

Behavioral Inference of Diving Metabolic Rate in Free-Ranging Leatherback Turtles

Corey J. A. Bradshaw^{1,2,*}

Clive R. McMahon^{1,2}

Graeme C. Hays²

¹School for Environmental Research, Institute of Advanced Studies, Charles Darwin University, Darwin, Northern Territory 0909, Australia; ²Department of Biological Sciences, Institute of Environmental Sustainability, University of Wales Swansea, Singleton Park, Swansea SA2 8PP, United Kingdom

Accepted 9/7/2006; Electronically Published 1/16/2007

ABSTRACT

Good estimates of metabolic rate in free-ranging animals are essential for understanding behavior, distribution, and abundance. For the critically endangered leatherback turtle (*Dermochelys coriacea*), one of the world's largest reptiles, there has been a long-standing debate over whether this species demonstrates any metabolic endothermy. In short, do leatherbacks have a purely ectothermic reptilian metabolic rate or one that is elevated as a result of regional endothermy? Recent measurements have provided the first estimates of field metabolic rate (FMR) in leatherback turtles using doubly labeled water; however, the technique is prohibitively expensive and logistically difficult and produces estimates that are highly variable across individuals in this species. We therefore examined dive duration and depth data collected for nine free-swimming leatherback turtles over long periods (up to 431 d) to infer aerobic dive limits (ADLs) based on the asymptotic increase in maximum dive duration with depth. From this index of ADL and the known mass-specific oxygen storage capacity (To_2) of leatherbacks, we inferred diving metabolic rate (DMR) as To_2/ADL . We predicted that if leatherbacks conform to the purely ectothermic reptilian model of oxygen consumption, these inferred estimates of DMR should fall between predicted and measured values of reptilian resting and field metabolic rates, as well as being substantially lower than the FMR predicted for an endotherm of equivalent mass. Indeed, our behaviorally derived DMR estimates (mean = 0.73 ± 0.11 mL O_2 min^{-1} kg^{-1}) were 3.00 ± 0.54 times the resting metabolic rate

measured in unrestrained leatherbacks and 0.50 ± 0.08 times the average FMR for a reptile of equivalent mass. These DMRs were also nearly one order of magnitude lower than the FMR predicted for an endotherm of equivalent mass. Thus, our findings lend support to the notion that diving leatherback turtles are indeed ectothermic and do not demonstrate elevated metabolic rates that might be expected due to regional endothermy. Their capacity to have a warm body core even in cold water therefore seems to derive from their large size, heat exchangers, thermal inertia, and insulating fat layers and not from an elevated metabolic rate.

Introduction

The interplay among physiology, environment, and behavior provides the quantitative framework necessary to understand the evolution of particular life histories under various environmental pressures (Bennett et al. 1990; Feder and Block 1991; Costa et al. 2001). Many advances have been made in recent years investigating these relationships in air-breathing marine vertebrates (i.e., penguins, marine mammals, and sea turtles), given the clear relationship between diving physiology and its limitations to subsurface diving behavior and prey acquisition (Thompson and Fedak 2001; Mori et al. 2002). Thus, obtaining robust estimates of the physiological limitations to diving is an essential element in quantifying the determinants of a species' behavior, distribution, and abundance (Feder and Block 1991; Costa et al. 2001; Jones et al. 2004).

For the critically endangered leatherback turtle (*Dermochelys coriacea*; Spotila et al. 2000), a potential complication to the understanding of its diving physiology is the long-standing debate over the metabolic rate of this species (Paladino et al. 1990). In short, do leatherbacks have a purely ectothermic reptilian metabolic rate or one that is elevated as a result of regional endothermy? To understand the arguments central to the debate, it is important first to define the terms relating to the thermal stasis continuum ranging from internal heat production resulting in relatively constant body temperature (homeothermy) to the fluctuation of internal temperature relative to an individual's surroundings (poikilothermy; Schmidt-Nielsen 1997). More specifically, "endothermic" animals can maintain high body temperature via internal heat production, whereas "ectotherms" depend on external heat sources (e.g., solar radiation) to maintain body temperature within a desired range

* Corresponding author; e-mail: corey.bradshaw@cdu.edu.au.

(Schmidt-Nielsen 1997). This terminology is generally used to distinguish the reptilian and piscine mode of temperature control from the metabolic control employed by birds and mammals (Schmidt-Nielsen 1997). However, a few species from groups considered to be ectothermic can produce heat from muscular activity so that internal body temperatures may be higher than that of the surrounding environment (e.g., the process often termed “regional endothermy” observed in some marine fish, such as lamnid sharks, billfish, and tuna; Holland et al. 1992; Dickson and Graham 2004).

Being ectotherms, reptiles generally have metabolic rates that are much lower than those of birds or mammals of equivalent mass (Kleiber 1975; Nagy 2005). Indeed, metabolic endotherms have metabolic rates that are more than an order of magnitude higher than those of ectotherms, given the endotherms’ reliance on metabolic control to maintain thermal stasis (Nagy 2005). In contrast, reptiles generally do not modify their metabolic rates to keep warm or cool; rather, they rely more on behavioral modification (e.g., basking) to modify their internal temperatures (behavioral thermoregulation; Huey 1974). However, some large-bodied reptiles demonstrate what has been termed “gigantothermy”: the maintenance of a relatively constant high body temperature via a large body and insulation by peripheral tissues (Paladino et al. 1990). The debate with respect to leatherback turtles is centered on which thermal model, higher metabolic rates associated with regional endothermy (perhaps even approaching those for metabolic endotherms) or strict ectothermy, contributes to the elevated temperatures observed.

The controversy began when Frair et al. (1972) found strong circumstantial evidence that leatherbacks can maintain deep body temperature much higher than that of the surrounding cold water in which they sometimes swim. Two explanations arose to explain the phenomenon: either the species demonstrates a type of metabolic endothermy previously unknown in other reptilians or its large size and the insulating properties of its subepidermal fat layer contribute to a higher efficiency in retaining the heat generated from muscular activity. Early anatomical studies indicated support for the latter hypothesis by the discovery of a countercurrent heat exchange system (Greer et al. 1973), but further work and discussion failed to find a clear resolution for the existence of metabolic endothermy (Neill and Stevens 1974; McNab and Auffenberg 1976). In a bid to resolve the issue of metabolic endothermy in this species, measurements of leatherback metabolic rate were eventually made (Paladino et al. 1990, 1996; Lutcavage et al. 1992), but these were done on animals that were nesting or restrained on the beach. As a result, these studies could not address the problem of determining the metabolic rate during the predominant stage of the life cycle, that is, the at-sea phase when individuals are free swimming. This inability to provide a confident range of metabolic rates prevented the appraisal of whether there was some metabolic control of internal body temperature in this species; therefore, the debate was sustained.

It is possible to measure metabolic rate in free-swimming individuals through the application of stable isotopes, such as doubly labeled water (DLW), that measure turnover rates of oxygen and hydrogen and allow for a quantification of CO₂ turnover (and hence, metabolic) rate (Speakman 1997; Speakman et al. 2001). However, obtaining DLW estimates of metabolic rate for free-swimming leatherbacks is an enormous challenge because of the logistics and high costs associated with dosing individuals with sufficient isotope, given their extremely large size relative to other reptiles, their high water turnover rates (Booth 2002), and the relatively long intervals between initial and final blood sampling dictated by their nesting periodicity (Lutz and Musick 1996). Yet these challenges have been successfully overcome recently in a groundbreaking study providing the first DLW estimates of metabolic rate for free-swimming leatherbacks during the internesting period (Wallace et al. 2005). Wallace et al. (2005) obtained four separate measurements of metabolic rate from three individual females, giving a mean of 1.20 mL O₂ min⁻¹ kg⁻¹ (coefficient of variation [CV] = 62%). However, obtaining a large sample size using this approach will be prohibitively expensive, and the high rate of water turnover will always lead to high individual variability, which perpetuates arguments regarding the role of metabolic control in heat production.

There are, fortunately, some indirect methods that can contribute to our understanding of metabolic rate in this and other diving species. An examination of diving profiles (maximum dive depth and duration) collected using archival or satellite technology (Costa et al. 2001) provides a quantitative framework for inferring the physiological limitations to diving. The main physiological determinants of diving capacity in air-breathing animals are (a) the available metabolic stores used during diving and (b) the rate at which these stores are metabolized (Kooyman 1989). The aerobic component of these metabolic stores is the major determinant of diving capacity (Kooyman et al. 1980; Kooyman 1989; Ponganis et al. 1997), giving rise to the concept of the aerobic dive limit (ADL). The ADL has been defined experimentally as the dive duration beyond which blood lactate levels begin to rise above resting levels (Costa et al. 2001), and it can be estimated by dividing an animal’s total oxygen stores by its metabolic rate while diving (Costa et al. 2001; Nagy et al. 2001).

Without detailed measurements of metabolic rate while diving, estimates of ADL are impossible using this method. However, previous studies have suggested approximate ranges of ADL for leatherbacks based on the distribution of surface intervals (Southwood et al. 1999) and visual estimates of dive duration ceilings from dive duration–maximum depth plots (Hays et al. 2004a). Thus, if an objective method for determining the approximate ADL of leatherbacks could be derived from the analysis of the relationship between dive duration and maximum dive depth, then maximum diving metabolic rates could be calculated by dividing known total oxygen stores by

these inferred ADL values. This approach requires a large sample of dive durations to be collected over a protracted period of time because animals will only rarely approach their ADL, perhaps not approaching it at all during certain life-history phases (e.g., during the breeding season, when dives are relatively short; Hays et al. 2004a). We have recently had success in equipping leatherback turtles for relatively long periods of time (>1 yr) with devices that relay dive profiles via satellite, thus providing the necessary large sample sizes. Therefore, in this article, we expand on the work of Hays et al. (2004a) by outlining an objective method of determining ADL from duration–maximum depth plots in free-swimming leatherback turtles so that diving metabolic rates can be calculated. Using this method, we aim to corroborate recent DLW measurements of leatherback turtle metabolic rates and hence resolve the debate on whether free-swimming leatherbacks have an essentially ectothermic reptilian metabolic rate or one that is elevated because of regional endothermy.

Methods

Data Collection

Satellite-relayed data loggers (SRDLs, manufactured by the Sea Mammal Research Unit, St. Andrews, United Kingdom) were attached to female turtles nesting at Levera Beach on the north shore of Grenada (Caribbean Sea; 12°12'N, 61°36'W) in July 2002 and again in May–July 2003. SRDL function and attachment procedures are summarized elsewhere (Hughes et al. 1998; Fedak et al. 2001; Hays et al. 2004a, 2004b; McMahan et al. 2005). SRDLs allowed dive profiles to be collected for up to 18 mo from deployment (Hays et al. 2004a). Dive profiles were collected throughout the sampled animals' foraging ranges. Detailed descriptions of the movement patterns are provided by Hays et al. (2004b, 2006) and McMahan and Hays (2006), although we provide a brief overview here. The nine female turtles were followed for between 181 and 431 d and dispersed widely to the north, northeast, and east of Grenada. Two turtles spent all their time in the tropical Atlantic between latitudes 0° and 20°N, while another traveled up to the northeastern coast of the United States (~40°N), overwintered near Bermuda, then traveled back to the northeastern coast of the United States. Yet another individual went northeast toward Africa and then toward the Cape Verde Islands (~15°N), while another went toward the Azores (~39°N) for the winter and then to the Bay of Biscay (France) the following spring. The most northerly position achieved by all turtles was just north of 50°N, for one turtle that subsequently moved toward the south immediately thereafter. In general, the five turtles traveling the farthest north achieved their most northerly latitudes in autumn before heading south at the start of the winter (Hays et al. 2004b, 2006; McMahan and Hays 2006).

Analysis

A visual inspection of dive duration–maximum depth plots for individual leatherback turtles suggests an asymptotic maximum dive duration with increasing depth (Hays et al. 2004a; Fig. 1a). We constructed an objective method for fitting a function to this asymptote by defining a type of nonlinear percentile regression, but we deliberately avoided using standard quantile regression techniques because values near the extremes of the edge were often highly influential on model fit. An examination of depth histograms (see “Results”) revealed few dives with maximum depths >200 m (1.6% of all dives). As a result, the determination of dive parameters was suspect beyond this depth value, so we excluded from our analyses all dives exceeding 200 m (as in Hays et al. 2004a). After removal of these dives, we created a series of depth bins (5-m increments) for each individual up to the maximum dive depth. We then determined the 97.5 percentile of the values for the ratio of duration (s) to depth (m) for each dive within each of the depth bins to determine the points that would be used to calculate the asymptotic relationship between duration and maximum depth (Fig. 1b). To this series of points we fitted a simple exponential-rise model of the form

$$t_{\max} = a(1 - e^{-bd}),$$

where t_{\max} = predicted dive duration in minutes, a and b are constants, and d = dive depth in meters (Fig. 1b). Of course, the choice of particular percentile thresholds to estimate the asymptotic property of duration–depth plots is somewhat arbitrary. For example, similar t_{\max} values could be derived by estimating anything between the ninety-fifth and ninety-ninth duration percentiles for set depth bins. We chose the 97.5 percentile on the basis of statistical convention (i.e., the 95% confidence interval).

Few dives exceeded the asymptotic duration estimated from the exponential-rise model, so we took that value (estimated as the predicted duration at 200 m) as an index of the ADL (ADL_i) for each individual. We then used these ADL_i to calculate the maximum diving metabolic rate (DMR) based on calculated values of total oxygen stores (To_2), where $DMR = To_2/ADL_i$ (Nagy et al. 2001). The published value of To_2 for leatherback turtles is 27 mL kg⁻¹ (Lutcavage et al. 1992). We then compared these estimates of DMR to previously published estimates of resting metabolic rate (RMR) and field metabolic rate (FMR) to examine the energetics of diving activity relative to inactivity and to appraise the effectiveness of duration–depth data for inferring metabolic rates. We can define these terms, essentially, as (1) RMR, the metabolic rate measured in adult leatherbacks resting on beaches after egg laying, (2) FMR, the integrated metabolic rate measured over a period of several days while at sea between nesting events during the breeding season, and (3) DMR, the diving metabolic rate inferred for

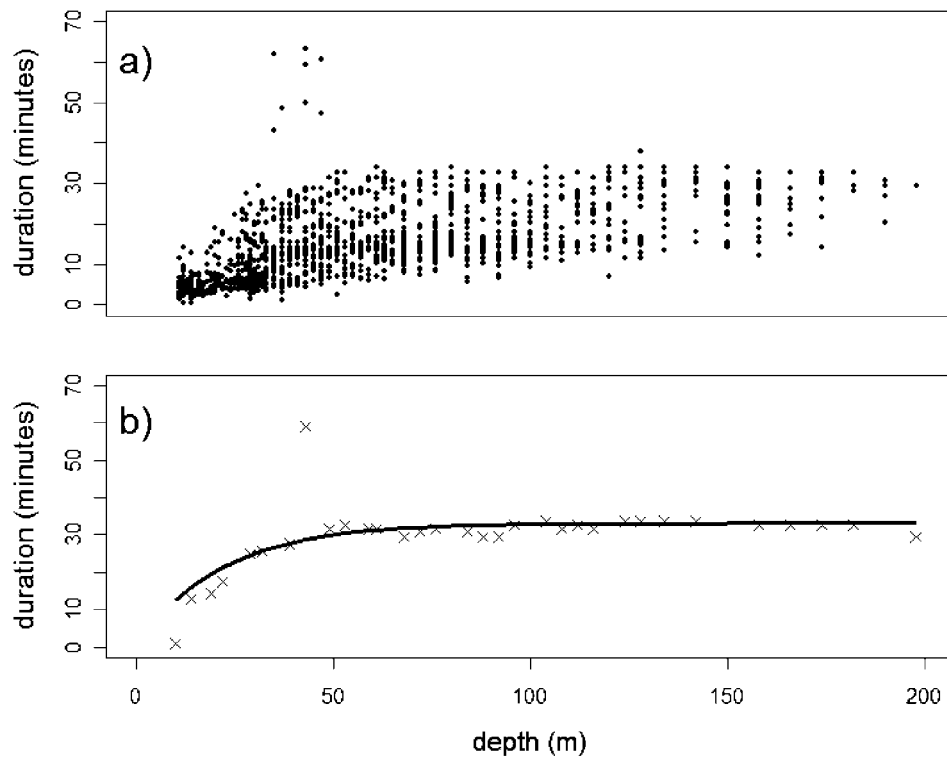


Figure 1. *a*, Example dive duration–maximum dive depth plot for an adult female leatherback turtle (turtle H). *b*, The 97.5 percentile dive durations per 5-m depth bin are shown as crosses, with the exponential-rise model (*line*) fitted to those points.

individual dives from the ceiling of dive duration versus depth. However, in addition to these empirical estimates of RMR and FMR, we used predictions of these values based on allometric scaling relationships. All calculated metabolic rates were expressed as milliliters of O_2 per minute using the following equations: 1 L O_2 consumed provides 4.7 kcal of energy, 1 kJ = 0.238846 kcal, and 1 kcal consumed per hour = 1.1622 W.

Results

We obtained a total of 17,618 dive profiles from nine adult female leatherback turtles (average of 1,958 dives individual⁻¹). Mean duration of the dive records was 341.8 ± 36.2 d (mean \pm SD); thus, the average number of dives recorded per day over all sampled individuals was 6.5 ± 1.2 . It should be noted that the dives recorded by SRDLs give only a portion of the total number of dives made each day because of the limited bandwidth of the Argos satellite network (Fedak et al. 2001). The deepest dive profile recorded for the nine individuals was 1,010 m, and the longest dive profile lasted 71 min; however, median depth was only 53 m, and median duration was 22 min (Fig. 2). After the 279 dives that exceeded 200 m in maximum depth were removed, median maximum dive depth and duration were 51 m and 22 min, respectively (Fig. 2*b*).

Mass and ADL

We were not able to measure mass directly, so we estimated mass based on curved-carapace length (CCL)-mass relationships previously established for leatherback turtles (Eckert et al. 1989; Southwood et al. 1999). The CCL measurements for our sample ranged from 1.46 to 1.58 m (mean = 1.53 ± 0.04 m), providing an estimated mass range of 279–337 kg (mean = 312 ± 18 kg). Mean maximum ADL_i was 37.6 ± 6.1 min (CV = 16%) and ranged from 19.2 to 48.1 min (Table 1; Fig. 3). Interestingly, the maximum dive duration was relatively invariant with depth after approximately 50 m (Fig. 3). The mean percentage of dives exceeding ADL_i was $3.6\% \pm 2.8\%$ (Table 1), and most of these occurred at depths shallower than 50 m (Fig. 3). There was no evidence for a relationship between log (estimated ADL_i) and log (body mass), possibly because of low sample size ($n = 9$).

To examine whether there was any evidence for anaerobic diving that could compromise the interpretation of the duration-depth relationship to estimate ADL_p , we examined the relationship between dive duration and postdive surface interval (PDSI). The accumulation of lactate resulting from anaerobic respiration requires an air-breathing diver to spend time recovering at the surface between long dives (Thompson and

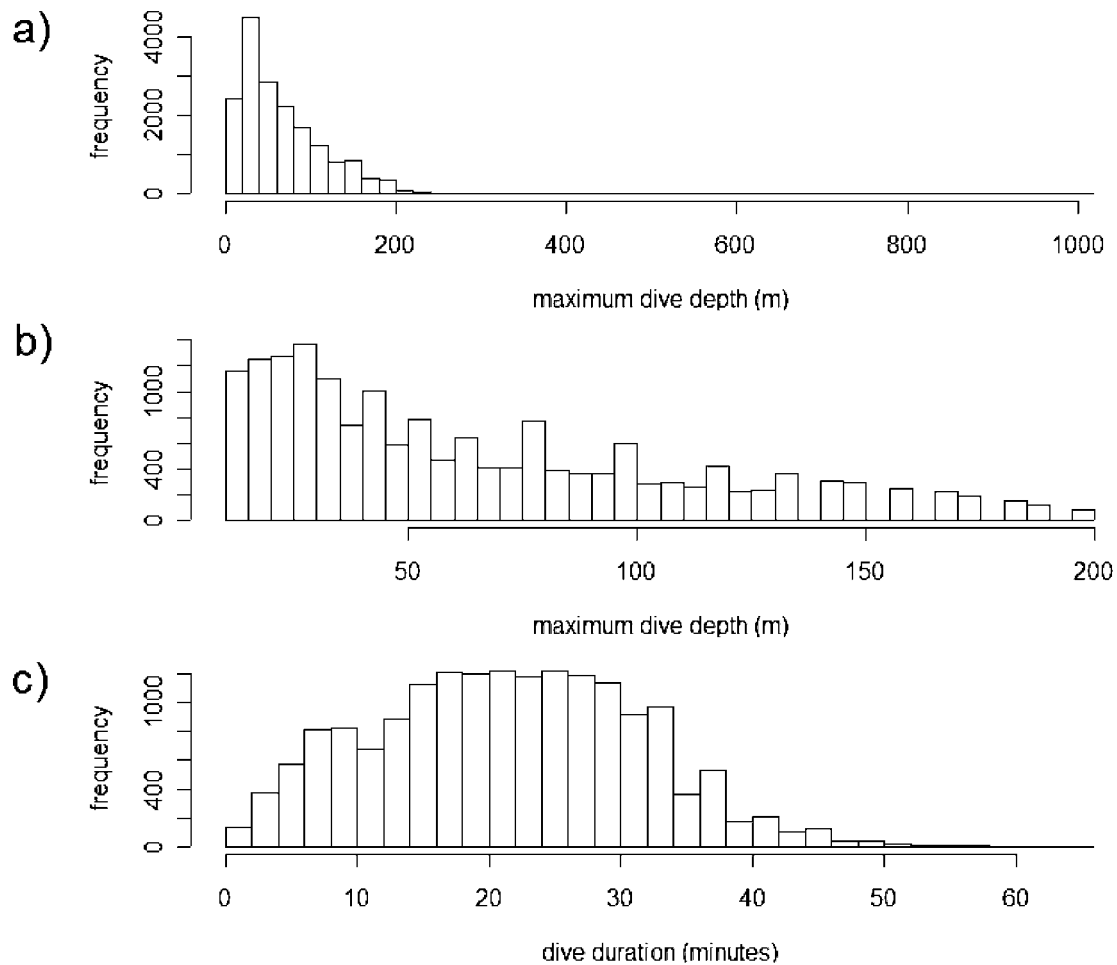


Figure 2. Frequency histograms for (a) maximum dive depth, (b) maximum dive depth excluding all dives of >200 m, and (c) dive duration for all nine leatherback turtles combined.

Fedak 1993), and this is expressed as a positive relationship between dive duration and PDSI (Costa et al. 2001). SRDLs provide several measures of diving behavior (described in detail in McMahon et al. 2007), but not all consecutive dive profiles are measured. Therefore, we identified all consecutive dives and calculated the PDSIs by subtracting the start time of dive $i + 1$ from the end time of dive i . When the median PDSI was plotted against 5-min interval bins of dive duration (Fig. 4), there was no obvious trend in PDSI relative to dive duration, and median PDSIs were consistently short relative to dive duration, suggesting that most dives were aerobic.

Inferred Diving Metabolic Rate

Mean DMR inferred from ADL, and estimated oxygen stores was $229.7 \pm 41.6 \text{ mL O}_2 \text{ min}^{-1}$ ($75.3 \pm 13.6 \text{ W}$), or $0.73 \pm 0.11 \text{ mL O}_2 \text{ min}^{-1} \text{ kg}^{-1}$ when expressed as mass-specific values (Table 1). Our DMR estimates lie at the low end of four separate

measurements of FMR from three individual females measured by Wallace et al. (2005; Table 1). Our DMR estimates are about 3 times the RMR values recorded for three restrained leatherback turtles on the beach by Lutcavage et al. (1990; Table 1).

We used allometric relationships to predict the ectothermic and endothermic FMRs for animals equal in size to our sample of adult leatherbacks (Fig. 5). For ectotherms, we used the equation reptile $\text{FMR} (\text{kJ d}^{-1}) = 0.196 \times \text{mass} (\text{g})^{0.889}$, which is based on species ranging in mass from 1.1 to 45,200 g (Nagy et al. 1999). For endotherms, we used the equation for mammals, $\text{FMR} (\text{kJ d}^{-1}) = 4.82 \times \text{mass} (\text{g})^{0.734}$, based on species ranging in mass from 7.3 to 99,000 g (Nagy et al. 1999). This comparison clearly indicates that the DMR we inferred for leatherbacks falls close to the predicted FMR for a reptile of equivalent size and is nearly an order of magnitude lower than the FMR predicted for a mammal of equivalent size (Fig. 5). In short, there is compelling evidence that leatherbacks have an ectothermic metabolic rate rather than one elevated because of regional endothermy.

Table 1: Summary dive and metabolic rate parameters for nine adult female leatherback turtles derived from dive depth–duration data (this study)

| ID | CCL (m) | Mass (kg) | ADL _i (min) | Dives >ADL _i (%) | MR (mL O ₂ min ⁻¹) | Mass-Specific MR (mL O ₂ min ⁻¹ kg ⁻¹) |
|-------------------------------------|------------|--------------|---------------------------|--------------------------------|--|---|
| Diving metabolic rate (this study): | | | | | | |
| A | 1.46 | 279 | 38.7 | 2.8 | 194.6 | .70 |
| B | 1.51 | 304 | 38.1 | 5.1 | 215.1 | .71 |
| C | 1.58 | 338 | 32.3 | 10.0 | 281.9 | .83 |
| D | 1.53 | 313 | 35.4 | 5.3 | 238.7 | .76 |
| E | 1.50 | 299 | 48.1 | 2.5 | 167.6 | .56 |
| F | 1.54 | 318 | 38.0 | 1.2 | 226.3 | .71 |
| G | 1.51 | 304 | 29.2 | 1.5 | 280.5 | .92 |
| H | 1.57 | 333 | 33.2 | 1.7 | 270.9 | .81 |
| I | 1.55 | 323 | 44.5 | 2.7 | 191.9 | .59 |
| FMR (Wallace et al. 2005): | | | | | | |
| W1 | ... | 270 | ... | ... | 609.2 ^a | 2.26 |
| W3a ^b | ... | 268 | ... | ... | 327.1 ^a | 1.22 |
| W3b ^b | ... | 268 | ... | ... | 196.1 ^a | .73 |
| W4 | ... | 308 | ... | ... | 188.0 ^a | .61 |
| RMR (Lutcavage et al. 1990): | | | | | | |
| L1 | ... | 280 | ... | ... | 64.4 | .23 |
| L2 | ... | 306 | ... | ... | 70.4 | .23 |
| L3 | ... | 328 | ... | ... | 95.2 | .29 |

Note. For comparison, the measured field metabolic rate (FMR) and resting metabolic rate (RMR) estimates for leatherback turtles from Wallace et al. (2005) and Lutcavage et al. (1990), respectively, are shown. Shown are turtle identification letter (ID), curved-carapace length (CCL), estimated mass, estimated aerobic dive limit (ADL), the percentage of dives greater than ADL, metabolic rate (MR), and mass-specific MR.

^a Calculated, from values in W kg⁻¹ given in table 1 of Wallace et al. (2005), using conversion values provided in “Methods.”

^b Two separate measurements from the same individual (Wallace et al. 2005).

Discussion

Our finding that free-swimming leatherback turtles have ADL-inferred DMRs (mean = 0.73 mL O₂ min⁻¹ kg⁻¹) close to both the predicted FMRs for reptiles of equivalent mass and the FMR values measured using DLW (Wallace et al. 2005) helps to resolve the regional endo-/ectothermy debate and our general understanding of diving physiology in this species. Adult leatherbacks seem to have an ADL of around 40 min that can be sustained only by an essentially ectothermic, reptilian metabolic rate. By contrast, we can calculate the much shorter ADL for an endotherm of the same size and oxygen stores. For example, applying an FMR for marine mammals of $FMR (W) = 30.43 \times \text{mass (kg)}^{0.524}$ (Boyd 2002) produces an estimated ADL of approximately 4.5 min, that is, nearly an order of magnitude shorter than the ADL_i we calculated. Even a quick visual examination of the dive duration–maximum depth plots reveals that leatherbacks cannot have an ADL close to 4.5 min because a large proportion of dives greatly exceed this duration. Our conclusion that leatherback turtles are ectothermic suggests that heat retention resulting from gigantothermy, heat exchangers, and insulation is responsible for the elevated body temperatures observed, rather than high heat production arising from a relatively high metabolic rate (Paladino et al. 1990).

It is arguable that a direct comparison of measured FMR to those predicted from allometric relationships may not be possible when the mass range used to establish the predicted mass-specific relationships does not overlap the mass of leatherback turtles (although it covers more than four orders of magnitude and should provide good inference). For example, Nagy’s reptile FMR–predictive equation, given above, covers only reptile species up to 45.2 kg, whereas our sample of leatherbacks had a mean mass of 312 kg and Wallace’s three individuals weighed a mean of 282 kg. However, regardless of the ability to extrapolate to such large species, the predicted FMR for mammals of equivalent mass (using the average mass for our sample) is 5.87 mL O₂ min⁻¹ kg⁻¹, a value much greater than the predicted FMR for reptiles of equivalent mass of 1.70 mL O₂ min⁻¹ kg⁻¹ (i.e., a difference of 4.17 mL O₂ min⁻¹ kg⁻¹) than the difference between the predicted reptile FMR and our DMR estimates (mean = 0.73 mL O₂ min⁻¹ kg⁻¹, i.e., a difference of 0.97 mL O₂ min⁻¹ kg⁻¹). We therefore conclude that the comparisons made are still informative in resolving the regional endo-/ectothermy debate for leatherback turtles.

Our main aim was not, however, to compare the measured and inferred metabolic rates of regionally endothermic leatherback turtles to those of endotherms of equivalent mass per

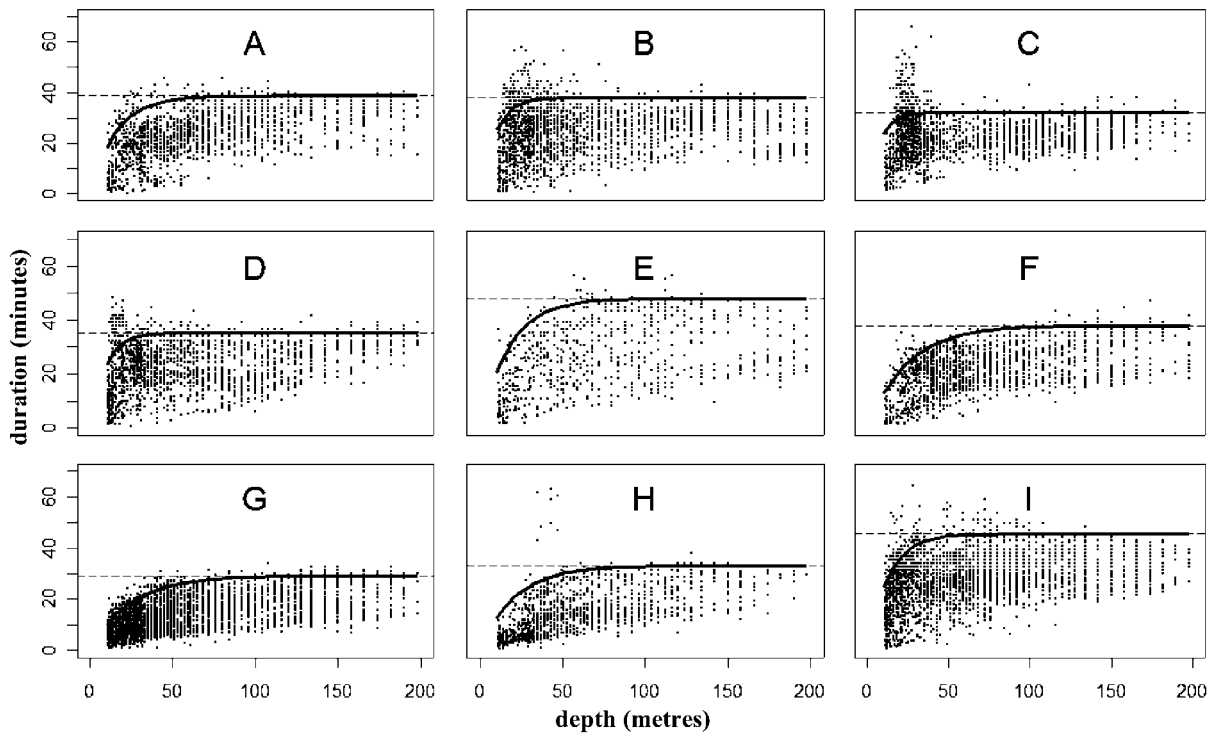


Figure 3. Dive duration–maximum dive depth plots for each individual (A–I), with the corresponding asymptotic exponential-rise model (solid lines) indicative of the aerobic dive limit index (ADL; dashed horizontal lines).

se; rather, we endeavored to examine whether there was sufficient evidence for elevated metabolic rates beyond those predicted for strictly ectothermic reptiles of equivalent mass. The predictive caveats aside, we have shown clearly that leatherback turtles have metabolic rates that fall neatly into the ranges expected for reptiles, and they do not, contrary to previous contentions, demonstrate patterns atypical for reptiles. There is some precedent for expecting elevated metabolic rates in ectothermic species demonstrating regional endothermy. For example, Sepulveda et al. (2003) established that strictly ectothermic fish (tribe Sardinii) closely related to regionally endothermic species (tribe Thunnini) within the same family (Scombridae) had relatively lower standard metabolic rates (defined therein as the minimum metabolic rate required for maintenance functions), even though both tribes have metabolic rates that are lower than those of endotherms of equivalent mass. However, the degree to which regionally endothermic leatherback turtles diverge from the normal ectothermic reptile mode of temperature regulation may never be fully determined until more controlled estimates of metabolic rates are obtained.

Our method for defining the upper limit of aerobic diving capacity (inferred ADL) requires a large sample size of dives since only occasionally does dive duration extend to the ADL. The multitude of dive profiles and the corresponding behaviors (movement, resting, foraging) they characterize, as represented

by the duration–depth plots, imply that most dives will be terminated before the individual achieves its ADL. For example, during foraging dives where no prey are found and consumed, the dive may be terminated earlier than would be expected had the diver pushed itself to the limits of its aerobic capacity (Thompson and Fedak 2001). This large range of dive types, commonly observed in diving vertebrates (Schreer et al. 2001), demonstrates the importance of obtaining large samples of dives for the estimation of ADL and maximum DMR. Only with sufficient sample sizes can the identification of this upper physiological (aerobic) limit be identified.

We observed that the maximum dive duration was relatively invariant with depth after 50 m (Fig. 3). Unlike other sea turtles with hard shells, the primary oxygen stores for leatherback turtles are the muscles and blood (Lutcavage et al. 1992), and little information is available on lung-regulated buoyancy control (James et al. 2005). Therefore, if oxygen stores vary little with depth, our observations might suggest that metabolic rates are likewise relatively constant over the deeper depth ranges. However, Southwood et al. (1999) found a negative relationship between heart rate and depth in leatherback turtles, and reductions in metabolic rate with depth have been found for many marine mammal species (Williams et al. 1999; Costa and Gales 2000; Hicks et al. 2004; Hastie et al. 2006). These observations lend weight to the hypothesis that there is some

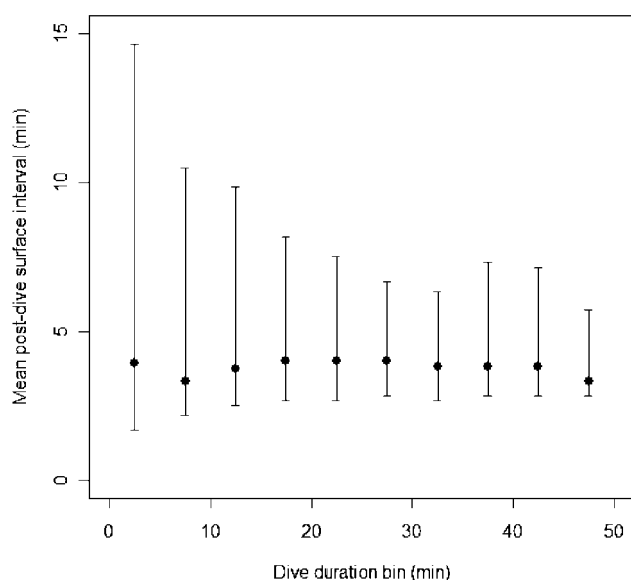


Figure 4. Median postdive surface interval (error bars indicate seventy-fifth and twenty-fifth percentiles) versus binned (5-min intervals) dive duration. The short postdive surface intervals relative to dive duration and the lack of a positive relationship suggest that most dives were aerobic (i.e., no extended postdive surface intervals were required to oxidize accumulated lactate expected if dives had had an anaerobic component).

modification of metabolic rate with depth in leatherback turtles, possibly in response to a reduction in oxygen stores as they dive (i.e., thus maintaining relatively constant maximum dive durations).

The method we employed is an objective technique for measuring variation in maximum DMRs among individuals, especially considering that other methods, such as DLW measurements, are often prohibitively expensive and logistically challenging (Wallace et al. 2005). To our knowledge, we are the first to employ this indirect technique, but we have confidence in our results, considering that there is now increasing evidence that DMR is generally lower than FMR, given various forms of diving hypometabolism in many diving vertebrates (Kooyman and Ponganis 1994; Hindell et al. 2000; Costa et al. 2001; Williams et al. 2001; Green et al. 2003). Our method was useful for estimating indices of maximum diving capacity such as the ADL because it reflects only the diving (and not the surface) component of oxygen utilization. Indeed, the direct measurement of blood lactate levels is difficult in free-swimming animals (Costa et al. 2001), and so an experimental determination of ADL (the point at which blood lactate rises above resting levels) is rarely possible. Using dive data as a surrogate inference of ADL is a novel method that takes only the diving component of metabolic estimation into account, thus potentially providing the best indirect assessment of ADL. We are also confident that the majority of the dives used to infer ADL were aerobic because

PDSIs were relatively short compared to dive duration, as would be expected after aerobic dives (Hays et al. 2000a); there was no evidence for a positive relationship between PDSI and dive duration (Fig. 4). Indeed, there is increasing evidence that when ADL is calculated correctly (i.e., removing the influence of surface metabolic rate), most diving vertebrates appear to exceed ADL only occasionally (Le Boeuf et al. 1988; Hindell et al. 1992, 2000; Nowicki et al. 1997; Hays et al. 2000a; Croll et al. 2001; Froget et al. 2004; Hansen and Ricklefs 2004), although there are some exceptions (Kooyman et al. 1980; Costa et al. 2001).

Of course, even the metabolic rates of ectotherms demonstrating some form of internal heat generation and retention are at least partially dependent on ambient temperature (Bennett and Dawson 1976), so comparisons to particular reference FMRs and RMRs will depend to some degree on the ambient temperatures experienced by the individuals being measured. Wallace et al. (2005) reported mean water temperatures experienced by foraging leatherback turtles at 26.6°C; however, making inferences about the “average” temperature conditions experienced by leatherback turtles is complicated by the temperature variability encountered during deep diving and long-distance movement (Hays et al. 2004a; McMahon et al. 2005).

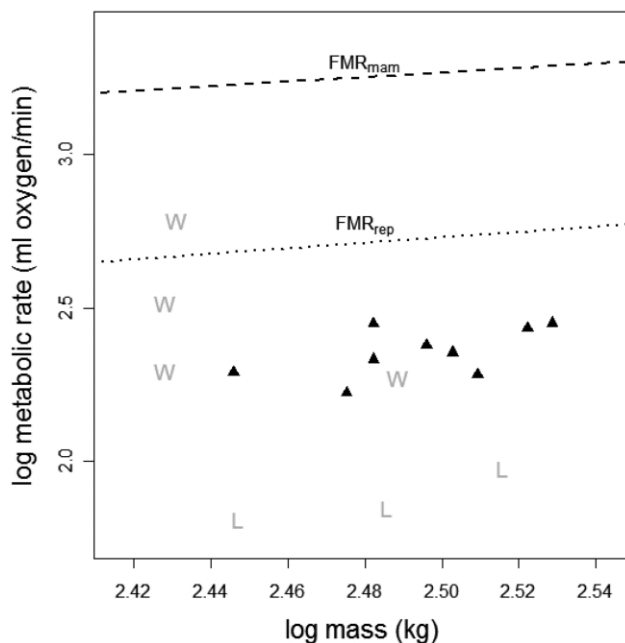


Figure 5. The diving metabolic rates we calculated for nine leatherback turtles (triangles) versus the predicted field metabolic rates (FMRs) for ectotherms (reptiles; dotted line, FMR_{rep}) and endotherms (mammals; dashed line, FMR_{mam}) predicted by the allometric relationships detailed by Nagy et al. (1999). Leatherbacks clearly fall nearer the ectothermic than the endothermic prediction. Also shown are the FMR measurements of Wallace et al. (2005; W) and the resting metabolic rate measurements of Lutcavage et al. (1990; L).

However, the environmental temperatures experienced by foraging turtles may be less important in dictating metabolic rates than their true core temperature, especially if body temperatures are relatively immune to short-term variation in ambient temperature. Indeed, leatherbacks modify their foraging behavior in colder waters by diving to shallower depths (Hays et al. 2006) and generally avoiding surface temperatures $<15^{\circ}\text{C}$ (McMahon and Hays 2006). In addition, recent evidence has shown that a leatherback turtle diving in cold water maintained higher-than-ambient body temperatures through efficient thermal inertia (James et al. 2006).

Variation in water temperature may also have important implications for interpreting the pattern of dives exceeding the inferred ADL. Even though this number was low (mean = 3.6%), most of these dives occurred at depths of <50 m. This observation could suggest that these relatively longer-duration dives resulted from lower metabolic rates associated with benthic resting dives, as has been suggested for other marine turtle species (Hays et al. 2000b). However, our deployments were made after nesting, and so we did not record interesting dives that might be benthic more frequently and have a resting function (Reina et al. 2005). Indeed, the majority of the dives occurred in deep, open-ocean habitat (Hays et al. 2004a, 2004b, 2006; McMahon and Hays 2006), suggesting that at least some of the shallow dives exceeding the inferred ADL may have resulted from lower temperatures experienced in colder waters.

It is arguable that our estimates of maximum DMR are applicable only to lower-latitude regions where warmer temperatures prevail. However, foraging leatherbacks demonstrate shallower dives of shorter duration when in higher latitudes (Hays et al. 2006), suggesting some behavioral adaptations to modify their thermal environment (James et al. 2005; Hays et al. 2006). As with DMRs, the ADL_i we estimated are likely to reflect the performance maxima of turtles in warmer water and so may not provide a universal ADL that applies throughout their range. Our samples were biased toward warmer waters, given that the deployment site was situated in the tropics; to obtain enough data from colder waters, additional deployments would have to be made at higher latitudes (as has recently begun; e.g., James et al. 2006).

In conclusion, the dives performed by an individual while foraging at sea provided a wealth of information for the detailed comparison of metabolically restricted diving capacity. The parameters derived using basic behavioral data provided an additional insight into the diving performance and metabolic constraints experienced by the largest diving marine reptile. It will be important to examine the duration-depth relationship at different latitudes (and different water temperatures) to summarize the variation in DMR across this species' range. We contend, therefore, that our method can be used to compare relative DMR and diving performance over time and space, especially in circumstances where direct measurements of metabolic rate are prohibitively expensive and logistically challenging.

Acknowledgments

Data were collected with funding from the Natural Environment Research Council and the Marine Conservation Society of the United Kingdom. The work was conducted with the permission and collaboration of the Grenadian Ministry of Agriculture, Forestry, Land, and Fisheries (including ethics approval). Additional funding was provided by the Australian Academy of Science Visiting Fellowship program to C.J.A.B. We thank M. Hays, J. Houghton, C. Isaacs, W. Kelly, R. King, C. Lloyd, P. Lovell, A. Myers, and B. Randig for assistance. S. Hochscheid and four anonymous reviewers provided helpful comments to improve the manuscript. G.C.H. conceived the study and collected the data. C.J.A.B. and G.C.H. did the analysis and wrote the article. C.R.M. provided intellectual content.

Literature Cited

- Bennett A.F., K.M. Dao, and R.E. Lenski. 1990. Rapid evolution in response to high-temperature selection. *Nature* 346:79–81.
- Bennett A.F. and W.R. Dawson. 1976. Metabolism. Pp. 127–223 in C. Gans and W.R. Dawson, eds. *Biology of the Reptilia*. Vol. 5. Academic Press, London.
- Booth D.T. 2002. The doubly-labeled water technique is impractical for measurement of field metabolic rate in freshwater turtles. *Herpetol Rev* 33:105–107.
- Boyd I.L. 2002. Energetics: consequences for fitness. Pp. 247–277 in A.R. Hoelzel, ed. *Marine Mammal Biology: An Evolutionary Approach*. Blackwell Science, Oxford.
- Costa D.P. and N.J. Gales. 2000. Foraging energetics and diving behavior of lactating New Zealand sea lions, *Phocartos hookeri*. *J Exp Biol* 203:3655–3665.
- Costa D.P., N.J. Gales, and M.E. Goebel. 2001. Aerobic dive limit: how often does it occur in nature? *Comp Biochem Physiol A* 129:771–783.
- Croll D.A., A. Acevedo-Gutierrez, B.R. Tershy, and J. Urban-Ramirez. 2001. The diving behavior of blue and fin whales: is dive duration shorter than expected based on oxygen stores? *Comp Biochem Physiol A* 129:797–809.
- Dickson K.A. and J.B. Graham. 2004. Evolution and consequences of endothermy in fishes. *Physiol Biochem Zool* 77: 998–1018.
- Eckert S.A., K.L. Eckert, P. Ponganis, and G.L. Kooyman. 1989. Diving and foraging behavior of leatherback sea turtles (*Dermochelys coriacea*). *Can J Zool* 67:2834–2840.
- Fedak M.A., P. Lovell, and S.M. Grant. 2001. Two approaches to compressing and interpreting time-depth information as collected by time-depth recorders and satellite-linked data recorders. *Mar Mamm Sci* 17:94–110.
- Feder M.E. and B.A. Block. 1991. On the future of animal physiological ecology. *Funct Ecol* 5:136–144.
- Frair W., R.G. Ackman, and N. Mrosovsky. 1972. Body tem-

- perature of *Dermochelys coriacea*: warm turtle from cold water. *Science* 177:791–793.
- Froget G., P.J. Butler, A.J. Woakes, A. Fahlman, G. Kuntz, Y. Le Maho, and Y. Handrich. 2004. Heart rate and energetics of free-ranging king penguins (*Aptenodytes patagonicus*). *J Exp Biol* 207:3917–3926.
- Green J.A., P.J. Butler, A.J. Woakes, and I.L. Boyd. 2003. Energetics of diving in macaroni penguins. *J Exp Biol* 206:43–57.
- Greer A.E., J.D. Lazell, and R.M. Wright. 1973. Anatomical evidence for a counter-current heat exchanger in the leatherback turtle (*Dermochelys coriacea*). *Nature* 244:181.
- Hansen E.S. and R.E. Ricklefs. 2004. Foraging by deep-diving birds is not constrained by an aerobic diving limit: a model of avian depth-dependent diving metabolic rate. *Am Nat* 163:358–374.
- Hastie G.D., D.A.S. Rosen, and A.W. Trites. 2006. The influence of depth on a breath-hold diver: predicting the diving metabolism of Steller sea lions (*Eumetopias jubatus*). *J Exp Mar Biol Ecol* 336:163–170.
- Hays G.C., C.R. Adams, A.C. Broderick, B.J. Godley, D.J. Lucas, J.D. Metcalfe, and A.A. Prior. 2000a. The diving behaviour of green turtles at Ascension Island. *Anim Behav* 59:577–586.
- Hays G.C., V.J. Hobson, J.D. Metcalfe, D. Righton, and D.W. Sims. 2006. Flexible foraging movements of leatherback turtles across the North Atlantic Ocean. *Ecology* 87:2647–2656.
- Hays G.C., S. Hochscheid, A.C. Broderick, B.J. Godley, and J.D. Metcalfe. 2000b. Diving behaviour of green turtles: dive depth, dive duration and activity levels. *Mar Ecol Prog Ser* 208:297–298.
- Hays G.C., J.D.R. Houghton, C. Isaacs, R.S. King, C. Lloyd, and P. Lovell. 2004a. First records of oceanic dive profiles for leatherback turtles, *Dermochelys coriacea*, indicate behavioural plasticity associated with long-distance migration. *Anim Behav* 67:733–743.
- Hays G.C., J.D.R. Houghton, and E.H. Myers. 2004b. Endangered species: pan-Atlantic leatherback turtle movements. *Nature* 429:522.
- Hicks J.L., R.J. O'Hara Hines, J.F. Schreer, and M.O. Hamill. 2004. Correlation of depth and heart rate in harbour seal pups. *Can J Zool* 82:1147–1156.
- Hindell M.A., M.A. Lea, M.G. Morrice, and C.R. McMahon. 2000. Metabolic limits on dive duration and swimming speed in the southern elephant seal *Mirounga leonina*. *Physiol Biochem Zool* 73:790–798.
- Hindell M.A., D.J. Slip, H.R. Burton, and M.M. Bryden. 1992. Physiological implications of continuous, prolonged and deep dives of the southern elephant seal (*Mirounga leonina*). *Can J Zool* 70:370–379.
- Holland K.N., R.W. Brill, R.K.C. Chang, J.R. Sibert, and D.A. Fournier. 1992. Physiological and behavioral thermoregulation in bigeye tuna (*Thunnus obesus*). *Nature* 358:410–412.
- Huey R.B. 1974. Behavioral thermoregulation in lizards: importance of associated costs. *Science* 184:1001–1003.
- Hughes G.R., P. Luschi, R. Menciacci, and F. Papi. 1998. The 7000-km oceanic journey of a leatherback turtle tracked by satellite. *J Exp Mar Biol Ecol* 229:209–217.
- James M.C., J. Davenport, and G.C. Hays. 2006. Expanded thermal niche for a diving vertebrate: a leatherback turtle diving into near-freezing water. *J Exp Mar Biol Ecol* 335:221–226.
- James M.C., R.A. Myers, and C.A. Ottensmeyer. 2005. Behaviour of leatherback sea turtles, *Dermochelys coriacea*, during the migratory cycle. *Proc R Soc B* 272:1547–1555.
- Jones D.R., A.L. Southwood, and R.D. Andrews. 2004. Energetics of leatherback sea turtles: a step toward conservation. Pp. 66–82 in M.S. Gordon and S.M. Barton, eds. *Experimental Approaches to Conservation Biology*. University of California Press, Berkeley.
- Kleiber M. 1975. *The Fire of Life: An Introduction to Animal Energetics*. Kreiger, Huntington, NY.
- Kooyman G.L. 1989. *Diverse Divers: Physiology and Behavior*. Springer, Berlin.
- Kooyman G.L. and P.J. Ponganis. 1994. Emperor penguin oxygen consumption, heart rate and plasma lactate levels during graded swimming exercise. *J Exp Biol* 195:199–209.
- Kooyman G.L., E.A. Wahrenbrock, M.A. Castellini, R.W. Davis, and E.E. Sinnett. 1980. Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J Comp Physiol B* 138:335–346.
- Le Boeuf B.J., D.P. Costa, A.C. Huntley, and S.D. Feldkamp. 1988. Continuous, deep diving in female northern elephant seals, *Mirounga angustirostris*. *Can J Zool* 66:446–458.
- Lutcavage M.E., P.G. Bushnell, and D.R. Jones. 1990. Oxygen transport in the leatherback sea turtle, *Dermochelys coriacea*. *Physiol Zool* 63:1012–1024.
- . 1992. Oxygen stores and aerobic metabolism in the leatherback sea turtle. *Can J Zool* 70:348–351.
- Lutz P.L. and J.A. Musick. 1996. *The Biology of Sea Turtles*. CRC, Boca Raton, FL.
- McMahon C.R., E. Autret, J.D. Houghton, P. Lovell, A.E. Myers, and G.C. Hays. 2005. Animal borne sensors successfully capture the thermal properties of ocean basins. *Limnol Oceanogr Methods* 3:392–398.
- McMahon C.R., C.J.A. Bradshaw, and G.C. Hays. 2007. Satellite tracking reveals unusual diving characteristics for a marine reptile, the olive ridley turtle (*Lepidochelys olivacea*). *Mar Ecol Prog Ser* (forthcoming).
- McMahon C.R. and G.C. Hays. 2006. Thermal niche, large-scale movements and implications of climate change for a critically endangered marine vertebrate. *Global Change Biol* 12:1–9.
- McNab B.K. and W. Auffenberg. 1976. The effect of large body

- size on the temperature regulation of the Komodo dragon, *Varanus komodoensis*. *Comp Biochem Physiol A* 55:345–350.
- Mori Y., A. Takahashi, F. Mehlum, and Y. Watanuki. 2002. An application of optimal diving models to diving behaviour of Brunnich's guillemots. *Anim Behav* 64:739–745.
- Nagy K.A. 2005. Field metabolic rate and body size. *J Exp Biol* 208:1621–1625.
- Nagy K.A., I.A. Girard, and T.K. Brown. 1999. Energetics of free-ranging mammals, reptiles, and birds. *Annu Rev Nutr* 19:247–277.
- Nagy K.A., G.L. Kooyman, and P.J. Ponganis. 2001. Energetic cost of foraging in free-diving emperor penguins. *Physiol Biochem Zool* 74:541–547.
- Neill W.H. and E.D. Stevens. 1974. Thermal inertia versus thermoregulation in “warm” turtles and tunas. *Science* 184:1008–1010.
- Nowicki S.N., I. Stirling, and B. Sjare. 1997. Duration of stereotyped underwater vocal displays by male Atlantic walrus in relation to aerobic dive limit. *Mar Mamm Sci* 13:566–575.
- Paladino F.V., M.P. O'Connor, and J.R. Spotila. 1990. Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature* 344:858–860.
- Paladino F.V., J.R. Spotila, M.P. O'Connor, and R.E. Gatten. 1996. Respiratory physiology of adult leatherback turtles (*Dermochelys coriacea*) while nesting on land. *Chelonian Conserv Biol* 2:223–229.
- Ponganis P.J., G.L. Kooyman, L.M. Winter, and L.N. Starke. 1997. Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions, *Zalophus californianus*. *J Comp Physiol B* 167:9–16.
- Reina R.D., K.J. Abernathy, G.J. Marshall, and J.R. Spotila. 2005. Respiratory frequency, dive behaviour and social interactions of leatherback turtles, *Dermochelys coriacea*, during the inter-nesting interval. *J Exp Mar Biol Ecol* 316:1–16.
- Schmidt-Nielsen K. 1997. *Animal Physiology*. Cambridge University Press, Cambridge.
- Schreer J.F., K.M. Kovacs, and R.J.O.H. Hines. 2001. Comparative diving patterns of pinnipeds and seabirds. *Ecol Monogr* 71:137–162.
- Sepulveda C.A., K.A. Dickson, and J.B. Graham. 2003. Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative of the tunas (family Scombridae). I. Energetics. *J Exp Biol* 206:2739–2748.
- Southwood A.L., R.D. Andrews, M.E. Lutcavage, F.V. Paladino, N.H. West, R.H. George, and D.R. Jones. 1999. Heart rates and diving behaviour of leatherback sea turtles in the eastern Pacific Ocean. *J Exp Biol* 202:1115–1125.
- Speakman J.R. 1997. *Doubly Labelled Water: Theory and Practice*. Chapman & Hall, London.
- Speakman J.R., G.H. Visser, S. Ward, and E. Król. 2001. The isotope dilution method for the evaluation of body composition. Pp. 56–98 in J.R. Speakman, ed. *Body Composition Analysis of Animals: A Handbook of Non-destructive Methods*. Cambridge University Press, New York.
- Spotila J.R., R.D. Reina, A.C. Steyermark, P.T. Plotkin, and F.V. Paladino. 2000. Pacific leatherback turtles face extinction. *Nature* 405:529–530.
- Thompson D. and M.A. Fedak. 1993. Cardiac responses of grey seals during diving at sea. *J Exp Biol* 174:139–164.
- . 2001. How long should a dive last? a simple model of foraging decisions by breath-hold divers in a patchy environment. *Anim Behav* 61:287–296.
- Wallace B.P., C.L. Williams, F.V. Paladino, S.J. Morreale, R.T. Lindstrom, and J.R. Spotila. 2005. Bioenergetics and diving activity of inter-nesting leatherback turtles *Dermochelys coriacea* at Parque Nacional Marion Las Baulas, Costa Rica. *J Exp Biol* 208:3873–3884.
- Williams T.M., J. Haun, R.W. Davis, L.A. Fuiman, and S. Kohin. 2001. A killer appetite: metabolic consequences of carnivory in marine mammals. *Comp Biochem Physiol A* 129:785–796.
- Williams T.M., J.E. Haun, and W.A. Friedl. 1999. The diving physiology of bottlenose dolphins (*Tursiops truncatus*). I. Balancing the demands of exercise for energy conservation at depth. *J Exp Biol* 202:2739–2748.