

Energy and Water Flux during Terrestrial Estivation and Overland Movement in a Freshwater Turtle

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ABSTRACT

The doubly labeled water (DLW) method for studying energy and water balance in field-active animals is not feasible for freshwater animals during aquatic activities, but several species of nominally aquatic reptiles leave wetlands for several critical and extended behaviors, where they face challenges to their energy and water balance. Using DLW, we studied energy and water relations during terrestrial estivation and movements in the eastern long-necked turtle (*Chelodina longicollis*), a species that inhabits temporary wetlands in southeastern Australia. Water efflux rates of 14.3–19.3 mL (kg d)⁻¹ during estivation were nearly offset by influx, indicating that turtles did not maintain water balance while terrestrial, though dehydration was slow. Estivation energy expenditure declined over time to 20.0–24.6 kJ (kg d)⁻¹ but did not indicate substantial physiological specializations. Energy reserves are predicted to limit survival in estivation to an estimated 49–261 d (depending on body fat), which is in close agreement with observed bouts of natural estivation in this population. The energy cost and water flux rates associated with overland movement behavior ranged from 46 to 99 kJ (kg d)⁻¹ and from 21.6 to 40.6 mL (kg d)⁻¹, respectively, for turtles moving 23–34 m d⁻¹. When a wetland dries, a turtle that forgoes movement to other wetlands can save sufficient energy to fuel up to 134 d in estivation. The increasing time in estivation with travel distance gained in this energy “trade-off” fits our previous observations that more turtles estivate when longer distances must be traveled to the nearest permanent lake, whereas emigration is nearly universal when only short distances must be traversed. The DLW method shows

promise for addressing questions regarding the behavioral ecology and physiology of freshwater turtles in terrestrial situations, though validation studies are needed.

Introduction

Energy is a limiting resource that features prominently in the behavioral ecology and life history of ectotherms (Congdon et al. 1982; Congdon 1989). Ectotherms must balance a finite energy intake against expenditures for maintenance as well as discretionary production (growth, reproduction, and storage), all of which could influence overall fitness. When energy expenditure surpasses intake, an individual is in negative energy balance, and with the exception of storage, energy allocated to one function is typically unavailable for others. Owing to these trade-offs, individuals must not only weigh the benefits of expenditure against intake but also respond in a way that results in the most optimum distribution of available energy among competing compartments. Individuals can exert some control over these energy allocations through their behavior. Some classic examples of energy trade-offs are for reproductive effort, where individuals may cease foraging and expend variable amounts of energy in parental care (Shine et al. 1997), attracting mates (Grafe 1996), defending territories (Grantner and Taborisky 1998), or migrating to breeding grounds (Kinnison et al. 2003).

Water is another resource that is vital for nearly all life processes, but access to it can be limited in terrestrial environments. Water generally makes up 65%–80% of an animal's body mass, but body water can fluctuate according to the availability of water in the environment and the individual's ability to physiologically or behaviorally regulate water balance through intake and loss. Physiological mechanisms to conserve water include metabolic depression (Guppy and Withers 1999), changes to skin permeability (Lillywhite 2006), and temporary storage of excretory wastes (Peterson 1996a). Behavioral regulation can include habitat choice and altered activity levels (Christian et al. 1996; Ligon and Peterson 2002), drinking and eating (Peterson 1996b), posture (Wygoda and Chmura 1990), and storage (Jorgensen 1998). Individuals that most successfully manage their energy and water budgets through behavioral or other allocation responses are expected to have the highest fitness (i.e., lifetime reproductive success) and thus be favored by natural selection.

Studying animals in the field, where they are free to respond in ways that influence their survival, would provide the most

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useful information with which to address behavioral ecology and evolutionary questions. The development of the doubly labeled water (DLW) technique (Lifson and McClintock 1966) has revolutionized the study of energy and water relations in animals (Nagy et al. 1999; Butler et al. 2004). Reptiles have proved to be tractable for the DLW method, but studies of turtles are limited to three terrestrial species and two sea turtles (Nagy and Medica 1986; Peterson 1996b; Henen 1997; Penick 2002; Wallace et al. 2005; Clusella Trullas et al. 2006; Jodice et al. 2006). The overall bias against turtles is puzzling, for most can be easily tracked and recaptured, and their ecology and life history has been otherwise well documented within a theoretical framework of energy and water that could be greatly advanced by field studies (Congdon et al. 1982; Mautz 1982; Minnick 1982; Congdon 1989). The bias against freshwater turtles stems from the intractability of the DLW method during aquatic activities (Booth 2002), but several critical aspects of their ecology occur in terrestrial habitats. For instance, nearly all freshwater turtles must leave the water to nest, and several make occasional or regular and extensive use of terrestrial habitats to move between wetlands, to overwinter, or to estivate (Semlitsch and Bodie 2003; Roe and Georges 2007, 2008). Terrestrial habitats feature perhaps most prominently in the ecology of turtles inhabiting temporary wetlands (Buhlmann and Gibbons 2001; Roe and Georges 2008).

The eastern long-necked turtle (*Chelodina longicollis*) inhabits both permanent and temporary wetlands and uses terrestrial habitats extensively for estivation and movements between wetlands (Roe and Georges 2007). The typical pattern of movement involves traveling from nutrient-poor permanent lakes to several highly productive temporary wetlands after flooding and then back to the lakes when the wetlands dry (Kennett and Georges 1990; Roe and Georges 2007). However, considerable behavioral variation among individuals in response to wetland drying is apparent because some immediately move to other wetlands while others estivate. We hypothesized that such behaviors are influenced by a weighing of the expected costs, risks, and benefits of overland movement versus those of estivation in the context of unpredictably fluctuating flood-dry cycles (Roe and Georges 2008). Energy and water costs are likely to factor into turtles' responses, but we currently have very little knowledge of these costs for freshwater turtles in the field.

Here, we measure energy use and water flux in a freshwater turtle (*C. longicollis*) during terrestrial behaviors using the DLW technique. We assess whether water or energy constrains the duration that individuals can survive in terrestrial estivation. We also estimate the energy and water flux rates associated with overland movements. These determinations allow us to compare the physiological costs of terrestrial estivation with those of movements between wetlands and ultimately arrive at a better understanding of the consequences of behavioral trade-offs in *C. longicollis* and perhaps other freshwater turtles.

Material and Methods

Study Site

Fieldwork was conducted in Booderee National Park (hereafter, Booderee), a 7,000-ha reserve located within the Commonwealth Territory of Jervis Bay on the southeast coast of Australia (150°43'E, 35°09'S). The site is characterized by several permanent dune lakes and a network of temporary wetlands within a matrix of forests and heath scrubland. Booderee has a temperate maritime climate with a long-term average annual rainfall of approximately 1,100–1,200 mm spread evenly throughout the year. In each month, there is typically >80 mm of rain, though the timing and intensity of rainfall can be highly variable. Summers are warm and winters are mild, with average minimum and maximum temperatures of 9°–15°C in July and 18°–24°C in January. Relative humidity typically exceeds 69% throughout the year. Climate statistics were obtained from the Australian Bureau of Meteorology. A more detailed description of the site can be found in articles by Kennett and Georges (1990) and Roe and Georges (2007, 2008).

Doubly Labeled Water Study Design

We studied the terrestrial movements, behavior, temperature, energy use, and water flux of two groups of turtles. The first group comprised seven individuals (four males and three females) that were, of their own accord, inactive under leaf litter in the forest adjacent to two temporary wetlands that had been dry for 43 and 65 d, respectively. Five of these animals were part of an ongoing radiotelemetry study (Roe and Georges 2007, 2008), and the other two were captured by searching under debris near the tagged turtles. These turtles were considered to be estivating, and we refer to them as the estivation group hereafter. The initial midline carapace length (CL) and mass of these turtles averaged 189.9 mm (range = 168.0–216.0 mm) and 612 g (range = 401–931 g), respectively.

The second group of turtles comprised 12 individuals (three males and nine females) captured from a nearby wetland. After capture, this group was kept at the University of Canberra field station for 2 d before being translocated to a terrestrial site approximately 4.5 km from the capture wetland. The release site was an extensive area of continuous forest and heathland in undulating dunes, very closely resembling the terrestrial landscape and vegetation that turtles traveled through when moving between wetlands. By taking turtles to a distant and unfamiliar site, we aimed to take advantage of their abilities to orient themselves and eventually initiate searching or homing behavior to return to wetlands (Graham et al. 1996) but without allowing a quick return to water. This group of turtles in simulated overland movement is hereafter referred to as the translocated group. The initial CL and mass of these turtles averaged 170.8 mm (range = 152.4–188.6 mm) and 482 g (range = 357–645 g), respectively.

Turtles in both groups were fitted with radiotransmitters (Sirtrack, Havelock North, NZ) according to protocols of Roe

and Georges (2007). We also attached temperature data loggers (Thermochron iButton; Dallas Semi-conductor) to the outer surface of the posterior marginal scutes of the carapace (opposite the transmitters) to approximate body temperatures. Data loggers were sealed using a fast-drying black rubber coating (Plasti Dip International, Blaine, MN) in a method identical to that of Grayson and Dorcas (2004). Data loggers were programmed to record temperature at 1-h intervals simultaneously for all turtles. None of the equipment impeded the movement of the hind legs and averaged a combined 5% of turtle body mass (range = 3.0%–7.8%). In the forest near each of the two temporary wetlands and the translocation site, we measured environmental temperatures at a location central to that used by the turtles. Data loggers were either buried under the leaf litter and sand or hung from a branch 1.5 m above the ground under dense canopy. Rain gauges were also placed in open areas at the three sites and checked daily.

Turtles were located every second day using a handheld receiver and antenna. We estimated the percentage of the turtles' carapace that was visible (hereafter, cover index) and determined their coordinate position using Global Positioning System units (GPS III Plus, Garmin, Olathe, KS; error of 1–7 m) or by distance and bearings from previous locations. We then plotted locations on habitat maps using ArcView GIS 3.1 (Environmental Systems Research Institute, Redlands, CA). We estimated movement distance along a straight-line path between sequential locations. This estimate is not likely to significantly overestimate actual movement distance because *Chelodina longicollis* typically travels in nearly linear paths with minimal sinuosity when moving overland (Stott 1987; Graham et al. 1996). Immediately following the final body water determinations, we flushed the turtles' stomachs to examine whether they had recently fed (Georges et al. 1986).

Field Metabolic and Water Flux Rate Measurements

We estimated field metabolic rate (FMR) and rates of water influx and efflux using the DLW technique (Lifson and McClintock 1966; Nagy 1980), a method that has been previously validated in several species of reptiles with an estimated accuracy of ~11% (Nagy 1989). The most precise estimates require that isotopes decline by approximately half of their starting enrichments without approaching background levels too closely (Nagy 1980).

On January 16–17, 2006, we took an initial body fluid sample and then injected each turtle intraperitoneally with DLW containing 200 μL 37 MBq ^3H and 250 μL 95% atoms excess H_2^{18}O . After a 4–5-h equilibration period in dry plastic bins in the laboratory (23°–24°C) and in the field for estivating turtles (22°–26°C), a second body fluid sample was taken. It was previously determined that isotopes take 2.5 h to come to equilibrium with body fluids at 22°C (Kilgour 1995). This period was adequate for isotope equilibration with body fluids in the translocated group, but total body water (TBW) calculated from isotope dilution space of initial fluid samples was overestimated

in estivating turtles (see details in "Calculations"), indicating insufficient equilibration time in this group. Following the equilibrium period and second fluid sample, each individual in the estivation group was released exactly where it was captured, while the translocated group was released at its designated site.

We measured FMR and water flux over three periods. Turtles were recaptured and additional fluid samples drawn on February 3–4, 2006 (ending period 1); February 21–22, 2006 (ending period 2); and March 11–12, 2006 (ending period 3). On the first recapture in early February, we reinjected turtles with volumes and isotope activities of DLW identical to those of the first injection, took another fluid sample after a 5-h equilibration period, and then released them. In late February, no reinjections were made, and at the final sampling in March, turtles were reinjected with 100–200 μL ^3H , and a final fluid sample was taken 5 h later for TBW estimates. Body fluid samples were drawn anterior to the hind leg, typically in the vicinity of the bridge. This region quickly yielded an adequate volume of extracellular fluid and blood. No leakage was observed at any injection, nor did any turtles void the contents of their bladders during handling. Before each fluid sample, turtles were brushed clean with a dry cloth, and body mass was measured to the nearest gram. All fluid samples (0.5–1.0 mL) were stored in plastic O-ring vials and refrigerated (3°C) until analysis.

Isotope Analyses

Water samples were extracted from body fluids by microdistillation under vacuum. Standard solutions of ^3H and ^{18}O injectate were prepared in volumetric flasks and analyzed along with the extracted water samples and the diluent used for the standard preparations. For tritium analysis, 20- μL subsamples of extracted water were pipetted into 3 mL of scintillation cocktail (Ultima Gold) and counted for 10 min in a Packard liquid scintillation counter (model 1600CA). Additional subsamples of extracted water were sent to Metabolic Solutions (Nashua, NH) for ^{18}O analyses within 8 mo of sample collection in the field.

Calculations

TBW was estimated using the isotope dilution space technique (Nagy 1980), either as ^{18}O dilution for DLW injections or as ^3H dilution for singly labeled water. TBW estimates from ^3H dilution were corrected by regression to reflect ^{18}O dilution because the use of ^{18}O yields more accurate estimates of TBW (Nagy 1980). For the February 21–22 sample, when no reinjections were made, TBW values were interpolated assuming linear change in TBW between samplings.

Our method was originally designed to measure rates of CO_2 production and water flux with the traditional two-sample technique, where rates of isotope turnover are determined from measurements of isotope activity in an initial (equilibrium) and a final fluid sample bracketing the sample period. We were alerted that equilibrium had not yet been reached in the initial

samples in the estivating turtles by their high TBW estimates (>100% as a percentage of body mass in several cases), whereas estimates in translocated turtles appeared to be in line with expectations for freshwater turtles (60%–80%; Minnich 1982; Crawford 1994). For this reason, we employed the one-sample (or single-sample) technique for both groups, where the equilibrium isotope enrichment in the animal was predicted from the isotope dilution space calculation rearranged to solve for isotope enrichment (Nagy et al. 1984; Webster and Weathers 1989). This method yields estimates of energy and water flux with reasonable reliability (i.e., errors of 5%–15%; Nagy et al. 1984; Webster and Weathers 1989). TBW estimates from the translocated turtles (the group that reached equilibrium) were used to establish TBW as a percentage of body mass at each sampling. For estivating turtles that had been out of water for 43–65 d and were thus presumably more dehydrated, we used the percentage body water established for the translocated turtles at the end of the 54-d study to estimate initial TBW for all sample periods. This assumption was supported by our observations that percentage body water in the translocated turtles (determined by the two-sample technique) initially declined in the first period but then became relatively stable for the duration of the study (see “Results”), a trend consistent with that found in Kilgour’s (1995) study of the same population. We then assessed the accuracy of the isotope enrichments predicted by the single-sample technique by comparing them with the actual postequilibrium measurements from the two-sample technique for the translocated turtles.

Rates of CO₂ production were calculated using equation (2) of Nagy (1980), and water efflux and influx rates were calculated using equations (4) and (6), respectively, of Nagy and Costa (1980). Net water movement was calculated as influx – efflux. We also calculated the fractional turnover rates for ³H (k_h) and ¹⁸O (k_o) as did Lifson et al. (1955) to assess the k_o/k_h ratio. To convert \dot{V}_{CO_2} to \dot{V}_{O_2} and units of energy, we assumed that turtles in both groups were fasting (see “Results”) and using either fat or mixed (fat : protein) energy stores. For fat catabolism, we used a thermal equivalent of 27.8 kJ L⁻¹ CO₂ and a respiratory quotient (RQ) of 0.71, and for a mixed metabolic substrate of fat and protein in equal proportions, we used 26.4 kJ L⁻¹ CO₂ and an RQ of 0.75 (Gessaman and Nagy 1988). We assumed that fat and protein yield 39.7 and 18.4 kJ g⁻¹ energy and 1.07 and 0.50 mL H₂O g⁻¹ metabolic water, respectively (Schmidt-Nielsen 1964; Gessaman and Nagy 1988).

Supplementary Observations

To determine whether behavior of translocated turtles was similar to that of turtles in natural overland movement, we quantified the movement rates of 41 turtles studied by radiotelemetry that moved overland between wetlands (for details, see Roe and Georges 2007). To compare temperatures of turtles estivating terrestrially with those of turtles active in wetlands, we equipped five additional aquatically active turtles with iButtons during summer (January–February) of 2006. We also assessed

shell temperatures of seven terrestrial and nine aquatic turtles during spring (October–November) of 2005. In both the spring and the summer sampling periods, water, terrestrial detritus, and air temperatures were also measured using iButtons.

Statistical Analyses

We performed statistical analyses with SPSS, version 14.0, and SAS, version 8.2. Where appropriate, we examined the assumptions of homogeneity of variances and normality; when data failed to meet assumptions, data were transformed to approximate normal distributions or equal variances. Statistical significance was accepted at the $\alpha \leq 0.05$ level except where stated otherwise. The Dunn-Sidak correction was applied to multiple related comparisons to constrain the experiment-wide Type I error to 0.05. All metabolic and water flux values are reported as mean \pm SE for a group of individuals (Speakman 1997; Butler et al. 2004).

Doubly Labeled Water

For comparisons of the various rate functions (CO₂ production, water influx and efflux) among animals of different body sizes, it is important to account for the effects of body mass. Our first approach was to examine relationships between log₁₀ body mass and log₁₀ rate for each sample period using regression. However, relationships between body mass and whole-body rate functions were weak, and none was significant after applying the Dunn-Sidak adjustment ($R^2 < 0.25$; $P \gg 0.008$ in all cases). Our inability to detect a mass effect for whole-body rate functions is not completely unexpected because small sample size, mass range, and behavioral variation among individuals often hide any underlying mass effects in field studies (Peterson 1996b). As a result, we used allometric relationships between FMR and water flux rates for reptiles in general because there is insufficient data for turtles alone (but see Jodice et al. 2006). The mass exponent for FMR in reptiles is 0.89 (Nagy et al. 1999), and for water flux in nontropical habitats, it is 0.91 (Nagy 1982). We used mass-specific values for FMR and water flux rates in all analyses, calculated accordingly: FMR/body mass^{0.89}; water flux/body mass^{0.91}.

To determine whether water influx and efflux rates differed within a sample period, we used paired *t*-tests. To examine changes in body water over time, we used repeated-measures ANCOVAs with sample period as the within-subjects factor and body mass as the covariate in a model with a compound symmetry covariance structure (PROC MIXED model, SAS, ver. 8.2). FMR and water efflux and influx rates (response variables) were also tested using the repeated-measures ANCOVA described previously but with group (estivation or translocated) as a between-subjects factor in the model. We then examined differences in rate functions calculated by the one- and two-sample techniques for the translocated turtles using ANOVAs. To assess whether shell temperature or movement rates (response variables) differed over the sample periods or between

groups, we used repeated-measures ANOVA with sample period as the within-subjects factor and group as the between-subjects factor. In the above analyses, FMR, water flux rates, body mass, and movement rates were \log_{10} transformed, while TBW (% body mass) was arcsine transformed.

Supplementary Observations

The relationship between distance and duration of interwetland movements was examined using linear regression, with the distance between wetlands as the independent variable and days in transit as the dependent variable. To determine whether turtle shell temperatures (dependent variable) during terrestrial estivation differed from those in wetlands in the two seasons (spring and summer), we used a two-way ANOVA with habitat, season, and their interaction as the independent variables.

Results

Doubly Labeled Water

All translocated turtles and six of seven estivating turtles were followed throughout monitoring, with none successfully returning to water. One estivating turtle died between days 18 and 36 (61–79 d after wetland drying), presumably of natural causes, and was not included in analyses. Another estivating individual began moving toward the nearest permanent lake. We retained this animal in the estivating group for all statistical analyses, but in some cases, we eliminate it when reporting energy and water flux rates characteristic of estivating turtles.

Translocated and estivating turtles differed behaviorally with respect to movements. Translocated turtles moved longer distances than did those in natural estivation during the three sample periods (ANOVA, period: $F_{2,32} = 3.94$, $P = 0.030$; group: $F_{1,16} = 14.63$, $P = 0.001$; period \times group: $F_{2,32} = 2.30$, $P = 0.127$; Table 1). Mean shell temperatures did not differ

between groups or among periods ($P > 0.272$ for group, period, and group \times period; Table 1), and cover index was also similar (Table 1). Rainfall was highest in the first sample period and declined thereafter (Table 1). At the end of the study, stomachs were empty, and the upper and lower surfaces of mouths were joined by a thick mucus.

Isotope turnover dropped to a level insufficient to confidently interpret CO_2 and water flux in the estivating turtles for the final two periods (Table 2). The k_o/k_h ratios were typically above 1.11 but fell to 1.09 in the translocated turtles for the final sample period (Table 2). Because of these potential limitations in the final sampling periods (Nagy 1980; Nagy and Costa 1980; Speakman 1997), we used the initial period and a final one that incorporated periods 2 and 3 into a single final period. We were able to combine periods 2 and 3 because no isotope reinjections were made between them. In doing so, we ensured that isotopes had declined by at least 41%–47% and that k_o/k_h ratios remained ≥ 1.11 . When we report results from periods 2 and 3 alone (i.e., Table 2; Fig. 1), we do so with the caution that estimates may be less precise.

Using the ^{18}O dilution space and the two-sample technique for translocated turtles, we found that TBW estimates ranged from an initial $64.3\% \pm 0.9\%$ of body mass to $62.6\% \pm 0.8\%$ by the end of the first sample period and then to $62.2\% \pm 2.0\%$ by the end of the study. TBW estimates determined by ^3H dilution were higher, ranging from 72.0% to 69.7%. However, changes in TBW (% body mass) were not significant (ANCOVA, $F_{1,21} < 1.04$, $P > 0.370$ for mass and period), even though water efflux was higher than influx (paired t -test, $t_{17} > 2.42$, $P < 0.027$ for all periods). Mean net water movement ranged from an initial -2.56 to -0.36 mL (kg d) $^{-1}$ in the final period for the translocated group and from -1.41 to -1.01 mL (kg d) $^{-1}$ in estivators. Water efflux and influx were 1.5–2.1 times higher in translocated than in estivating turtles. Water flux rates declined in both groups from the first

Table 1: Summary of behavior, temperature, and rainfall for *Chelodina longicollis*

Sample Period	Duration (d)	Movement (m d $^{-1}$)	Cover Index (% visible) ^a	Shell Temperature (°C)	Rainfall (mm)
Estivation:					
1	17.81	3.0 \pm 1.5	13.7 \pm 10.5	21.9 \pm .2	25.3
2	17.96	3.5 \pm 2.2	15.8 \pm 12.8	21.7 \pm .3	20.5
3	17.96	2.6 \pm 1.7	21.5 \pm 15.5	21.5 \pm .3	.3
2–3 ^b	35.83	2.7 \pm 1.9	18.7 \pm 14.1	21.6 \pm .2	10.4
Translocated:					
1	17.75	34.1 \pm 6.0	20.2 \pm 4.3	22.0 \pm .2	37.4
2	17.97	35.2 \pm 9.6	19.7 \pm 5.9	21.9 \pm .2	16.7
3	17.97	10.9 \pm 2.6	7.7 \pm 3.4	21.7 \pm .3	0
2–3 ^b	35.59	23.1 \pm 6.0	13.7 \pm 4.4	21.8 \pm .2	8.4

Note. Data gathered during terrestrial estivation and movements following translocation. Values are either means or means \pm SE.

^a An estimate of the percentage of the carapace that was visible.

^b Interval 2–3 reflects temperature and behavior through both of the final two periods, combined to yield more robust energy and water turnover estimates.

Table 2: Summary of water flux and field metabolic rate (FMR) for *Chelodina longicollis*

Sample Period	Mass (g)	Water Efflux (mL [kg d] ⁻¹)	Water Influx (mL [kg d] ⁻¹)	FMR (mL CO ₂ [g h] ⁻¹)	FMR ^a (mL O ₂ [g h] ⁻¹)	³ H Turnover (%)	¹⁸ O Turnover (%)	k _v /k _h
Estivation:								
1	634 ± 60	19.3 ± 3.1	17.8 ± 2.7	.095 ± .008	.126 ± .010	41	47	1.23
2	620 ± 62	17.2 ± 2.2	16.2 ± 1.8	.063 ± .007	.084 ± .009	39	43	1.16
3	605 ± 68	13.1 ± 2.0	12.1 ± 1.6	.039 ± .008	.052 ± .011	31	32	1.11
2–3 ^b	606 ± 65	15.3 ± 2.2	14.3 ± 1.8	.043 ± .008	.057 ± .011	57	61	1.11
Translocated:								
1	456 ± 23	40.6 ± 1.8	38.1 ± 1.7	.148 ± .012	.196 ± .016	68	72	1.15
2	447 ± 21	25.9 ± .9	25.5 ± .8	.102 ± .007	.136 ± .009	54	58	1.16
3	445 ± 21	17.7 ± .8	17.3 ± .8	.040 ± .007	.053 ± .009	42	44	1.09
2–3 ^b	440 ± 21	22.0 ± .7	21.6 ± .6	.072 ± .004	.097 ± .006	73	77	1.13

Note. Studied using doubly labeled water during natural terrestrial estivation ($n = 6$) and terrestrial movements after translocation ($n = 12$). Values are either means or means ± SE.

^a Assuming a respiratory quotient of 0.75.

^b Interval 2–3 reflects isotope declines through both of the final two periods.

to the second sampling periods, but the decline was much more pronounced in the translocated turtles (Tables 2, 3). FMR also declined in both groups but remained 1.6–1.7 times higher in translocated than in estivating turtles, with the exception of period 3, in which FMR of translocated turtles was similar to that of estivators (Tables 2, 3; Fig. 1).

Postequilibrium ³H enrichments predicted from the one-sample technique differed from two-sample determinations by an average of +0.3% (range = -4.2% to 7.0%) for the first period and -2.2% (range = -5.6% to 2.9%) for the second, while ¹⁸O enrichments differed by an average of +0.5% (range = -3.7% to 10.7%) for the first period and -0.5% (range = -4.8% to 7.2%) for the second. These differences did not translate into significant variation between the one- and two-sample techniques for estimates of water efflux, influx, or FMR (water flux: $P > 0.206$; FMR: $P = 0.158$).

Supplementary Observations

Movements of translocated turtles were relatively straight, with minimal sinuosity and typically in the direction of familiar wetlands (Fig. 2), and were drawn out over several days and interrupted by periods of extended inactivity buried in detritus. This behavior is similar to that of turtles undertaking overland movements during the course of their natural activities. Travel time for turtles moving between wetlands separated by an average of 427 m (range = 40–1,470 m; Roe and Georges 2007) was 15 ± 2.8 d (range = 1.5–44 d), increasing with distance according to the following equation: time (d) = $0.024 \times$ distance (m) + 5.447 ($F_{1,39} = 12.85$, $P = 0.001$, $R^2 = 0.25$). These turtles traveled at an average rate of 27.4 m d⁻¹, which is comparable to that of the translocated turtles (Table 1).

Shell temperatures during terrestrial estivation were lower than those of aquatically active turtles by 2.0°C and 2.5°C in spring and summer, respectively, and lower in spring than in summer for turtles in both habitat types (ANOVA, habitat: $F_{1,23} = 20.00$, $P < 0.001$; season: $F_{1,23} = 36.16$, $P < 0.001$,

habitat \times season: $F_{1,23} = 0.33$, $P = 0.570$; Fig. 3). Shell temperatures matched the temperatures of the surrounding substrates, generally tracking maximum detritus and minimum water temperatures while in terrestrial and aquatic habitats, respectively (Fig. 3).

Discussion

Rates of water flux and energy expenditure in *Chelodina longicollis* were consistent with those of other terrestrial turtles

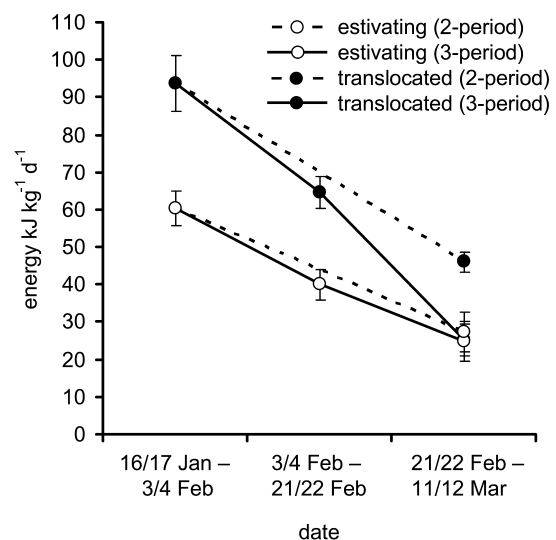


Figure 1. Field metabolic rates (FMRs) for *Chelodina longicollis* estivating in terrestrial habitats (mass range = 469–910 g) compared with those of turtles during overland movement after translocation to a site distant from water (mass range = 351–629 g). A thermal equivalent of 26.6 J/L CO₂ was used to reflect the catabolism of protein and fat. We report FMRs for all three periods separately (3-period) and for an initial and a final period (2-period), where the final period reflects isotope declines through the final two periods combined (February 3–March 12).

Table 3: Results of repeated-measures ANCOVA for the effects of body mass, sample period, and group (translocated and estivating) on field metabolic and water flux rates for turtles studied with doubly labeled water

Variable	df	F	P
Log ₁₀ water efflux:			
Log ₁₀ mass	1, 15	10.95	.005
Period	1, 15	136.97	<.001
Group	1, 16	11.56	.004
Period × group	1, 15	26.77	<.001
Log ₁₀ water influx:			
Log ₁₀ mass	1, 15	12.85	.003
Period	1, 15	153.46	<.001
Group	1, 16	16.08	.001
Period × group	1, 15	29.08	<.001
Log ₁₀ FMR (CO ₂ production):			
Log ₁₀ mass	1, 15	9.35	.008
Period	1, 15	45.96	<.001
Group	1, 16	4.62	.047
Period × group	1, 15	.69	.418

(Tables 2, 4), suggesting that the DLW method shows promise in its application to freshwater turtles during terrestrial behaviors. Water flux and metabolic rates varied considerably between estivating turtles and those moving overland, indicating that these two behaviors have very different physiological consequences. Next, we explore these physiological consequences and limitations in the context of variable behaviors in response to wetland drying.

Terrestrial Estivation

When a turtle estivates, survival depends on its ability to maintain water and osmotic balance and to support energy requirements until wetlands relood. Turtles can survive on minimal inputs of energy and water and tolerate prolonged osmotic imbalances in body fluids (Peterson 1996a; Henen 1997; Peterson and Stone 2000; Ligon and Peterson 2002), while some nominally aquatic turtles can also forage on land to replenish energy stores (Scott 1976). For those that cannot feed while terrestrial, water or energy will ultimately limit the duration they can survive out of water.

Estivation is a behavioral strategy to reduce energy and water demands with or without accompanying physiological adjustments (Seidel 1978). The lower temperatures of estivating turtles compared with those of turtles in wetlands (Fig. 3) would allow for some energy savings on top of lowered activity, but a physiological adjustment that can further reduce energy use during estivation is metabolic depression. A reduction of 70%–80% below standard metabolic rate (SMR) at high temperatures is typical of estivating ectotherms (Guppy and Withers 1999), but depression to this extent in freshwater turtles has been

demonstrated only in *Kinosternon flavescens* (Seidel 1978) and *Chelodina rugosa* (Kennett and Christian 1994), though such a capacity may be population specific (see Grigg et al. 1986; Peterson and Stone 2000; Ligon and Peterson 2002). If we assume that SMR in our study population of *C. longicollis* is 0.026 mL O₂ (g h)⁻¹ (Chessman 1978), a depression of 70%–80% should have yielded metabolic rates in the range of 0.005–0.008 mL O₂ (g h)⁻¹. Even though FMR in estivating *C. longicollis* declined over time, we found no evidence of metabolic depression to this extent after at least 97 d in estivation (time since wetlands dried). After excluding the individual that became active, the lowest FMR during estivation was 0.042–0.049 mL O₂ (g h)⁻¹ and 20.0–23.4 kJ (kg d)⁻¹ (catabolism of protein and fat) or 0.047–0.055 mL O₂ (g h)⁻¹ and 21.0–24.6 kJ (kg d)⁻¹ (catabolism of fat only). Although these values are more than 70% below FMR predictions for reptiles during their active seasons (Nagy et al. 1999), they are similar to those for freshwater crocodiles in dry-season estivation (Christian et al. 1996) and terrestrial tortoises during drought (Table 4; Peterson 1996b).

It is not surprising that *C. longicollis* did not feed in terrestrial habitats because chelid turtles use a “strike, gape, and suck” method of prey capture (Parmenter 1976) that is not well suited for feeding out of water. Consequently, metabolic demands must be met by energy stores, the largest of which are primarily in the form of fat and protein in reptiles (Derickson 1976). To calculate the length of time that energy stores could last requires knowledge of reserve amount and substrates catabolized. We were unable to directly determine either of these in our study animals, so we report an energy expenditure range based on catabolism of fat only and a mixed substrate of fat and protein

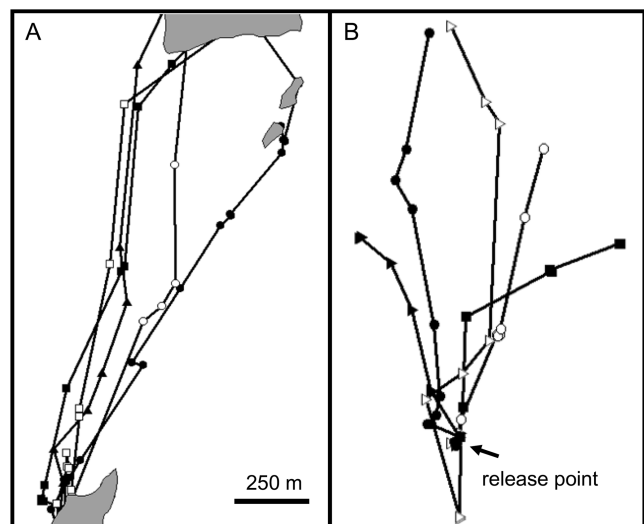


Figure 2. Similarity of terrestrial movements of turtles (A) naturally traveling between wetlands and (B) after translocation to stimulate movements back to the wetland. To avoid obscuring individual movement paths, not all individuals are shown.

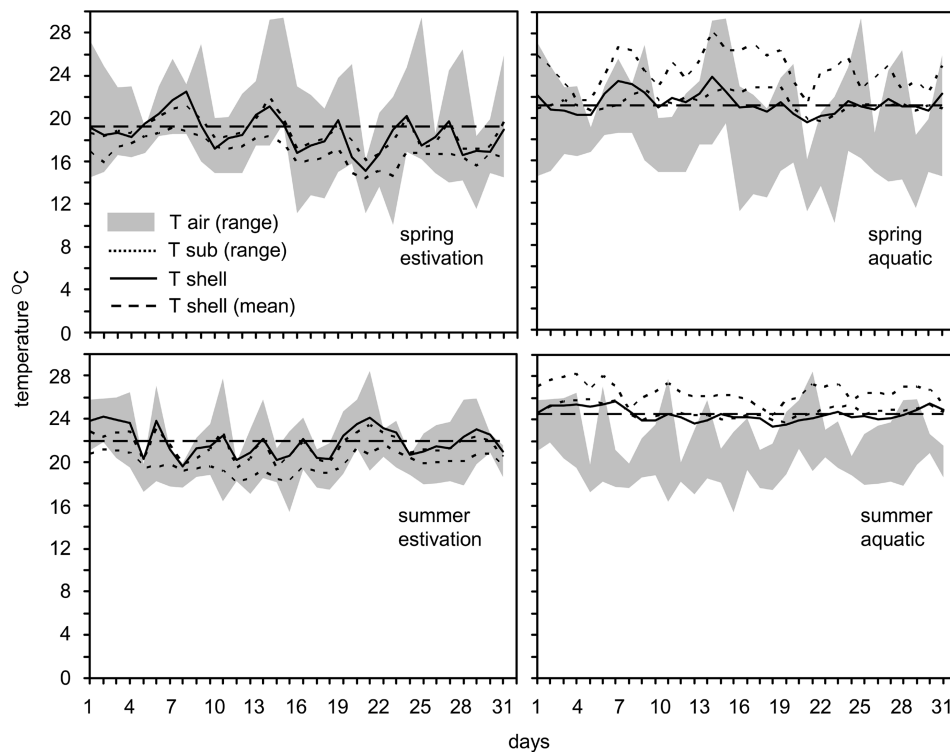


Figure 3. Environmental and shell temperature (T_{shell}) variation for turtles terrestrially estivating and aquatically active in spring (October 27–November 26) and summer (January 27–February 26). Substrate temperatures (T_{sub}) for terrestrial turtles reflect sand/detritus temperatures and water temperature at 0.2- and 1.2-m depths for aquatic turtles. The horizontal dashed line is mean shell temperature through the sampling period.

in equal proportions (Crawford 1994). For reserve amounts, we used previous determinations of lipids in freshwater turtles, the most thorough of which was that of Congdon et al. (1982), who determined that *Chrysemys picta* was, on average, 3% lipid by weight, but some individuals were up to 9%–10% lipid. Other studies in small- to medium-sized freshwater turtles document a similar level of variability in lipid amounts (Belkin 1965; McPherson and Marion 1982; Crawford 1994; Kennett and Christian 1994), though one (*K. flavescens*) may have much higher reserves (Long 1985). On the basis of expenditures of 20.0–24.6 kJ (kg d)⁻¹, a 500-g *C. longicollis* with 15–45 g (3%–9%) of fat could survive for 49–261 d (Fig. 4). Survival times for any given initial fat reserve will likely be toward the higher end of this range because turtles typically burn both protein and fat during long-term fasting (Crawford 1994; Henen 1997).

Chelodina longicollis is known for its ability to store and reabsorb water from the cloacal bladder, adjust uric acid excretions, and limit cutaneous water loss, all of which are adaptations to combat desiccation (Rogers 1966; Chessman 1984). Our study is the first to examine the ability of *C. longicollis* to maintain water balance in the field. Even though water balance was not maintained while *C. longicollis* were terrestrial, two lines of evidence suggest that they were slow to dehydrate during extended periods of terrestrial occupancy. First, TBW (%)

body mass) had not dropped significantly after 54 d terrestrial in the translocated turtles. This does not mean that water was not lost but instead that net water loss was in concert with starvation-associated decline in body dry components, such that percentage body water remained constant over time (i.e., Christian et al. 1996). Second, turtles in both groups took in water at rates to nearly offset efflux (Table 2). Fasting animals can take in water by drinking, absorbing from the environment, using water stores, or producing metabolic water. Metabolic water accounted for only 4.2%–9.6% of influx during any sample period (assuming both energy substrate scenarios), and water influx was beyond what turtles could have stored before leaving wetlands if they were capable of storing water in folds of skin or other reservoirs outside of the body water pool. Contact with moist microhabitats may allow for some water uptake through the skin or via respiration, but this ability has never been demonstrated in *C. longicollis*. The most likely route of water intake is through drinking. We observed several *C. longicollis* emerging from their terrestrial refuges and drinking from pooled water in the leaf litter during heavy rainfall (Roe 2008), and *C. longicollis* can “drink” when the cloaca is in contact with water (Chessman 1978). Through a combination of behavioral and physiological means, *C. longicollis* estivating

Table 4: Water flux and field metabolic rates (FMRs) for free-ranging terrestrial turtles in mid- to late summer

Species (sex)	Mass (g) ^b	Water Flux ^a		FMR ^a		Source
		mL d ⁻¹	mL (kg d) ⁻¹	kJ d ⁻¹	kJ (kg d) ⁻¹	
<i>Chelodina longicollis</i> (M, F): ^c						
Terrestrial estivation	612 (401–931)	9–12	14–19	15–41	23–60	This study
Terrestrial movement	482 (357–645)	10–20	22–41	22–46	46–94	This study
<i>Gopherus agassizii</i> (M, F)	900 (500–2,200)	4–15	5–17	33–52 ^d	36–57 ^d	Nagy and Medica 1986
<i>G. agassizii</i> (M)	3,000 (2,500–3,500)	3–36	1–13	30–69	11–26	Peterson 1996b
<i>G. agassizii</i> (F)	1,500 (1,350–1,750)	NR ^e	NR	42–49	29–34	Henen 1997
<i>Gopherus polyphemus</i> (M, F)	3,400 (2,500–4,300)	31–93	11–30	77–188	34–62	Jodice et al. 2006
<i>Terrapene carolina</i> (M, F)	400 (383–426)	11	24	26 ^d	58 ^d	Penick et al. 2002

^a Mass-specific water flux and metabolic rates were calculated as (water flux)/(body mass)^{0.91} and (FMR)/(body mass)^{0.89}.

^b Mass is as an estimated mean value and reported range.

^c Does not include energy and water flux estimates from period 3 alone, where doubly labeled water estimates were less robust.

^d Where FMR is reported as mL CO₂ in other studies, we assume a thermal equivalent of 21.7 J mL⁻¹ CO₂.

^e NR = not reported.

in terrestrial habitats achieves rates of water flux on par with those of other strictly terrestrial turtles (Table 4).

The consistently negative net water movements for *C. longicollis* would eventually lead to their reaching lethal dehydration limits in terrestrial habitats. In turtles, lethal dehydration generally occurs at a loss of 30%–35% of initial body mass from water (Seidel 1978; Mautz 1982; Peterson and Stone 2000; Ligon and Peterson 2002). After we excluded the individual that became active, estivating turtles lost water at a rate of -1.41 to -0.66 mL (kg d)⁻¹ during the first and final sampling periods. At these rates, a 500-g *C. longicollis* would have lost <5% of its body mass from water after 54 d and could survive 455 d in estivation before reaching vital dehydration limits. Because this survival time is considerably longer than that predicted for starvation (Fig. 4), it appears that depletion of energy reserves constrains the length of time *C. longicollis* can estivate, a conclusion supported by our observations of estivation in this population (Fig. 4). For instance, a typical turtle estivates for 64 d before dying or moving back to water (Roe and Georges 2007), a duration consistent with our projections for turtles with 3% body fat (Fig. 4). Ninety-five percent of turtles estivated for no longer than 216 d, a duration consistent with projections for turtles with 9% body fat (Fig. 4). The longest-known estivation was 480 d (Roe and Georges 2007), but this particular turtle was the only one that estivated over an entire overwintering and early spring period (June–November), when energy demands are expected to be substantially reduced because of low temperatures.

Several studies have examined how energy and water may limit survival in freshwater turtles, but responses are generally measured in turtles kept in confinement at constant temperature and humidity and deprived of food or water (or both) in the laboratory. *Sternotherus minor* survived for 164–270 d in water with no food (Belkin 1965), a time very similar to our predictions and field observations for *C. longicollis* (Fig. 4).

Several species of *Kinosternon* deprived of water reach critical dehydration limits within 25–80 d (Seidel 1978; Peterson and Stone 2000; Ligon and Peterson 2002), and on the basis of measures of evaporative water loss, Chessman (1978, 1984) proposed that *C. longicollis* could survive only a few months out of water in natural situations. Although such laboratory studies are valuable for determining physiological capacities and tolerance limits, they are limited in their ability to mimic the complex conditions that turtles respond to in the wild. We found no evidence that water loss in *C. longicollis* had approached vital limits after several weeks in natural estivation, which is due in large part to their ability to access water in

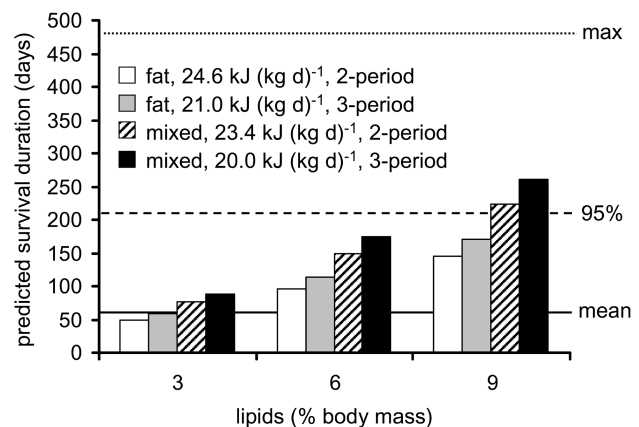


Figure 4. Range of predicted survival times (bars) for a 500-g turtle in terrestrial estivation, assuming catabolism of fat only or protein and fat (mixed). Energy expenditures are the lowest mean field metabolic rates measured at 21°–22°C for estivating turtles (469–908 g) estimated from division of sampling into either two or three sample periods (see “Results” for a detailed explanation). The horizontal lines are the mean, 95%, and maximum durations that turtles are known to estivate at the study site (Roe and Georges 2007).

Table 5: Energy trade-offs between terrestrial estivation and one- and two-way movements to other wetlands for a 500-g turtle

Travel Distance ^a (m)	One-Way Movement				Two-Way Movement			
	Total Energy ^b (kJ)	Total Time Estivating (d)	Energy Profit from Trade-off ^b (kJ)	Extra Time in Estivation (d)	Total Energy ^b (kJ)	Total Time Estivating (d)	Energy Profit from Trade-off ^b (kJ)	Extra Time in Estivation (d)
50	109–114	11	43–45	4	218–228	22	86–90	8
500	284–298	28	112–117	12	568–596	56	224–234	24
1,000	478–502	48	188–198	19	956–1,004	96	376–396	38
1,500	672–705	67	265–278	26	1,344–1,410	134	530–556	52

Note. A 500-g turtle would have between 608 and 2,614 kJ expendable energy assuming both energy substrate alternatives and 3%–9% fat.

^a Distances are representative of the range of required overland travel distances at the site (Roe and Georges 2007).

^b Energy values represent the range assuming catabolism of mixed substrates (fat and protein) or fat only.

terrestrial environments. Maintaining energy balance during terrestrial behavior is more limiting because individuals must rely solely on diminishing reserves that cannot be replenished without returning to wetlands.

Terrestrial Movements

Much like in studies of estivation, the costs associated with terrestrial movement in aquatic animals are often measured under conditions that completely remove them from the challenges they face in natural environments. The only measurements of aerobic costs of terrestrial activity in freshwater turtles have been estimated from respirometry during short bouts of activity (e.g., Stockard and Gatten 1983; Baudinette et al. 2000). In reality, animals must contend with soft substrates (e.g., leaf litter and sand), irregularities in slope, obstacles to traverse (e.g., rocks, woody debris), resistance from vegetation, and exposure to high temperatures, all of which could add substantially to movement costs (van Marken Lichtenbelt et al. 1993). Movements may also require bouts of intense activity, wherein some energy demands are met anaerobically (Stockard and Gatten 1983; Congdon and Gatten 1989). Because the DLW technique measures the sum of all demands on metabolism and water over long periods of natural behavior (i.e., costs of physical displacement, maintaining awareness and posture, circadian cycles, recovery from oxygen debt, and SMR), our measures provide the most ecologically relevant estimates yet reported for the energy expenditure and water flux during terrestrial movements for freshwater turtles. Even though our measures were not taken from turtles moving overland during the course of their natural behaviors, our protocol simulated as closely as possible these natural movements.

FMRs for translocated turtles moving 34 m d⁻¹ were 94–99 kJ (kg d)⁻¹ (assuming both energy substrate scenarios), nearly identical to predictions for active reptiles of similar size (Nagy et al. 1999). Water influx and efflux rates of 38–41 mL (kg d)⁻¹ during this period were nearly double those predicted for other reptiles from arid and semiarid regions but in line with those

from the tropics (Nagy 1982). FMR and water flux of turtles moving 23 m d⁻¹ were 46–48 kJ (kg d)⁻¹ and 22 mL (kg d)⁻¹, respectively, both of which are similar to those for active box turtles and gopher tortoises (Table 4; Penick et al. 2002; Jodice et al. 2006) and consistent with water flux for other nontropical reptiles (Nagy 1982). However, as was the case for estivation, overland movement must be supported by stored energy reserves that cannot be replenished until returning to water, whereas hydration levels can be more easily maintained.

Our estimates of the energy costs of terrestrial behavior fill some important gaps in the knowledge of field energetics in *C. longicollis*. By comparing FMR between estivation and overland movement behaviors, we estimate that terrestrial movement is 1.6–1.7 times more energetically expensive than estivation. This value is toward the lower end of the spectrum for other active reptiles (range = 1.1–5.1, mean = 2.6; Christian et al. 1997), but this is to be expected because our inactivity estimates based on FMR are higher than if we had used the more typical SMR (Neiwarowski and Waldshmidt 1992), and our activity estimates are of animals that are only sporadically active. This difference in energy expenditure between behaviors most likely reflects the longer movement distances of the translocated turtles because shell temperature and cover index did not differ between groups (Table 1). Together with our equation relating movement time to distance, these estimates allow us to explore the energy consequences and trade-offs of estivation and movement in the context of wetland flood-dry cycles (Table 5).

We estimate that a 500-g turtle with 3%–9% body fat would have 608–1,787 kJ expendable energy assuming fat catabolism or 889–2,614 kJ assuming that mixed fuels (fat and protein) are burned in equal proportions until all fat is catabolized. At a drying temporary wetland 1,500 m from the nearest body of water, a turtle faces a movement cost of 672–705 kJ, which is 265–278 kJ more than estivation costs over the same time frame. By not moving, this energy could instead be used (i.e., a trade-off) to fuel the demands for an extra 26 d of estivation, allowing for a total of 67 d at the dry wetland to await reflooding

(Table 5). If the turtle is to make a two-way movement (i.e., to a permanent lake and back when the wetland refloods), these costs and their equivalent days in estivation gained in the trade-off would be doubled. Assuming that all else is equal, should the temporary wetland reflood at 134 d (4.5 mo) after drying, both strategies would be energetically equivalent if a distance of 1,500 m must be traveled. For earlier reflooding, estivation would be the more economic strategy, but if the wetland remains dry beyond this time, it would have been better to move to a permanent lake, where at least some energy needs could be met before staging a return when temporary wetlands reflood. Our model demonstrates that the energy costs of movement and potential gains in extended estivation time in the trade-off will be high when long distances must be traveled but will decrease with decreasing distance between wetlands (Table 5). When wetlands are only 50 m apart, turtles that forgo movement would gain only eight additional days in estivation, which is a short window of time to expect a wetland to reflood. In this case, a trade-off may be of little value, and movement to another wetland would almost always be more economical. This change in value of the trade-off with distance fits with our previous behavioral observations that more turtles estivate and for more extended periods when longer distances must be traveled to the nearest permanent lake, whereas movement to other wetlands is the near-universal response when only a short distance must be traversed (Roe and Georges 2008). We realize that other factors can influence behavior (e.g., predation risk and social interactions) and that our energy models are oversimplified by not including other energy allocations (e.g., reproduction; Congdon and Tinkle 1982). However, our aim was not to present an ecological energy budget but only to explore the relative costs of energy and water for estivation and terrestrial movement.

Future Applications in Freshwater Turtles

Despite the commonly cited problems of the DLW technique for studying aquatic turtles, it has recently been applied to sea turtles (Wallace et al. 2005; Clusella Trullas et al. 2006), and here we demonstrate its potential utility for freshwater turtles during terrestrial behaviors. However, the DLW technique is not without some potentially serious drawbacks, even for terrestrial studies of freshwater turtles. The high water flux rates and relatively low metabolism of *C. longicollis* during estivation resulted in a low washout ratio of isotopically labeled oxygen to hydrogen (k_o/k_h ; Table 2). When this ratio falls below 1.1, estimates of CO_2 production will lack sufficient precision because any errors or deviations from assumptions will become more influential (Speakman 1997). Because our ratios were never much above this critical minimum and actually dropped below it during the third sample period in one group, it is apparent that we were operating at the limits of the DLW technique. Problems may also arise from the differential rate of diffusion of H-labeled and O-labeled water across wet skin,

which tends to overestimate FMR (Nagy 1980). We did not quantify the moisture content of terrestrial refuges, though refuge sites were typically in well-drained sandy soil, and turtles were never observed submerged in water. Without knowing the contribution of cutaneous exchange to overall water flux, we cannot discount the possibility that some of the observed decline in FMR estimates over time (Fig. 1) resulted from decreased cutaneous exchange as terrestrial refuges became drier with less rainfall. Validation studies, where CO_2 production measured directly by respirometry is compared with DLW results, would provide some resolution on the accuracy of our FMR estimates.

We demonstrate how field measures of energy and water flux can greatly contribute to our understanding of animal behavior beyond that which can be achieved in laboratory studies alone. By examining the physiological limitations of estivation and costs of overland movement, we were able to construct models that were remarkably consistent with observed behaviors of *C. longicollis* in response to wetland drying (Roe and Georges 2007, 2008). The physiological ecology of terrestrial activities is a largely unexplored frontier in freshwater turtle biology that promises to yield important insights into their behavioral ecology and evolution. Behavioral responses to wetland drying have been relatively well documented (Gibbons et al. 1983; Buhlmann and Gibbons 2001), but examining the underlying physiological responses to water and energy limitations under natural conditions could provide a richer understanding of species' range limits, habitat associations, and local adaptations (Peterson and Stone 2000; Ligon and Peterson 2002). Reproduction often involves long overland movements (Congdon et al. 1983, 1987) and extended stays in terrestrial habitats (Burke et al. 1994; de Solla and Fernie 2004), and several turtles move between wetlands in search of alternate resources and for dispersal (Roe and Georges 2007), a behavior that is particularly characteristic of males (Morreale et al. 1984; Gibbons et al. 1990; Tuberville et al. 1996). Such movements are undertaken with expected gains in resource acquisition (i.e., multiple matings for males) that also have associated costs. Long-distance and extended terrestrial movement in the contexts just discussed are typically assumed to exact a high cost on energy and water budgets, yet these costs have never been quantified in the field before now. Though our specific results may be of limited direct applicability to other systems, our framework and methods could help to address some of these and other unresolved issues in freshwater turtle ecology.

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Literature Cited

- Baudinette R.V., A.M. Miller, and M.P. Sarre. 2000. Aquatic and terrestrial locomotory energetics in a toad and a turtle: a search for generalizations among ectotherms. *Physiol Biochem Zool* 73:672–682.
- Belkin D.A. 1965. Reduction of metabolic rate in response to starvation in the turtle *Sternotherus minor*. *Copeia* 1965:367–368.
- Booth D.T. 2002. The doubly-labeled water technique is impractical for measurements of field metabolic rate in freshwater turtles. *Herpetol Rev* 33:105–107.
- Buhlmann K.A. and J.W. Gibbons. 2001. Terrestrial habitat use by aquatic turtles from a seasonally fluctuating wetland: implications for wetland conservation boundaries. *Chelonian Conserv Biol* 4:115–127.
- Burke V.J., J.W. Gibbons, and J.L. Greene. 1994. Prolonged nesting forays by common mud turtles (*Kinosternon subrubrum*). *Am Midl Nat* 131:190–195.
- Butler P.J., J.A. Green, I.L. Boyd, and J.R. Speakman. 2004. Measuring metabolic rate in the field: the pros and cons of the doubly labeled water and heart rate methods. *Funct Ecol* 18:168–183.
- Chessman B.C. 1978. Ecological Studies of Freshwater Turtles in South-eastern Australia. PhD diss. Monash University, Victoria.
- . 1984. Evaporative water loss from three south-eastern Australian species of freshwater turtle. *Aust J Zool* 32:649–655.
- Christian K., B. Green, and R. Kennett. 1996. Some physiological consequences of estivation by freshwater crocodiles, *Crocodylus johnstoni*. *J Herpetol* 30:1–9.
- Christian K.A., R.V. Baudinette, and Y. Pamula. 1997. Energetic cost of activity by lizards in the field. *Funct Ecol* 11:392–397.
- Clusella Trullas S., J.R. Spotila, and F.V. Paladino. 2006. Energetics during hatchling dispersal of the olive ridley turtle *Lepidochelys olivacea* using doubly labeled water. *Physiol Biochem Zool* 79:389–399.
- Congdon J.D. 1989. Proximate and evolutionary constraints on energy relations of reptiles. *Physiol Zool* 62:356–373.
- Congdon J.D., G.L. Breitenbach, R.C. van Loben Sels, and D.W. Tinkle. 1987. Reproduction and nesting ecology of snapping turtles (*Chelydra serpentina*) in southeastern Michigan. *Herpetologica* 43:39–54.
- Congdon J.D., A.E. Dunham, and D.W. Tinkle. 1982. Energy budgets and life histories of reptiles. Pp. 233–271 in C. Gans and F.H. Pough, eds. *Biology of the Reptilia*. Vol. 13. Academic Press, New York.
- Congdon J.D. and R.E. Gatten. 1989. Movements and energetics of nesting *Chrysemys picta*. *Herpetologica* 45:94–100.
- Congdon J.D. and D.W. Tinkle. 1982. Reproductive energetics of the painted turtle (*Chrysemys picta*). *Herpetologica* 38:228–237.
- Congdon J.D., D.W. Tinkle, G.L. Breitenbach, and R.C. van Loben Sels. 1983. Nesting ecology and hatching success in the turtle *Emydoidea blandingi*. *Herpetologica* 39:417–429.
- Crawford K.M. 1994. Patterns of energy substrate utilization in overwintering painted turtles, *Chrysemys picta*. *Comp Biochem Physiol* 109A:495–502.
- Derickson W.K. 1976. Lipid storage and utilization in reptiles. *Am Zool* 16:711–723.
- De Solla S.R. and K.J. Fernie. 2004. Is the cost of locomotion the reason for prolonged nesting forays of snapping turtles, *Chelydra serpentina*? *Can Field-Nat* 118:610–612.
- Georges A., R.H. Norris, and L. Wensing. 1986. Diet of the freshwater turtle *Chelodina longicollis* (Testudines: Chelidae) from the coastal dune lakes of the Jervis Bay Territory. *Aust Wildl Res* 13:301–308.
- Gessaman J.A. and K.A. Nagy. 1988. Energy metabolism: errors in gas-exchange conversion factors. *Physiol Zool* 61:507–513.
- Gibbons J.W., J.L. Greene, and J.D. Congdon. 1983. Drought-related responses of aquatic turtle populations. *J Herpetol* 17:242–246.
- . 1990. Temporal and spatial movement patterns of sliders and other turtles. Pp. 201–215 in J.W. Gibbons, ed. *Life History and Ecology of the Slider Turtle*. Smithsonian Institution Press, Washington, DC.
- Grafe T.U. 1996. Energetics of vocalization in the African reed frog (*Hyperolius marmoratus*). *Comp Biochem Physiol* 114A:235–243.
- Graham T., A. Georges, and N. McElhinney. 1996. Terrestrial orientation by the eastern long-necked turtle, *Chelodina longicollis*, from Australia. *J Herpetol* 30:467–477.
- Grantner A. and M. Taborsky. 1998. The metabolic rates associated with resting, and with the performance of agonistic, submissive, and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *J Comp Physiol* 168B:427–433.
- Grayson K.L. and M.E. Dorcas. 2004. Seasonal temperature variation in the painted turtle (*Chrysemys picta*). *Herpetologica* 60:325–336.
- Grigg G.C., K. Johansen, P. Harlow, L.A. Beard, and L.E. Taplin. 1986. Facultative estivation in a tropical freshwater turtle *Chelodina rugosa*. *Comp Biochem Physiol* 83A:321–323.
- Guppy M. and P. Withers. 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol Rev* 74:1–40.
- Henen B.T. 1997. Seasonal and annual energy budgets of female desert tortoises (*Gopherus agassizii*). *Ecology* 78:283–296.
- Jodice P.G.R., D.M. Epperson, and G.H. Visser. 2006. Daily

- energy expenditure in free-ranging gopher tortoises (*Gopherus polyphemus*). *Copeia* 2006:129–136.
- Jorgensen C.B. 1998. Role of urinary and cloacal bladders in chelonian water economy: historical and comparative perspectives. *Biol Rev* 73:347–366.
- Kennett R. and K. Christian. 1994. Metabolic depression in estivating long-neck turtles. *Physiol Zool* 67:1087–1102.
- Kennett R. and A. Georges. 1990. Habitat utilization and its relationship to growth and reproduction of the eastern long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae), from Australia. *Herpetologica* 46:22–33.
- Kilgour S.L. 1995. Water and Energy Relations of the Eastern Long Necked Turtle *Chelodina longicollis* during Terrestrial Migration. Honors thesis. University of Canberra.
- Kinnison M.T., M.J. Unwin, and T.P. Quinn. 2003. Migratory costs and contemporary evolution of reproductive allocation in male chinook salmon. *J Evol Biol* 16:1257–1269.
- Lifson N., G.B. Gordon, and R. McClintock. 1955. Measurement of total carbon dioxide production by means of $D_2^{18}O$. *J Appl Physiol* 7:704–710.
- Lifson N. and R. McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. *J Theor Biol* 12:46–74.
- Ligon D.B. and C.C. Peterson. 2002. Physiological and behavioral variation in estivation in mud turtles (*Kinosternon* spp.). *Physiol Biochem Zool* 75:283–293.
- Lillywhite H.B. 2006. Water relations of tetrapod integument. *J Exp Biol* 209:202–226.
- Long D.R. 1985. Lipid utilization during reproduction in female *Kinosternon flavescens*. *Herpetologica* 41:58–65.
- Mautz W.J. 1982. Patterns of evaporative water loss. Pp. 443–481 in C. Gans and F.H. Pough, eds. *Biology of the Reptilia*. Vol. 12. Academic Press, London.
- McPherson R.J. and K.P. Marion. 1982. Seasonal changes of total lipids in the turtle *Sternotherus odoratus*. *Comp Biochem Physiol* 71A:93–98.
- Minnich J.E. 1982. The use of water. Pp. 325–395 in C. Gans and F.H. Pough, eds. *Biology of the Reptilia*. Vol. 12. Academic Press, London.
- Morreale S.J., J.W. Gibbons, and J.D. Congdon. 1984. Significance of activity and movement in the yellow-bellied slider turtle (*Pseudemys scripta*). *Can J Zool* 62:1038–1042.
- Nagy K.A. 1980. CO_2 production in animals: analysis of potential errors in the doubly labeled water method. *Am J Physiol* 238:466–473.
- . 1982. Field studies of water relations. Pp. 483–501 in C. Gans and F.H. Pough, eds. *Biology of the Reptilia*. Vol. 12. Academic Press, London.
- . 1989. Field bioenergetics: accuracy of models and methods. *Physiol Zool* 62:237–252.
- Nagy K.A. and D.P. Costa. 1980. Water flux in animals: analysis of potential errors in the tritiated water method. *Am J Physiol* 238:454–465.
- Nagy K.A., I.A. Girard, and K.T. Brown. 1999. Energetics of free-ranging mammals, reptiles, and birds. *Annu Rev Nutr* 19:247–277.
- Nagy K.A., R.B. Huey, and A.F. Bennet. 1984. Field energetics and foraging mode of Kalahari lacertid lizards. *Ecology* 65:588–596.
- Nagy K.A. and P.A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. *Herpetologica* 42:73–92.
- Niewiarowski P.H. and S.R. Waldshmidt. 1992. Variation in metabolic rates of a lizard: use of SMR in ecological contexts. *Funct Ecol* 6:15–22.
- Parmenter C.J. 1976. The Natural History of the Australian Freshwater Turtle *Chelodina longicollis* Shaw (Testudinata, Chelidae). PhD thesis. University of New England, Biddeford, ME.
- Penick D.N., J.D. Congdon, J.R. Spotila, and J.B. Williams. 2002. Microclimates and energetics of free-living box turtles, *Terrapene carolina*, in South Carolina. *Physiol Biochem Zool* 75:57–65.
- Peterson C.C. 1996a. Anhomeostasis: seasonal water and solute relations in two populations of the desert tortoise (*Gopherus agassizii*) during chronic drought. *Physiol Zool* 69:1324–1358.
- . 1996b. Ecological energetics of the desert tortoise (*Gopherus agassizii*): effects of rainfall and drought. *Ecology* 77:1831–1844.
- Peterson C.C. and P.A. Stone. 2000. Physiological capacity for estivation of the Sonoran mud turtle, *Kinosternon sonoriense*. *Copeia* 2000:684–700.
- Rogers L.J. 1966. The nitrogen excretion of *Chelodina longicollis* under conditions of hydration and dehydration. *Comp Biochem Physiol* 18:249–260.
- Roe J.H. 2008. *Chelodina longicollis* (eastern long-necked turtle): drinking behavior. *Herpetol Rev* 39:212–213.
- Roe J.H. and A. Georges. 2007. Heterogeneous wetland complexes, buffer zones, and travel corridors: landscape management for freshwater reptiles. *Biol Conserv* 135:67–76.
- . 2008. Maintenance of variable responses for coping with wetland drying in freshwater turtles. *Ecology* 89:485–494.
- Schmidt-Nielsen N. 1964. *Desert Animals: Physiological Problems of Heat and Water*. Oxford University Press, London.
- Scott A.F. 1976. Aquatic and Terrestrial Movements of Farm Pond Populations of the Eastern Mud Turtles (*Kinosternon subrubrum*) in East-Central Alabama. PhD diss. Auburn University.
- Seidel M.E. 1978. Terrestrial dormancy in the turtle *Kinosternon flavescens*: respiratory metabolism and dehydration. *Comp Biochem Physiol* 61A:1–4.
- Semlitsch R.D. and J.R. Bodie. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conserv Biol* 17:1219–1228.
- Shine R., T.R.L. Madsen, M.J. Elphick, and P.S. Harlow. 1997. The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. *Ecology* 78:1713–1721.
- Speakman J.R. 1997. *Doubly Labeled Water: Theory and Practice*. Chapman & Hall, London.

- Stockard M.E. and R.E. Gatten. 1983. Activity metabolism of painted turtles (*Chrysemys picta*). *Copeia* 1983:214–221.
- Stott P. 1987. Terrestrial movements of the freshwater tortoise *Chelodina longicollis* Shaw as monitored with a spool tracking device. *Aust Wildl Res* 14:559–567.
- Tuberville T.D., J.W. Gibbons, and J.L. Greene. 1996. Invasion of new aquatic habitats by male freshwater turtles. *Copeia* 1996:713–715.
- Van Marken Lichtenbelt W.D., R.A. Wesselingh, J.T. Vogel, and K.B.M. Albers. 1993. Energy budgets in free-ranging green iguanas in a seasonal environment. *Ecology* 74:1157–1172.
- Wallace B.P., C.L. Williams, F.V. Paladino, S.M. Morreale, R.T. Lindstrom, and J.R. Spotila. 2005. Bioenergetics and diving activity of internesting leatherback turtles *Dermochelys coriacea* at Parque Nacional Marino Las Baulas, Costa Rica. *J Exp Biol* 208:3873–3884.
- Webster M.D. and W.W. Weathers. 1989. Validation of single sample doubly labeled water method. *Am J Physiol* 256:572–576.
- Wygoda M.L. and C.M. Chmura. 1990. Effects of shell closure on water loss in the Sonoran mud turtle, *Kinosternon sonoriense*. *Southwest Nat* 35:228–229.