, not necessarily breathing or basking (Eckert, 2002), we only included surface events of >12 s and ⩽20 min in calculation of post-dive surface intervals. We excluded less than 6% of all surface intervals using these criteria.

We used least-squares linear regressions to analyze relationships between mean dive variables and Student's *t*-tests to compare dive variables between treatment groups (DLW *vs* LTD turtles; SPSS 11.5.1, Chicago, USA), and accepted significance at P=0.05 level. We arcsine transformed percentage data and values are presented as means ± 1 S.D. unless otherwise noted. We conducted all procedures under permits 288-2002-OFAU and 273-2003-OFAU from the Costa Rican Ministerio del Ambiente y Energía (MINAE) and Drexel University IACUC Approval 02183 and 02185.

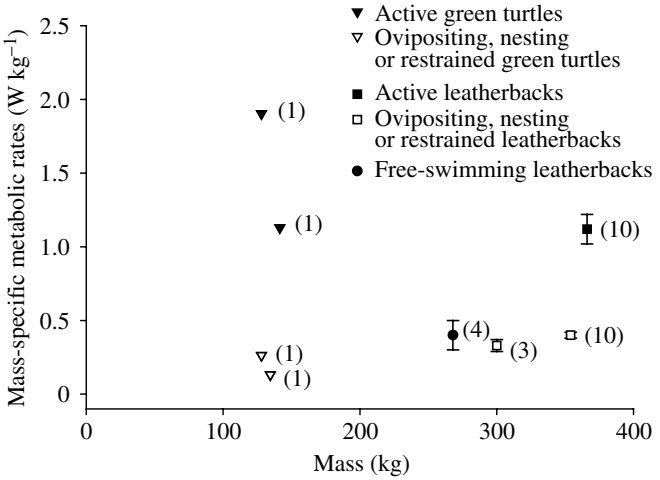

Fig. 2. Mass-specific metabolic rates of adult female leatherback and green turtles. The field metabolic rates reported in this study for internesting leatherbacks (filled circle) were similar to metabolic rates measured during oviposition, nest construction or restraint for leatherbacks (open squares; Paladino et al., 1990, 1996) and slightly higher than those for green turtles (open inverted triangles; Prange and Jackson, 1976; Jackson, 1985). The FMRs were lower than metabolic rates measured during vigorous nest covering or walking along the beach for green turtles (filled inverted triangles; Prange and Jackson, 1976; Jackson, 1985) and leatherbacks (filled square; Paladino et al., 1990, 1996). Values are means ± 1 S.E.M.; numbers in parentheses indicate sample size.

Results

Field metabolic rates and water turnover rates

We measured four FMRs for three free-ranging internesting leatherbacks (Table 1). We obtained two FMRs for Female 3, one for the first 3 days of her internesting period, and one for her entire 14 day period. This turtle came ashore and attempted to nest after 3 days but did not lay any eggs, and we obtained a blood sample at that point. The turtle's field metabolic rates (FMRs) and diving behavior were similar to those of the other turtles (Tables 1 and 2). The FMRs for the three turtles (range: 0.20–0.74 W kg^{-1}) were similar to MRs for nesting female leatherbacks and slightly higher than MRs of nesting green

Table 1. *Mass, water turnover rates, and field metabolic rates of adult female leatherback turtles*

Turtle	Mass (kg)	Study duration (days)	N_O (O^{18}dilution space; mol)	%TBW (O^{18})	k_D:k_O	ml H_2O day^{-1}	%TBW day^{-1}	FMR (W kg^{-1})	Reptilian RMR[1] (W kg^{-1})	Mammalian RMR[2] (W kg^{-1})
1	270	14.7	10275	68.5	0.70	28696	15.5	0.74	0.146	0.826
2	196	11.2	7976	73.3	1.04	39391	27.2	ND	0.154	0.895
3	268	14.1	11036	74.1	0.86	45825	21.5	0.40	0.146	0.828
3 (3 days)	268	3.1	11036	74.1	0.92	62465	29.9	0.24	0.146	0.828
4	308	12.8	14105	82.4	0.93	58408	23.6	0.20	0.143	0.800
5	298	12.7	11402	68.9	ND	ND	ND	ND	0.144	0.806
Mean	268			73.9			23.5	0.40	0.147	0.830
S.D.	44			5.7			5.5	0.20		

We obtained four FMRs for three leatherback turtles during the internesting period (one for the first 3-day interval and one for the entire 14-day interval for Turtle 3).

Leatherback FMR values are intermediate between allometric predictions of resting metabolic rates (RMRs) for reptiles[1] (RMR=0.378$M_b^{-0.17}$) and mammals[2] (RMR=3.35$M_b^{-0.25}$; equations from Paladino et al., 1990, 1996).

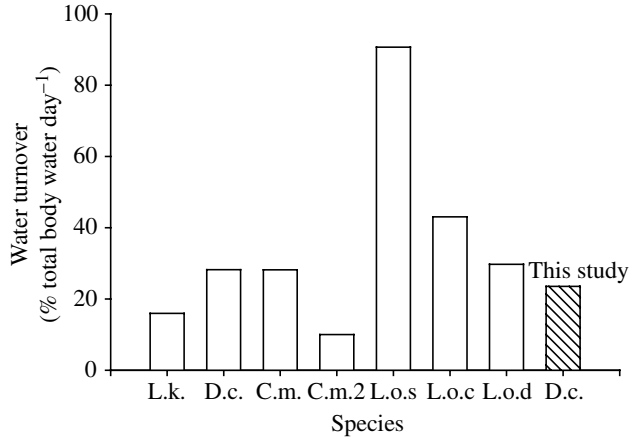

Fig. 3. Water turnover rates (% TBW day^{-1}) for marine turtles. Water turnover rates measured by DLW in this study (mean=24% TBW day^{-1}, range=16–30% TBW day^{-1}) are within the range of published values for marine turtles. *x*-axis labels from left to right, with the method by which water turnover rates were derived: L.k., *Lepidochelys kempii* adults, deuterated water (D_2O) (Ortiz et al., 2001); D.c., *D. coriacea* hatchlings, lachrymal gland secretions (Reina et al., 2002b); C.m.1, *C. mydas* hatchlings, lachrymal gland secretions (Reina, 2000); C.m.2, *C. mydas* juveniles, DLW (Jones et al., in press); L.o., *Lepidochelys olivacea* hatchlings (s, swimming, c, crawling, d, digging) DLW (Clusella Trullas et al., in press); D.c. (striped bar): *D. coriacea* adults, DLW, this study.

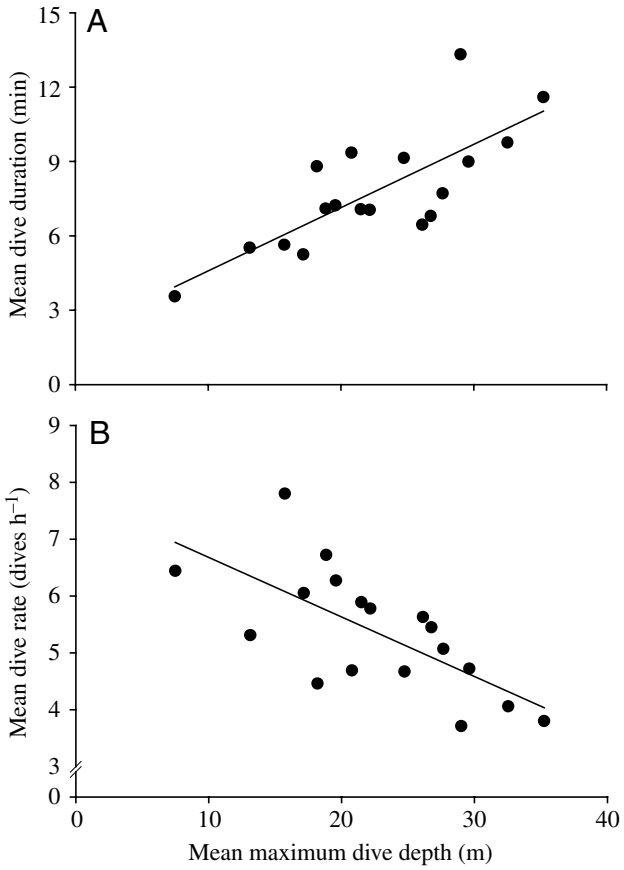

Fig. 4. Mean maximum dive depth *vs* (A) mean dive duration and (B) mean dive rate for internesting leatherback turtles. Increases in mean maximum dive depth resulted in increased mean dive durations (A; y=2.033+0.2553x, r^2=0.588, P<0.001) and decreases in mean dive rates (B; y=7.727–0.1048x, r^2=0.469, P=0.002) of 18 adult female leatherbacks.

turtles *Chelonia mydas* obtained by analyses of respiratory gases during oviposition (Fig. 2). We were unable to measure FMRs for two of the study turtles (Females 2 and 5).

Calculated total body water (TBW) for five study female leatherbacks (ranging in mass from 196 to 308 kg) was 73.9±5.7% (range: 68.5–82.4%; Table 1). Water turnover rates (rH_2Os) of internesting leatherbacks (including the rH_2O during the 3-day interval for Turtle 3) ranged from 16% to 30% of TBW day^{-1} (mean: 24±5.5% TBW day^{-1}), which were within the range of published rH_2O values for leatherbacks and other species of marine turtles (Fig. 3). Female 2 exhibited the highest rH_2O for her entire 11-day internesting period (27.2% TBW day^{-1}), while Female 3 had a higher rH_2O during the first 3 days of her internesting period (29.9% TBW day^{-1}).

Diving activity during the internesting period

We recorded diving activity of four of five DLW turtles and 14 'control' (LTD) turtles, totaling 23 402 total dives. Individual turtles demonstrated different diving patterns in terms of mean dive variables and water temperature T_w (Table 2). Across all turtles, mean maximum dive depth was 22.6±7.1 m, with mean dive depth 14.6±4.6 m and mean dive duration 7.8±2.4 min. The deepest single dive was 200 m (Turtle 16) and the longest was 44.9 min (Turtle 2). Turtles reached maximum depths of ⩽20 m on approximately 60% of all dives, and approximately 43% of all dive durations were ⩽5 min. The mean water temperature leatherbacks encountered was 26.6°C, while the minimum encountered was 13.6°C (Turtle 16).

Including all dives for all turtles, post-dive surface intervals increased as the duration of the preceding dive increased (Spearman Rank Correlation, r^2=0.159, P<0.001) As mean maximum depth increased for all turtles, mean dive duration (r^2=0.588, P<0.001; Fig. 4A), mean bottom time (r^2=0.590, P<0.001), and proportion of U-dives (r^2=0.750, P<0.001) increased while mean dive rate decreased (r^2=0.469, P=0.002; Fig. 4B). Additionally, increased mean maximum depth resulted in turtles spending an increased proportion of time in T_w⩽24°C (r^2=0.424, P=0.003); a stronger relationship existed with percentage of time spent in T_w⩽20°C (r^2=0.595, P<0.001).

Turtles with LTDs attached that underwent DLW experiments had significantly longer internesting periods than turtles that only had LTDs attached (Student's t-test: t_{17}=7.951, P<0.001; DLW turtles: 13.1±1.4 days, LTD turtles: 9.1±0.8 days). Additionally, DLW turtles spent a significantly higher proportion of time in T_w⩽24°C than LTD turtles (t_{16}=3.165, P=0.006; DLW: 16.0±2.6%, LTD: 7.6±5.0%), especially during the early phase of the internesting period, which directly followed nesting and the restraint portion of the experiment

(t_{16}=3.508, P=0.003; DLW: 25.5±4.5%, LTD: 10.4±6.9%). Furthermore, DLW turtles made significantly more total dives (t_{16}=3.325, P=0.004; DLW: 1671±324, LTD: 1194±233), and particularly more U-dives during the internesting period than LTD turtles (t_{16}=5.125, P<0.001; DLW: 1206±242, LTD: 750±130).

FMRs, diving physiology and activity

Although we had a small sample size, (1 FMR for Turtles 1 and 4, 2 FMRs for Turtle 3), we used statistical analyses (Pearson Product–Moment Correlation) to examine the relationships between FMR, diving physiology and activity. We found several suggestive of strong positive relationships between FMRs and mean dive durations (r^2=0.991), bottom times (r^2=0.992), percentage of time spent in $T_w \leqslant 24$°C (r^2=0.990), and surface interval (r^2=0.999, P=0.027).

Combining the FMRs acquired by us with the value for adult leatherback total O_2 stores reported by Lutcavage et al. (1992), cADLs for internesting female leatherbacks were between 11.7 min and 44.3 min (Table 3A).

Discussion

Free-swimming leatherbacks have low internesting metabolic rates, and thus spend little energy while active at sea between nesting events. The DLW-derived FMRs that we measured for free-swimming, internesting leatherbacks were similar to leatherback MRs measured during restraint on the beach, oviposition or nest chamber construction, and lower than leatherback MRs obtained by analyses of respiratory gases during vigorous nest-covering and walking on the beach (Paladino et al., 1990, 1996). In addition, our data are consistent with the findings of Southwood et al. (1999), who reported that diving heart rates of leatherbacks were more

Table 2. *Dive variables for internesting female leatherback turtles at Parque Nacional Marino Las Baulas, Costa Rica, 2002-03 and 2003-04*

						Mean dive variables						
Turtle	Treatment	Period (days)	Total dives	Total U-dives	Total V-dives	Duration (min)	Surface interval (min)	Depth (m)	Maximum depth (m)	Rate (dives h^{-1})	Bottom time (min)	% Time in $T_w \leqslant 24$°C
1	DLW/LTD	14.7	1652	1321	331	9.0	3.6	19.6	29.6	4.7	3.4	19.0
2	DLW/LTD	11.2	1222	860	362	8.8	3.7	12.1	18.2	4.5	3.2	17.0
3	DLW/LTD	14.1	1952	1411	541	7.2	2.8	13.0	19.6	6.3	2.7	13.0
3 (3 days)	DLW/LTD	3.1	404	318	86	6.8	2.5	15.2	22.9	6.5	2.5	14.1
4	DLW	12.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	DLW/LTD	12.8	1856	1234	622	6.8	3.0	17.2	26.8	5.5	2.3	15.0
Mean (DLW turtles)		13.1[a]	1671[b]	1207[c]	464	8.0	3.2	15.5	23.5	5.2	2.9	16.0[d]
S.D.		1.4	324	242	140	1.1	0.4	3.5	5.5	0.8	0.5	2.6
6	LTD	9.2	1044	751	293	9.1	3.6	15.7	24.7	4.7	3.2	7.8
7	LTD	9.0	1642	874	768	5.3	2.9	10.8	17.2	6.1	1.7	7.2
8	LTD	9.8	1536	486	1050	3.6	4.2	4.9	7.5	6.4	1.3	0.2
9	LTD	8.0	1233	783	450	6.5	3.0	16.7	26.1	5.6	2.2	10.9
10	LTD	10.0	880	742	138	13.3	4.5	17.9	29.0	3.7	3.7	7.1
11	LTD	7.7	984	613	371	7.1	4.1	14.2	22.2	5.8	2.4	5.4
12	LTD	9.0	1273	972	301	7.7	2.6	17.6	27.7	5.1	2.7	7.9
13	LTD	8.0	1333	776	557	5.6	3.1	10.3	15.7	7.8	2.0	1.1
14	LTD	8.2	1302	610	692	5.5	3.6	8.2	13.1	5.3	1.8	2.9
15	LTD	10.0	1146	853	293	9.8	3.0	21.2	32.5	4.1	3.7	15.7
16	LTD	9.0	791	637	154	11.6	3.9	23.0	35.2	3.8	4.4	17.5
17	LTD	9.9	1087	836	251	9.4	3.5	14.1	20.8	4.7	3.6	7.7
18	LTD	9.1	1185	714	471	7.1	3.4	12.8	18.8	6.7	2.8	10.9
19	LTD	10.0	1284	859	425	7.1	3.4	12.9	21.5	5.9	1.9	3.8
Mean (LTD turtles)		9.1[a]	1194[b]	750[c]	444	7.8	3.5	14.3	22.3	5.4	2.7	7.6[d]
S.D.		0.8	233	130	253	2.6	0.5	4.9	7.6	1.2	0.9	5.0
Mean (all turtles)		10.1	1300	852	448	7.8	3.4	14.6	22.6	5.4	2.7	9.5
S.D.		2.1	319	248	229	2.4	0.5	4.6	7.1	1.1	0.8	5.8

ND, no data. There were no dive data for Turtle 4 because she did not have a data logger.

DLW/LTD, turtles used for doubly labeled water/data logger experiments; LTD, turtles with only data loggers attached. Data from the 3-day period for Turtle 3 were not included in calculation of means.

Significantly different pairs denoted by common superscript: [a]P<0.001; [b]P=0.004; [c]P<0.001; [d]P=0.006.

similar to heart rates during oviposition than relatively higher heart rates measured during active nest covering or walking on the beach. In a broader context, leatherback FMRs were intermediate between allometric expectations of reptilian and mammalian RMRs (Paladino et al., 1990, 1996; Table 1). The FMRs were slightly higher than the metabolic rates for green turtles during oviposition but lower than the metabolic rates of green turtles during activity (Prange and Jackson, 1976; Jackson, 1985; Fig. 2). These FMRs also indicate that swimming is more energetically efficient than walking for leatherbacks as it is for other vertebrates (Schmidt-Nielsen, 1972).

Adult female leatherbacks in this study exhibited high rH_2O values (16–30% TBW), but within the range of values for leatherbacks and other marine turtle species obtained with isotopically labeled water (Clusella Trullas et al., in press; Ortiz et al., 2001; Jones et al., in press) and by analyses of lachrymal gland secretion rates (Reina, 2000; Reina et al., 2002a; Fig. 3). The rH_2O values for female leatherbacks reported here are understandable considering that internesting leatherbacks produce massive egg clutches, which contain large amounts of water. Indeed, the female leatherbacks in this study laid subsequent clutches of approximately 3–8 kg in mass (B. P. Wallace, unpublished data). Moreover,

Table 3. *Calculated aerobic dive limits (cADLs), mean and maximum dive durations for leatherback turtles*

A

Turtle	Mass (kg)	FMR (ml O_2 min^{-1})	O_2 stores (ml)	cADL (min)	Mean duration (min)	Maximum duration (min)	Ratio of mean duration to cADL	% Dives exceeding cADL
1	270	625	7290	11.7	9.0	22.2	0.77	32.3
2	196	ND	5292	ND	8.8	44.9	ND	ND
3	268	351	7236	20.6	7.2	22.2	0.35	0.05
3 (3 days)	268	233	7236	31.0	6.8	16.4	0.22	0.0
4	308	188	8316	44.3	ND	ND	ND	ND
5	298	ND	7992	ND	6.8	21.0	ND	ND

Among DLW turtles, mean dive durations were all lower than cADLs, and turtles rarely exceeded their cADLs.
We used total oxygen stores reported by Lutcavage et al. (1992) to calculate O_2 stores for each turtle.

B

	Duration (min)		Ratio of mean duration to:		% Dives exceeding:	
Turtle	Mean	Maximum	cADL=11.7 min	cADL=44.3 min	cADL=11.7 min	cADL=44.3 min
1	9.0	22.2	0.77	0.20	32.26	0
2	8.8	44.9	0.75	0.20	36.66	0.08
3	7.2	22.2	0.62	0.16	16.60	0
3 (3 days)	6.8	16.4	0.58	0.15	12.87	0
4	ND	ND	ND	ND	ND	ND
5	6.8	21.0	0.58	0.15	17.89	0
6	9.1	26.4	0.78	0.21	33.91	0
7	5.3	18.3	0.45	0.12	8.47	0
8	3.6	27.6	0.30	0.08	4.43	0
9	6.5	21.1	0.55	0.15	17.52	0
10	13.3	31.0	1.14	0.30	60.11	0
11	7.1	30.0	0.60	0.16	20.43	0
12	7.7	24.1	0.66	0.17	23.10	0
13	5.6	19.2	0.48	0.13	7.73	0
14	5.5	25.7	0.47	0.12	14.75	0
15	9.8	23.9	0.83	0.22	47.64	0
16	11.6	26.6	0.99	0.26	50.19	0
17	9.4	29.6	0.80	0.21	31.65	0
18	7.1	25.8	0.61	0.16	21.94	0
19	7.1	24.6	0.60	0.16	17.21	0
Mean	7.8	25.3	0.66	0.18	25.02	0
S.D.	2.4	6.4	0.20	0.05	15.62	0.02

Mean dive durations for all turtles in this study were within cADLs (11.7–44.3 min; based on FMRs). Overall, only one dive duration (44.9 min; Turtle 2) exceeded the maximum cADL.

leatherbacks possess highly effective osmoregulatory capabilities that allow them to drink seawater without incurring negative water and ionic balance (Reina et al., 2002b). Turtle 3 exhibited a higher rH_2O during the first 3 days of her internesting period than for the entire 14 days period (Table 2), which corresponded to the high frequency of water/prey ingestion events during the first few days after nesting reported by Southwood et al. (2005).

Efficacy of DLW method in studies of marine turtle energetics

While the rH_2O values for all turtles in this study were within the range of water fluxes for other marine turtles (Fig. 3), we were unable to calculate FMRs for Females 2 and 5 using DLW. Female 2 turned over 27.2% of her TBW daily, or nearly 3 times during her 11-day internesting period, while Female 5 completely washed out the deuterium isotope, also indicating a high rH_2O. In other studies using isotopically labeled water to calculate rH_2O values for olive ridley (*Lepidochelys olivacea*; Clusella Trullas et al., in press), Kemp's ridley (*L. kempii*; Ortiz et al., 2001), and green turtles (Jones et al., in press), no animals turned over their TBW more than 2.5 times during the study period. Apparently, there exists a threshold ratio of rCO_2 to rH_2O (where rH_2O must be <27.2% TBW day^{-1}) necessary for the DLW method to be able to measure rCO_2 in leatherbacks and perhaps marine turtles in general.

Validation experiments of the accuracy of the DLW method have been performed for several animal species over a wide size range (reviewed in Speakman, 1997). Performing simultaneous metabolic measurements (DLW and respirometry, for example) on adult marine turtles is extremely difficult, due to factors such as their marine lifestyle, large size, endangered status, and the high cost of the large volume of enriched DLW required. In general, validations indicate that although individual variation might account for serious discrepancies between DLW measurements and those acquired by reference methods, the DLW method tends to overestimate rCO_2 by less than 5% among different animal clades (Butler et al., 2004). Only one truly simultaneous validation study has been performed for marine turtles to date, which reported that the global mean of DLW-derived rCO_2 values for juvenile green turtles was not significantly different and only varied by 5.2% from that obtained by gas respirometry for the same period from the same animals (Jones et al., in press).

A ratio of deuterium to oxygen-18 isotopic washout (k_D:k_O) that exceeds 0.9 implies that ~90% of oxygen elimination is tied to water losses, and therefore the DLW method might not accurately quantify CO_2 production (Speakman, 1997). In this study, the combination of long study durations, high rH_2Os and low metabolic rates resulted in insufficient divergence (Female 2; k_D:k_O=1.04) or complete washout (Female 5) of the isotopes, rendering the DLW method unable to calculate rCO_2 (Butler et al., 2004) for these two leatherbacks. The other k_D:k_O ratios that we calculated ranged from 0.70–0.93, slightly above or within the recommended range (Speakman, 1997). The relatively high water turnover rates and k_D:k_O ratios that we measured indicate the need for caution when interpreting our results (Speakman, 1997; Butler et al., 2004). However, Jones et al. (in press) reported k_D:k_O ratios between 0.84–0.92 for juvenile green turtles, and the DLW-derived MRs were not significantly different from MRs obtained by respirometry in that study. Considering existing DLW validation information (Jones et al., in press) and the fact that our FMRs fell within the range of measured MRs for leatherbacks during various activities (Paladino et al., 1990, 1996), we conclude that our measurements were accurate and biologically realistic, despite the lack of simultaneous validation data *via* respirometry.

Internesting diving activity

Dives tended to be shorter and shallower for Pacific Costa Rican leatherbacks (mean durations ≈7–8 min, mean depths ≈15–19 m; Southwood et al., 1999, 2005; this study) than dives for leatherbacks in the Caribbean near St Croix (mean durations ≈10–15 min, mean depths ≈60–100 m; Eckert et al., 1986, 1989; Eckert, 2002). Dive variables from leatherbacks in the South China Sea off Malaysia were intermediate (mean durations ≈8–12 min, mean depths ≈26–45 m; Eckert et al., 1996). These differences were probably due to relatively shallower depths available to internesting leatherbacks on the continental shelf near PNMB, Costa Rica, relative to other sites (Morreale, 1999; Southwood et al., 1999). Increasing mean maximum dive depths were associated with increased mean dive duration and decreased mean dive rates (Fig. 4), similar to trends reported for leatherbacks worldwide (Eckert et al., 1986, 1996; Southwood et al., 1999; Reina et al., 2005). Similar trends were reported for New Zealand sea lions *Phocarctos hookeri* (Costa and Gales, 2000) and Australian sea lions *Neophoca cinerea* (Costa and Gales, 2003). Therefore, while leatherback diving activity patterns appear to be constrained primarily by different depths encountered in different internesting habitats (Eckert et al., 1996; Morreale, 1999; Southwood et al., 1999), some general patterns in dive behavior exist globally between and among taxa of diving animals.

We found that post-dive surface intervals lengthened with increased dive duration for all dives across all turtles, contrary to some findings (Eckert et al., 1989, 1996; Southwood et al., 1999; but see Reina et al., 2005). However, this relationship had a low r^2 value (0.159), indicating >80% of the variance in post-dive surface interval durations that was not explained simply by preceding dive durations. Given the relatively short dive durations and surface intervals of leatherbacks in this study, turtles were probably not using the post-dive surface interval to recover from CO_2 accumulation during diving apnea.

Leatherbacks occasionally exhibited extremely long surface intervals (maxima 21.8–108.3 min). These long surface intervals represented periods of traveling within the upper few meters of the water column, and not resting or basking behavior, as previously hypothesized (Eckert et al., 1986, 1989; Southwood et al., 1999). This is supported by swim speed and location data off Playa Grande (Southwood et al.,

2005) and St Croix (Eckert, 2002) and video footage (Reina et al., 2005) for internesting leatherbacks. Moreover, Penick (1996) measured minimal blood flow to the carapace surfaces of nesting leatherbacks, indicating that leatherbacks would have limited ability for heat gain while basking.

Leatherbacks that had undergone the experimental handling required by the DLW methodology had significantly longer internesting periods, made significantly more U-dives, and spent more time in $T_w \leqslant 24$°C than leatherbacks to which we only attached data loggers. This was especially evident during the early third of the internesting period. Corticosteroid hormone concentrations increase in response to stress related to prolonged handling (Gregory and Schmid, 2001), and this can inhibit various physiological functions, including egg production (Owens, 1997; Rostal et al., 2001; Milton and Lutz, 2003). Such hormonal inhibition of reproductive function would account for the extended internesting period of the DLW turtles (13.1 days) relative to the LTD turtles (9.1 days). Turtle 3 re-emerged 3 days after nesting, which was probably another manifestation of the hormonal inhibition of the natural egg production process during the internesting period in response to this prolonged stress. This turtle went through the entire nesting process, but did not lay eggs. Although this was a rare occurrence in this population, the turtle's field metabolic rates (FMRs) were similar to the others we obtained (Table 1) and her diving behavior was similar to the behavior of other turtles (Table 2). Furthermore, Turtle 3 returned to nest successfully 11 days later (14 days after the previous nesting), which was her fifth and final nest of the season. A season total of five nests is within the normal range for nesting leatherbacks in this population (see Reina et al., 2002b).

According to Reina et al. (2005), U-dives chiefly serve a resting purpose, and almost 20% of these dives involve the turtles remaining stationary on the ocean bottom for up to 1 min. Furthermore, Southwood et al. (2005) reported decreases in body temperature T_b during relative inactivity on or near the ocean bottom, indicating physiological heat dissipation. For all turtles in our study, as the number of U-dives that turtles made increased, so did the percentage of time spent in $T_w \leqslant 24$°C (r^2=0.311, P=0.016). However, DLW turtles made significantly more U-dives than LTD turtles (Table 2), thus accounting for the increased percentage of time that DLW turtles spent in colder waters than LTD turtles. We hypothesize that the prolonged restraint in cargo nets and sequential blood-drawing procedures caused the experimental turtles to incur elevated corticosteroid levels and increased heat loads, which resulted in protracted internesting periods and compensatory thermoregulatory behavior during the first few days at sea after the experiment. What effects, if any, the experimental stress had on the FMR values themselves is unknown, especially since DLW-derived FMRs are integrations of metabolism during the entire study period, not for particular activities. However, DLW turtles performed within the range of dive variables for all turtles (Table 2), indicating that their diving activities (and presumably their FMRs) were representative of internesting leatherbacks in general. It is important to point out that DLW turtles returned to nest successfully and also resumed normal internesting periods after the experiment. While the prolonged restraint was necessary and the DLW experiment likely imposed stress on leatherbacks, it did not interfere with their long-term reproduction or behavior. Nonetheless, these factors should be taken into consideration when conducting this type of experiment with marine turtles because of their endangered status and the unavoidably stressful nature of the experiment.

FMRs, diving physiology and activity

Our proposed upper limit on leatherback cADL (44.3 min) corroborates the findings of Hays et al. (2004a), who reported an apparent ceiling on leatherback migratory dive durations at around 40 min. Mean dive durations for all four DLW turtles were below the cADL range (11.7–44.3 min), and DLW turtles exceeded their cADL on only 0–33% of their dives (Table 3A). We then calculated the percentage of dives for all 18 turtles exceeding the lower (11.7 min) and upper limits (44.3 min) of the FMR-derived cADLs. All turtles regularly dived below or within the cADL range, and on the average 25% of dives exceeded the lower limit (Table 3B, Fig. 5). Only one dive exceeded the upper limit (44.9 min; Turtle 2), but none exceeded the upper limits proposed by Lutcavage et al. (70 min; 1990) and Southwood et al. (67 min; 1999).

According to Thompson and Fedak (2001), an air-breathing, diving animal should regularly approach and often exceed its ADL when foraging conditions are advantageous, and doing so would allow it to better exploit such conditions. Many marine mammals and birds routinely exceed cADLs while actively foraging (Costa et al., 2001). Conversely, if potential foraging success is low, an animal should limit its energy expenditure and dive well within its ADL. The fact that

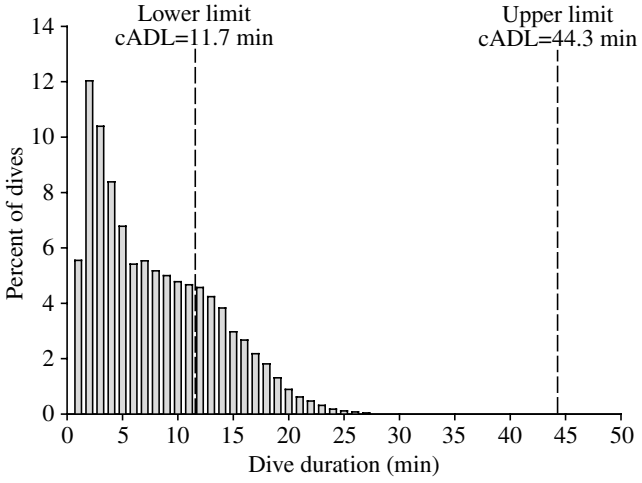

Fig. 5. Frequency distribution of dive durations for all turtles in relation to calculated aerobic dive limits (cADLs). Approximately 43% of all dives were ⩽5 min long, and 25% of all dives exceeded the lower limit of the cADLs (11.7 min) that we calculated from field metabolic rates for free-swimming, internesting leatherbacks. Only one dive duration exceeded the upper limit (44.3 min) of cADLs.

internesting leatherbacks rarely approached their cADL values suggests that prey availability off Parque Nacional Marino Las Baulas was low and turtles were not actively foraging (Thompson and Fedak 2001; Hays et al., 2004a). Considering the weak relationship between dive durations and post-dive surface interval (r^2=0.159), these analyses further support the conclusion that internesting leatherbacks maintain relatively low activity levels, conserve energy by diving well-within physiological limits, and are rarely, if ever, anaerobic during the internesting period (Jones et al., 2004).

We found positive relationships between FMRs and mean dive durations, bottom times, surface intervals, and the proportion of time turtles spent in $T_w \leqslant 24°C$ (all $r^2>0.99$). These results raise interesting questions about leatherback thermoregulation, diving physiology and behavior. Leatherbacks might dive more actively, thereby increasing metabolic rates (since increased muscle activity automatically results in higher metabolism) in order to exploit colder waters, presumably to forage. Eckert et al. (1986, 1989, 1996) hypothesized that diurnal differences in dive patterns represented foraging activity following the deep-scattering layer (DSL). However, Hays et al. (2004a) pointed out that leatherbacks migrate great distances away from nesting grounds to increase foraging success because prey abundance is presumably greater on pelagic foraging grounds than along tropical coasts. In addition, Reina et al. (2005) did not observe any feeding activity in video footage of the first day after nesting, and Southwood et al. (2005) found no relationship between ingestion events and diel dive patterns, which were previously thought to be related to vertical movements of the DSL (Eckert et al., 1986, 1989, 1996). If leatherbacks were actively foraging, an increase in FMR over RMR of 10–30% might be expected due to specific dynamic action (Withers, 1992). However, the fact that FMRs that we obtained were not significantly elevated relative to nesting leatherback MRs (Fig. 2) renders the possibility that leatherbacks were foraging during the internesting period highly unlikely.

According to the gigantothermy model, leatherbacks must maintain low MRs and increase blood flow to peripheral tissues to dissipate heat generated internally to avoid overheating in the tropics (Paladino et al., 1990). Southwood et al. (2005) recorded subcarapace and gastrointestinal tract temperatures of internesting leatherbacks and surmised that their measured gradients between T_b and T_w for leatherbacks in the tropics supported the predictions of the gigantothermy model. While data on blood flow adjustments by leatherbacks at sea are not available, the relatively low FMR values that we report in the present study for internesting leatherbacks reinforce the conclusions of Paladino et al. (1990) and Southwood et al. (2005). Furthermore, we found relationships between FMRs, increased activity levels (mean maximum depth, dive duration, bottom time) and proportion of time spent in $T_w \leqslant 24°C$. This suggests that leatherbacks with increased activity levels (and perhaps higher metabolic rates) might avoid overheating while in the tropics by increasing the proportion of time spent in cool water, thus behaviorally moderating their body temperatures by using cooler water as a heat sink (Fig. 6). Southwood et al. (2005) reported T_b values that were consistently elevated above T_w, but T_b could be affected by modifications in swimming and diving activity and fluctuating T_w. Tuna also experience limitations on activity in warm water in the tropics (Blank et al., 2004), and modulate heat transfer both physiologically and behaviorally (Dewar et al., 1994). Leatherbacks in this study spent the highest percentage of time in cooler waters in the early third of the internesting period after nesting, implying that increased heat loads incurred during increased activity associated with nesting necessitated shuttling to colder T_w. Southwood et al. (2005) recorded frequent ingestion events during this segment of the internesting period, potentially indicating internal heat dissipation through ingestion of cooler water and/or prey. It is plausible that dive patterns of leatherbacks foraging in cold waters would be opposite to those for internesting leatherbacks, in that turtles might spend

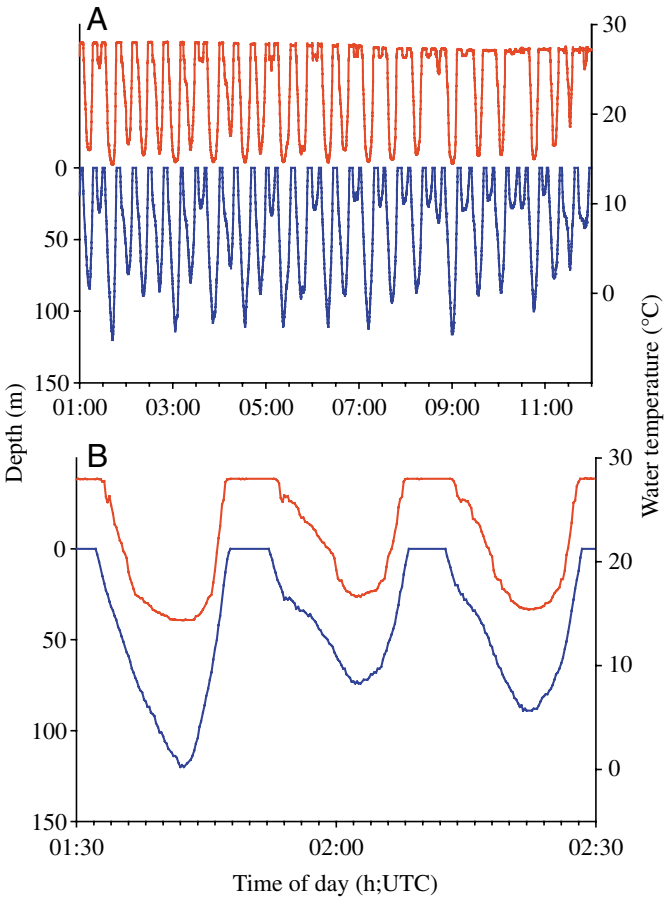

Fig. 6. Depth (blue trace) *vs* temperature (red trace) diving profiles for (A) 12 h and (B) 1 h. Electronic archival dive data displayed were recorded during day 3 of the internesting period for Turtle 1 and include her deepest dive (120 m), longest dive duration (22.2 min), and coldest water temperature experienced (14.4°C). Water temperatures experienced by Turtle 1 during this 12 h period ranged from 28.1 to 14.4°C.

more time at the surface in warmer water in response to prolonged periods submerged in colder water, as documented in bigeye (Holland et al., 1992) and bluefin tuna (Gunn and Block, 2001). Additional experiments simultaneously measuring leatherback body temperatures, metabolism and diving activity in cold water are needed to distinguish between these possibilities.

In order for female leatherbacks to reproduce, they must harvest and store sufficient energy to facilitate nest construction, egg production and survival at sea between nesting events. Of those components, only energy expenditure during the internesting period can be flexible, since compromises in egg production and nest construction would decrease reproductive success. The relatively low FMRs reported here, almost exclusively aerobic diving, and apparent thermal constraints on activity imposed by warm tropical water exhibited by leatherbacks in this study, suggest minimized energy expenditure during the internesting period. This might facilitate increased energy allocation to egg production and nesting, as reported for internesting green turtles (Hays et al., 2000). Future studies should incorporate more data on metabolism, body temperatures and diving behavior of migrating and foraging turtles in cooler waters in order to understand how environmental and life history demands affect marine turtle energetics and activity.

List of abbreviations

ADL	aerobic dive limit
APE	atom percent excess
cADL	calculated aerobic dive limit
DLW	doubly labeled water
DSL	deep-scattering layer
FMR	field metabolic rate
k	isotopic washout
LTD	light–temperature–depth recorder
MR	metabolic rate
rCO_2	CO_2 production
rH_2O	water turnover rate
RMR	resting metabolic rate
SAG	shelled albumen gob
T_b	body temperature
T_w	water temperature
TBW	total body water

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