

Allometric scaling of lung volume and its consequences for marine turtle diving performance

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Abstract

Marine turtle lungs have multiple functions including respiration, oxygen storage and buoyancy regulation, so lung size is an important indicator of dive performance. We determined maximum lung volumes (V_L) for 30 individuals from three species (*Caretta caretta* $n=13$; *Eretmochelys imbricata* $n=12$; *Natator depressus* $n=5$) across a range of body masses (M_b): 0.9 to 46 kg. V_L was 114 ml kg^{-1} and increased with M_b with a scaling factor of 0.92. Based on these values for V_L we demonstrated that diving capacities (assessed via aerobic dive limits) of marine turtles were potentially over-estimated when the V_L -body mass effect was not considered (by 10 to 20% for 5 to 25 kg turtles and by >20% for turtles ≥ 25 kg). While aerobic dive limits scale with an exponent of 0.6, an analysis of average dive durations in free-ranging chelonian marine turtles revealed that dive duration increases with a mass exponent of 0.51, although there was considerable scatter around the regression line. While this highlights the need to determine more parameters that affect the duration-body mass relationship, our results provide a reference point for calculating oxygen storage capacities and air volumes available for buoyancy control.

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1. Introduction

Aquatic animals that breathe air are constrained in their underwater activities by the necessity to return regularly to the surface to re-supply their oxygen stores and discharge accumulated carbon dioxide. The time an individual air breather can spend underwater depends on the amount of oxygen stored in the body (respiratory system, blood, muscles), the rate at which oxygen is consumed, and, if necessary, any anaerobic capacity (Kooyman, 1989). If the magnitude of these physiological parameters is known, the performance of diving animals can be evaluated and predicted (Ponganis et al., 1993; Carbone et al., 1996).

The lungs can potentially store a large amount of oxygen to diving animals, but this store may be rendered largely unavailable when lungs collapse during deep dives or due to restricted

perfusion of the lung tissue (Burggren, 1988). In fact, most diving mammals have similar lung sizes to terrestrial mammals, but aquatic species have an increased capacity to store oxygen elsewhere via increased blood volume and higher haemoglobin and myoglobin concentrations (Snyder, 1983). Another considerable problem for an animal that dives with air in its lungs is the extra effort and energy required to overcome the resulting positive buoyancy during descent. For example, diving ducks (*Aythya* spp.), must work harder against buoyancy caused by air in the respiratory tract and trapped in the plumage than against drag, leading to the conclusion that they may never become negatively buoyant at their typical diving depths (Lovvorn et al., 1991).

Although there are some general differences in the distribution and efficiency of oxygen-storing tissues among diving mammals, birds and reptiles, the degree to which air is made available in the lungs is not determined a priori by phylogeny; rather, it appears that diving behaviour and the depth range used by a given species determine this distribution (Kooyman, 1989; Boyd, 1997). For

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this reason, Snyder (1983) differentiated between long-diving whales and seals that exhale before submerging, versus short-diving dolphins and porpoises that dive on inspiration. Diving reptiles demonstrate another oxygen-storing strategy by relying primarily on the lungs which they access via intermittent perfusion. Increased blood and tissue oxygen storage capacity has been reported only for the leatherback turtle (*Dermochelys coriacea*), which is the deepest-diving and only extant soft-shelled marine turtle species (Lutcavage et al., 1992).

A wide range of tidal lung volumes have been determined for marine turtles, spanning 4 ml kg⁻¹ to 187 ml kg⁻¹ across species (for a review see Lutcavage et al., 1997). However, the use of different methods and variability in body size and position make these volumes difficult to compare. Maximum lung capacities of 7.9 and 8.9% of body mass were reported for two loggerhead turtles (*Caretta caretta*), which can hold up to 72% of the total oxygen store (Lutz and Bentley, 1985). Using their data for maximum lung volume and some published data regarding O₂ saturation of inspired air and circulatory system, blood volume and myoglobin concentration, Lutz and Bentley (1985) calculated a total oxygen store of 22.2 ml kg⁻¹ for the loggerhead turtle. The total oxygen store is often used to calculate the time that a diving animal can stay underwater consuming all its oxygen reserves. Originally, dive performance was assessed by comparing dive durations to the aerobic dive limit (ADL), i.e., the time spent in submersion before a rise in blood lactic acid above baseline values occurs (Kooyman et al., 1980). Since this is difficult to measure, particularly in freely diving animals, the ADL is often calculated (cADL) by the ratio of the total amount of oxygen stored in the body to the diving metabolic rate (Ponganis et al., 1993; Schreer et al., 1997; Costa et al., 2001). Such calculations have formed the basis for interpreting the diving behaviour of diverse species. For marine turtles, some authors have calculated aerobic dive limits of 33 min (Lutz and Bentley, 1985) and of 63 to 427 min (Hochscheid et al., 2005) in loggerhead turtles, and of 5 to 70 min (Lutcavage et al., 1992), 11.7 to 44.3 min (Wallace et al., 2005) and 19.2 to 48.1 min (Bradshaw et al., 2007) in leatherback turtles.

However, many of the values used in the calculations above were given as mass-specific values or percentages of body mass in the original literature. Since the use of percentages and mass-specific indices to present physiological data does not control for effects of body size on the measured parameter, it is not known if there are also ontogenetic changes in the lung capacity or other oxygen storing compartments (Packard et al., 1999). To address this problem, we measured maximum lung volumes for a range of different-sized marine turtles to establish the scaling exponent of the lung volume-body mass relationship within this taxon. We then used measured lung volume data to predict the oxygen-storing capacity of marine turtles of various sizes, and examined the consequences for the assessment of diving performance.

2. Methods

2.1. Maximum lung volume

Maximum lung volumes (V_L) were determined during dissections of deceased loggerhead, flatback (*Natator depress-*

sus) and hawksbill (*Eretmochelys imbricata*) turtles using a method modified from Lutz and Bentley (1985) which involved filling the lungs with a known volume of water (as described below). In May 2005 we acquired 13 hawksbill turtles, with body masses ranging between 1.5 and 46.0 kg, from a frozen storage facility (Crocodylus Park, Darwin, Northern Territory, Australia). The smaller individuals were from two cohorts collected as eggs from Groote Eylandt in the Gulf of Carpentaria, Australia in late 2002 and incubated to hatching at Crocodylus Park as a part of another study examining growth trends and aging techniques. Two larger individuals collected several years earlier that had died in captivity had been frozen since their death. We also had access to five flatback turtles (2 to 13 kg) that had also been housed at the same park and had subsequently died (and again, frozen). The dissections of 18 loggerhead turtles, ranging in body mass between 0.9 and 45.5 kg, were made at the Stazione Zoologica Anton Dohrn in Naples (Italy), where stranded dead specimens collected from the Campania region regularly undergo necropsy to determine possible causes of death. Only fresh carcasses were chosen for the lung volume measurements, and dissections were usually made on the day the carcass was found and brought to the facility in Naples. Lung volumes of turtles for which apparent lung pathologies were detected during the necropsy were not measured.

Frozen specimens were thawed for a period of 12–36 h depending on size and then weighed to the nearest 0.1 kg with a 10- or 100-kg Pesola balance (Pesola AG, Switzerland). In Naples, we weighed loggerheads to the nearest 0.01 kg with a digital crane scale (model MCW60-HD, Tamagnini, Parma, Italy). The dead turtles were placed on their carapace and opened from the ventral side by removal of the plastron (Wyneken, 2001). The gastrointestinal tract, heart and liver were removed to expose the lungs fully so that these could be examined and observed during the filling process. We refrained from removing the lungs completely as was done by Lutz and Bentley (1985) because there was a high risk of damaging the lung tissue which would have compromised filling. Although such volumetric measurements are better done with the lungs submerged in water to provide equal filling pressures for all lung sizes, the in-water method is somewhat problematic in that it is more difficult to detect leaks. Heatwole and Seymour (1975) found no consistent difference between lung volumes of sea snakes (e.g. *Aipysurus* spp.; *Hydrophis* spp.) obtained from either in-air or in-water measurements. Therefore, we followed the authors' recommendations and used only lungs in good condition where the tissue was intact and lung's surface tension reasonably taut when filled with liquid.

We cut the trachea just caudal to the neck region well above the bronchial bifurcation. The oesophagus was passed through the bronchial bifurcation to assist visual inspection of the filling process. A measuring cylinder was filled with a known quantity of freshwater and coloured with blue dye. Colouring the water (with food dye) allowed us to detect leaks and to determine maximum fill capacity. Next, a plastic tube was inserted into the open end of the trachea and a clamp was placed over one bronchus to permit the filling of each lung independently. A funnel was then placed over the distal end of the tube and the

coloured fluid was poured gently into the funnel (one person holding the tube and funnel, and a second person maintaining the seal between the tube end and trachea and monitoring the filling process).

The lung was filled to its maximum volume when water no longer descended into the lung and either remained in the bronchus or ascended into the trachea. The lungs were usually collapsed at the beginning of the filling process, but in case some air was trapped, it was slowly replaced by the water (which could be verified by air bubbles ascending into the trachea). In such cases the lung filling was always terminated when there was no more formation of air bubbles. For greater accuracy we withdrew the water from the trachea/bronchi using a syringe and returned this water to the measuring cylinder before determining the total volume held by the lung. A clamp was placed onto the first bronchus and the procedure was repeated for the second lung. The two volumes were then combined to provide an estimate of total lung volume. However, there were a few occasions when this delicate and precise dissection procedure resulted in one lung being damaged (usually small cuts or tears), thus precluding an accurate volume measurement. See below for how we estimated total lung volume in individuals for which only one lung's volume was measured.

2.2. Analysis

For some individuals only one lung volume could be measured, so we first tested whether there was a bias in lung volume between sides to estimate total lung volume in those individuals with only one measurement. We applied a series of five generalized linear mixed-effects models (GLMM) to the data (after removing all individuals with only one lung volume measurement), coding \log_{10} (single) lung volume as the response variable, and *side*, *species*, and the *side*species* interaction as fixed effects. The term *individual* was coded as a random effect to account for repeated measurements (right and left) per individual. Models were contrasted using an index of Kullback–Leibler (K–L) information loss which assigns relative strengths of evidence to each model (Burnham and Anderson, 2002). We used Akaike's Information Criterion corrected for small sample sizes (AIC_c) because of our small sample ($n=30$). AIC_c provides a measure of model parsimony to identify those model(s) from a set of candidate models that minimize K–L information loss (Burnham and Anderson, 2004), with the relative likelihoods of candidate models assessed using AIC_c weights (Burnham and Anderson, 2002). Thus, the weight ($wAIC_c$) of any particular model varies from 0 (no support) to 1 (complete support) relative to the entire model set. Model goodness-of-fit was assessed by calculating the per cent deviance explained (%DE) by a model relative to the null.

We considered two procedures for estimating the relationship between lung volume and body mass: (1) a simple least-squares linear regression of the \log_{10} -transformed data. The relative evidence for a non-negative slope model was tested by contrasting it to the null (intercept-only) model using AIC_c , with the relative evidence ratio calculated as the $wAIC_c$ of the slope model $\div wAIC_c$ of the intercept-only model. Model goodness-

of-fit was calculated using the least-squares r^2 value. We expanded on this approach to test for any hypothesized differences in the volume-mass relationship among the three species investigated. To this end, we constructed another model set (three linear models) considering the *mass*, *species*, and *mass*species* terms (response=lung volume). *Mass* and *volume* variables were again \log_{10} -transformed. Models were contrasted using the information-theoretic AIC_c as described above.

(2) Although the most-commonly used regression technique employs least squares to minimize the squared vertical deviations from the fitted regression line, there are many cases when there is likely to be some error contained in both the x and the y variables; indeed, such variation is common in biological data. For these situations, Model II regression (also known as 'reduced major axis' or 'geometric mean' regression; Sokal and Rohlf, 1981) is preferred (Riggs et al., 1978; Webb et al., 1981; Harvey and Pagel, 1991) because least-squares regression tends to underestimate the true slope of the regression line. We used a randomised bootstrap procedure (10,000 iterations with replacement) to select \log_{10} lung volume– \log_{10} mass pairs from the dataset, and then calculated the geometric mean regression slope as the square root of the sum of squared differences of $y \div$ the sum of squared differences of x (Sokal and Rohlf, 1981) for each iteration. The distribution of slopes was used to determine the probability that the true slope of the relationship was equal to 1 (perfect allometry). A slope <1 indicates that lung volume is relatively smaller in larger turtles, and a slope >1 indicates that lung volume is relatively larger in larger turtles.

2.3. Evaluation of dive performance

We estimated the effect of the allometric slope constant being <1 on turtle dive performance by calculating aerobic dive limits (cADL) for a range of turtle masses (from 5 to 100 kg) based on previous work (see Hochscheid et al., 2005). Briefly, we first calculated the expected metabolic rate (\dot{V}_{O_2}) at 25 °C (given the temperature-dependency of marine turtle metabolic rates – Hochscheid et al., 2005) for this mass range as:

$$\log_e \dot{V}_{O_2} = -2.87 + 0.168 T_w + 0.035 \log_e M_b$$

based on data for loggerhead turtles (Hochscheid et al., 2005). The mass-specific oxygen capacity (lung O_2) of the lungs was then estimated as:

$$\text{lung } O_2 = 0.174(113.6 \cdot M_b^{\text{slope}})$$

where 0.174 is the proportion of the inspired air that is oxygen at the start of a dive (Berkson, 1966), and slope was set to 1 or the value we estimated <1 (see Results). These values were added to the estimated tissue (blood and muscles) oxygen content using the value of 6.7 ml O_2 kg^{-1} for marine turtles (Lutz and Bentley, 1985) to provide total body oxygen content (T_{O_2}). Finally, cADL was calculated as:

$$cADL = \frac{T_{O_2}}{\dot{V}_{O_2}}$$

We evaluated the relationship between both estimates of cADL and body mass using least-squares regression as described above.

To evaluate the effect of body mass on marine turtle diving performance, we compiled data from previous studies that had recorded the diving behaviour of free-ranging marine turtles. Data were selected from papers that gave information on average duration and body mass or carapace length for individual turtles (a summary of these data and references is given in Table 1). Leatherback turtles were excluded from the analysis because they have much shorter dive durations than similar-sized hard-shelled marine turtles. Because of the temperature dependence of dive duration, only those data describing dives occurring in waters between 22 and 30 °C were selected or if not specified, when turtles were studied in a summer neritic habitat (e.g., nesting areas). Temperatures below this range can cause large depressions in metabolic rate, giving rise to long dive durations (Hochscheid et al., 2005). When body masses were not given, turtle lengths (usually given as curved carapace or straight carapace length) were taken instead and body masses were calculated from known mass-length relationships (Hays et al. (2000) for green turtles). We tested for a relationship between log-transformed average dive duration and mass using simple least-squares and Model II regression as described above.

3. Results

3.1. Body mass and lung volumes

Lung anomalies or diseases were detected in six turtles (5 loggerhead and 1 hawksbill) during dissection, so that lung

Table 1
Average dive duration of four species of marine turtle measured via time-depth recorders or satellite telemetry

Species	<i>n</i>	Average duration (min)	M_b range (kg)	Reference
<i>Caretta caretta</i>	2	29.4	50–60 ^a	Houghton et al. (2002)
	6	26.1	62.5–82	Minamikawa et al. (2000)
	1	53.6	92.6	Minamikawa et al. (1997)
<i>Chelonia mydas</i>	1	24.1	52	Hochscheid et al. (2005)
	2	10.2	63–86 ^b	Hochscheid et al. (1999)
	2	32.3	150.6–236.5	Hays et al. (2000)
	6	33.3	141–205 ^b	Hays et al. (2004)
	33	1.4	0.035–0.07 ^c	Salmon et al. (2004)
	2	24.9	12.3–24	Brill et al. (1995)
	6	13.1	11.6–21.6	Southwood et al. (2003)
<i>Eretmochelys imbricata</i>	4	19.2	3.7–17.2	van Dam and Diez (1996)
	3	67.5	72.5–80.5	Storch et al. (2005)
<i>Lepidochelys olivacea</i>	1	49.8	39	Beavers and Cassano (1996)

Water temperatures in these studies ranged from 22 to 30 °C. *n*=number of turtles used in the corresponding study.

^a Estimated by J.D. Houghton, personal communication.

^b Estimated using the equation: M_b (kg) = $-349.22 + 4.585$ CCL (cm) which describes the relationship between body mass and curved carapace length (CCL) established for *C. mydas* by Hays et al. (2000).

^c Only mean M_b of different age groups was given.

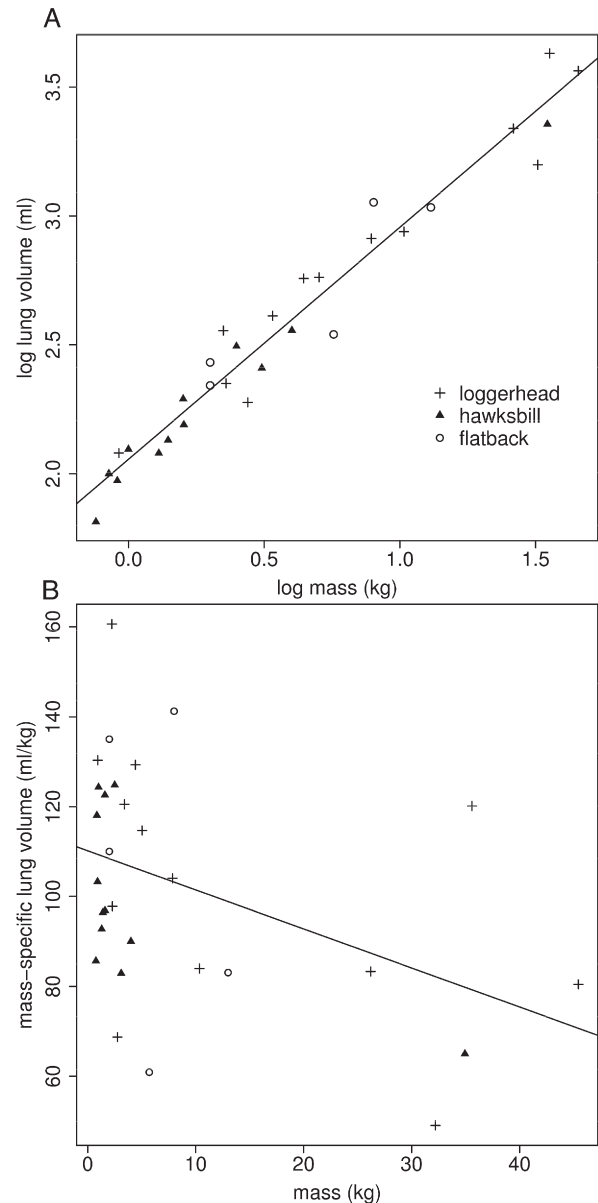


Fig. 1. Relationship between (A) log-transformed body mass (M_b) and absolute lung volume (V_L) and (B) body mass and mass-specific lung volume, observed for 30 hard-shelled sea turtles from three genera. Key to species is given in the figure legend. Data for lung volume presented in panel A were bias-corrected (see main text for more information on how the correction was done). The least-squares lines of best fit were: (A) $\hat{y} = 2.0552 + 0.9008x$; $r^2 = 0.9531$ and (B) $\hat{y} = 110.1531 - 0.8689x$; $r^2 = 0.1410$. Least-squares r^2 values and lines of best fit equations do not take into account uncertainty (variation) in the *x*-axis values as does Model II regression (see Methods), so they are only approximate.

volumes could not be measured for these individuals. Therefore, we obtained the lung volume for a total of 30 individuals (12 hawksbills, 5 flatbacks and 13 loggerheads). For these turtles, mean overall body mass was 9.7 kg (range 0.76–45.5 kg) and the mean body mass for each species was 4.5 kg for hawksbills (range: 0.76–34.9 kg), 6.1 kg for flatbacks (range: 2.0–13.0 kg), and 13.8 kg for loggerheads (range: 0.9–45.5 kg). Total lung volumes ranged from 0.033 to 2.140 l.

There were some evidence for a bias in lung volume between sides: the most parsimonious generalized linear mixed-effects

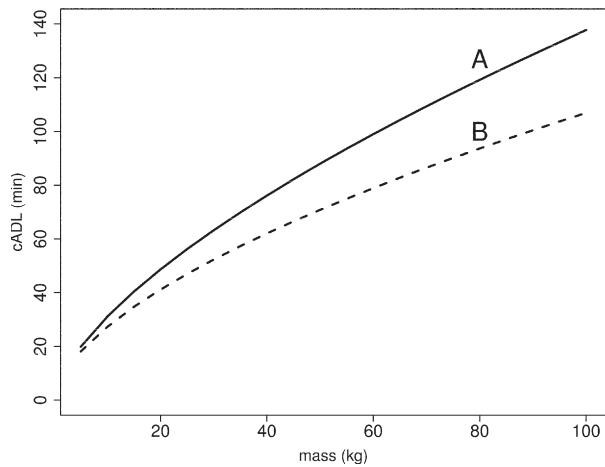


Fig. 2. The relationship between body mass and calculated aerobic dive limits (cADL) in hard-shelled marine turtles at a temperature of 25 °C. A: fitted curve with V_L -mass slope = 1; B: fitted curve with V_L -mass slope of 0.923.

model included only the *species* term ($wAIC_c=0.64$; %DE = 13.4%), with the model including the *side* and *species* terms following with $wAIC_c=0.25$ (%DE = 14.5%). The effect of *species* was expected given that we tended to sample relatively larger loggerhead turtles compared to the other two species. The *side* term by itself explained 1.1% of the deviance in volume, with the right-side measurements being on average 7.2% larger than the left-side measurements ($\bar{x}_r=446$ ml; $\bar{x}_l=416$ ml; based only on turtles with both sides measured). There was little evidence for an interaction effect ($wAIC_c=0.04$), suggesting that there was no species specificity in the bias. Therefore, we considered two subsequent estimates of total lung volume for those missing one or the other lung volume measurement: (1) the first dataset ignored the weak possible bias by simply doubling the lung volume that was measured and (2) the second dataset was corrected for this possible bias by multiplying left lung volumes by 1.072 and adding those values to the measured value for the left side, or dividing the right lung volumes by 1.072 and adding those values to the measured value for the right side. All subsequent results are presented for both datasets.

The least-squares linear regression revealed a strong linear relationship for the bias-uncorrected data (AIC_c evidence ratio [ER] = 8.1×10^{19} , $r^2=0.955$) and a slope of $0.904 \pm SE=0.029$). For the bias-corrected data, the relationship provided a slope of $0.901 \pm SE=0.037$; $r^2=0.953$; ER = 4.2×10^{19}) (Fig. 1). The test for species-specific relationship between volume and mass revealed little support for the model containing the *species* * *mass* interaction for both uncorrected and corrected datasets (uncorrected and corrected: $wAIC_c=0.008$), and there was <0.5% increase in the %DE with the addition of the *species* or interaction term. We conclude therefore that there was no evidence for a species bias in the volume-mass relationship.

With this result in hand, we estimated the range of the Model II regression slope to account for variance in both x and y variables and to determine the amount of bias in the estimated allometric scaling exponent brought about by relying solely on least-squares regression. For the uncorrected dataset, the mean slope was estimated as 0.925 (95% bootstrapped confidence

interval: 0.852 to 1.002). The probability that the slope was ≥ 1 according to the bootstrapped distribution was 0.028. For the bias-corrected dataset, the mean slope was 0.923 (95% bootstrapped confidence intervals: 0.848 to 0.998), and a probability of being ≥ 1 equal to 0.022). This provides relatively convincing evidence that the true slope was < 1 , indicating that larger turtles have relatively smaller lung volumes (Fig. 1B).

So, the mean bias-corrected Model II slope gives:

$$\log_{10} V_L = 2.055 + 0.923 \times \log_{10} M_b$$

or

$$V_L [\text{ml}] = 113.6 \times M_b^{0.923}$$

3.2. Dive performance

Aerobic dive limits without considering the allometry of lung volume compared to those where the non-unity allometry was taken into account overestimated the diving capabilities of turtles weighing 5 to 25 kg by 10 to 20%, and by >20% for turtles with body masses of ≥ 25 kg (Fig. 2). Ignoring or including the allometric scaling exponent calculated above resulted in the following relationships between cADL and mass:

$$\text{cADL} = 6.999 M_b^{0.647} \quad (V_L\text{-mass scaling exponent} = 1)$$

$$\text{cADL} = 6.956 M_b^{0.593} \quad (V_L\text{-mass scaling exponent} = 0.923)$$

The least-squares regression indicated strong evidence for a relationship between average dive duration and body mass (ER = 66.4), but there was considerable scatter around the line of best-fit ($r^2=0.24$; Fig. 3). The least-squares regression provided

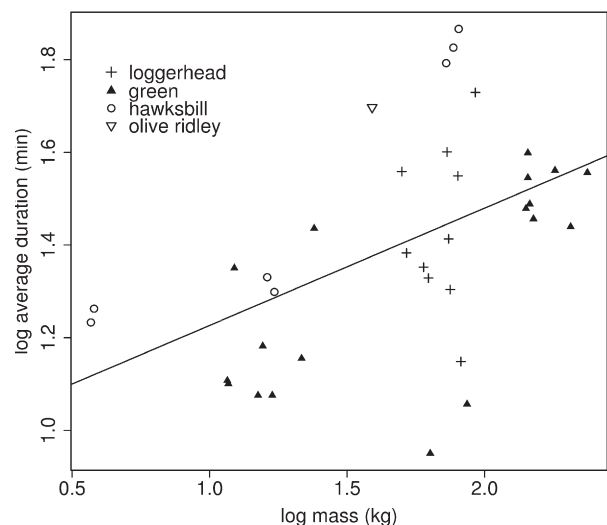


Fig. 3. Average dive duration in relation to body mass for different hard-shelled marine turtle species. The solid line describes the relationship between body mass and mean dive duration for all chelonian marine turtles (see also main text). Mean dive durations, body masses and references for each species are given in Table 1. The least-squares line of best fit is described as $\hat{y} = 0.9733 + 0.2532x$; $r^2=0.2369$. Least-squares r^2 value and line of best fit equation do not take into account uncertainty (variation) in the x -axis values as does Model II regression (see Methods), so they are only approximate.

a scaling exponent of 0.253, but given the error in both x and y variables, we applied the bootstrap Model II regression which revealed that the slope of the exponent of the above relationship was larger at 0.505 (95% bootstrapped confidence interval: 0.364 to 0.683).

4. Discussion

We are aware of only one other study examining the allometry of lung volumes in reptiles (Tenney and Tenney, 1970) where the calculated cross-species scaling exponent was 0.75. However, Tenney and Tenney's (1970) measurements were based on a few individuals (23) across several taxonomic groups (turtles, lizards and snakes), and the data were considerably more variable. It is therefore possible, that scaling exponents within taxonomic groups may differ from this average 3/4 exponent. Our data for marine turtles show that with a scaling factor of 0.92, ontogenetic increases in lung volume are only slightly inferior to the concomitant increases in body mass.

Maximum lung volumes were slightly larger than previous estimates for marine turtles which range from 0.094 to 0.12 l kg⁻¹ (thus 9.4 to 12% of M_b) for *C. caretta* (Lutz and Bentley, 1985) and *C. mydas* (Berkson, 1966; Tenney et al., 1974), respectively, but they are still lower mass-specific lung volumes than those calculated for other reptiles that have volumes of approximately 20% of body mass (Tenney and Tenney, 1970). Within the turtles only (including terrestrial and freshwater turtles), marine turtles have the lowest mass-specific lung volumes (Patterson, 1973). On the other hand, sea snake lungs can occupy at least 19% of the body and even up to 58% in *Pelamis platurus* (Heatwole and Seymour, 1975). These large volumes may be advantageous for snakes that float at the water surface head down while scanning below for prey (Heatwole and Seymour, 1975). Although marine turtle lungs can hold up to 72% of the oxygen needed during diving (Lutz and Bentley, 1985), they do not increase relative lung volume to increase oxygen stores. This does not appear to be a general trait in diving mammals either because lung volumes are comparable among terrestrial and aquatic species (Snyder, 1983).

Why then do marine turtles have smaller relative lungs than their freshwater and terrestrial relatives, even though the former rely on their lungs as oxygen store for diving? One hypothesis is that smaller lungs can reduce buoyancy, thereby lowering the energy consumed during the descent phase of a dive or during bottom-feeding while diving shallowly in neritic habitats (for studies on buoyancy, diving behaviour and energetic costs see Wilson et al., 1992; Skrovan et al., 1999; Sato et al., 2002; Hays et al., 2007). Additionally, marine turtles have an enlarged respiratory surface provided by secondary bronchi, so the reduction in total lung volume is not necessarily disadvantageous for gas exchange. On the other hand, marine turtle lungs can still hold enough air, and hence oxygen, to provide them with respiratory autonomy for dives lasting up to several hours (Hochscheid et al., 2005), albeit as a result of depressed metabolic rate in low-temperature environments.

Our results also demonstrate that a single mass-specific lung volume cannot be applied across a range of body sizes. By

determining the allometric scaling component of lung volume in this taxon, we have highlighted an important aspect of models that attempt to estimate oxygen limitation to diving in marine turtles. Neither did we find evidence that mass-specific lung volumes varied among the three species studied. This suggests either that the lung volume-mass relationship we observed is a product of the co-evolutionary constraints imposed by the marine environment, with hard-shelled turtles appearing to have adapted similarly across taxa. Alternatively, this observation may result from the lungs of marine turtles acting as both the major oxygen store and buoyancy organ, or simply that any subtle differences among species with respect to this relationship may require far greater sample sizes to discern the small potential effect size.

The slight deviation from isometric growth of the lungs may also be explained in part by the different ecological and behavioural characteristics of the oceanic juvenile and neritic adult life stages of marine turtles (Bolten, 2003). Generally speaking, post-hatchling and young immature turtles spend their first years of life in the pelagic zone (Bolten, 2003) (but not in flatbacks, see Walker and Parmenter, 1990), where a proportionally larger lung may provide enough buoyancy for the young to rest and breathe at the surface without the need for active swimming. As a point of comparison, other marine animals such as sea otters (*Enhydra lutra*) and sea snakes (*Pelamis platurus*) have oversized lungs to aid buoyancy (Tarasoff and Kooyman, 1973; Graham et al., 1975). Apart from this, small marine turtles also consume much more oxygen per unit mass than larger individuals (Prange and Jackson, 1976; Hochscheid et al., 2005), and hence, an increased oxygen reservoir in the lungs may be an advantage to offset this extra metabolic demand.

Although the relative difference in lung size between smaller and larger individuals appears small, estimates of aerobic dive limits indicate that this allometry has nonetheless important consequences for the evaluation of dive performance in marine turtles (Fig. 2). We did not account for a body mass effect on muscle mass and haemoglobin concentrations, but these appear to be independent of size (Schmidt-Nielsen, 1984) and are more likely to vary with individual body condition and health status. However, it might be worth investigating the scaling of blood and tissue oxygen stores in a future study to see if there has been some sort of trade-off between lung and blood/tissue stores. Regardless of the outcome of such a study, it appears that turtle metabolic rate scales in general with a lower exponent than oxygen stores, causing a consequent increase in dive duration with body size. Accordingly, the calculated aerobic dive limit, which is affected by the scaling of both metabolic rate and body oxygen store, increased with body mass with a mean scaling exponent of approximately 0.6.

As for any possible limitations or biases associated with the particular methods we employed to estimate lung volume, we concede that a water proxy for total gaseous lung volume may not necessarily emulate true physiological conditions. However, we filled the lungs while they were still attached to the carapace in the supine position so that the water distended the lungs only towards the ventral (hence, upwards) and lateral sides. We did not observe any

unnatural distortions of the lungs during the filling process as reported by Lutz and Bentley (1985), so we are confident that the lungs were filled equally and to maximum holding capacity irrespective of turtle size. As such, our relative allometric comparisons should remain intact even if a small systematic bias did result.

From our results we predict that if dive duration in free-ranging turtles is predominantly influenced by the same parameters used to calculate aerobic dive limits, then duration also scales with a mean exponent of approximately 0.6. In their extended review on the diving capacity of air-breathing vertebrates, Schreer and Kovacs (1997) had too few data to calculate allometric equations for dive durations in marine turtles; however, there has been a recent proliferation of studies using time-depth recorders to measure dive parameters of turtles in their natural habitat. Re-examination of empirical data for average dive durations available in the literature revealed that dive duration increases with body mass at an exponent of 0.505. There is obviously considerable scatter around the fitted line because there are various other parameters such as dive depth, temperature, and activity level in addition to body mass that affect aerobic diving capacity. Hence, the large 95% confidence interval suggests that the scaling exponent can equally be anywhere between 0.364 and 0.683. Turtles appear to conform to the general paradigm among endothermic divers of increased dive performance in larger individuals (Schreer and Kovacs, 1997), but the increase in dive duration is not, as we predicted, directly proportional to increases in body mass. The prevailing dataset needs to be complemented, as soon as more data on dive durations become available, to narrow the confidence interval of the scaling exponent and determine with more certainty whether dive duration scales as predicted by our cADL-mass relationship.

In conclusion, we have shown that lung volume does not increase proportionally to body mass in marine turtles; consequently, previous predictions based on proportional lung volumes for the calculation of oxygen stores may over-estimate diving capacity in this taxon. It still has to be evaluated whether the relatively smaller lung sizes of larger individuals pose any constraints on their diving behaviour. It is probable that most dives are made with the lungs at only a proportion of maximum lung capacity, which means that practically speaking, aerobic dive 'limits' are shorter than the theoretical values considered here. Indeed, many air-breathing divers modify the pre-dive inspiration volume in anticipation of particular behaviours (e.g., Wilson, 2003; Hays et al., 2004). Our results therefore apply to the upper physiological limit of diving performance based on maximum lung volumes (cf. Bradshaw et al., 2007). To date, the dive performance of free-ranging marine turtles does not seem to correspond to volumetric limitations of their major oxygen storage organ, but this can only be further addressed once true lung volumes achieved during diving are measured reliably. Nonetheless, our results provide a useful reference point for maximum oxygen storage capacities as well as for the maximum depth range over which marine turtles of various sizes can regulate their buoyancy via the volume of inspired air in the lungs.

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