

The dual function of the lung in chelonian sea turtles: buoyancy control and oxygen storage

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Abstract

Chelonian sea turtles use their lung as a buoyancy organ and as the major oxygen store when diving, and hence, buoyancy regulation and oxygen consumption can be expected to interact. The buoyancy of seven juvenile loggerhead turtles, *Caretta caretta*, was determined by measuring directly their underwater weight (M_{uw}) while they were resting on a freely suspended weighing platform at a depth of 80 cm. Underwater weight was recorded continuously for 2 days for each turtle, followed by another 2 days measurements during which the turtles carried lead weights attached to their carapace, and finally, a last day of measurement after the weights had been removed. Total duration of resting dives (t_r), buoyancy (F_B), total resulting force acting on the resting turtle (F_{res}) and body volumes were derived from the M_{uw} data. Turtles were slightly negatively buoyant when resting ($F_{res} = -0.2943$ to -0.981 N kg⁻¹) and M_{uw} increased significantly throughout each apnoeic period, meaning that the turtles progressively lost buoyancy. Pulmonary gas loss was calculated from the rate at which buoyancy decreased, which was significantly slower during the first half of the dive than during the second half of the dive. Resting oxygen consumption rates (V_{O_2}) were calculated from these data assuming that the pulmonary gas loss represents oxygen consumption from the lung. The V_{O_2} obtained in this way (1.69–4.86 ml O₂ min⁻¹) corresponded well with other published and V_{O_2} measured previously on loggerhead turtles in the same facility. Using oxygen from the lung affects buoyancy and may have impacts on the diving behaviour.

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Abbreviations: F_B , buoyancy force; M_b , body mass; M_{uw} , underwater weight; M_{uw_0} , M_{uw} at beginning of dive; t_r , resting dive time; T_w , water temperature; V_{O_2} , oxygen consumption rate; ΔM_{uw} , difference between M_{uw} at the beginning and M_{uw} at the end of the dive.

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1. Introduction

When air-breathing animals dive, they have some air enclosed in the respiratory system and/or entrapped in the plumage or fur (Wilson et al., 1992; Fish, 2000; Fish et al., 2001). For this reason, they are often positively buoyant at the surface and have to overcome this buoyancy by forceful swimming during the first phase of the descent, particularly when they inhale prior to submergence (Skrovan et al., 1999; Minamikawa et al., 2000; Glen et al., 2001; Sato et al., 2002). As depth increases, the air then becomes compressed and buoyancy is reduced until the animal reaches a depth at which it is neutrally buoyant provided that its body density is higher than that of the surrounding water but see Wilson et al. (1992) for examples of diving animals less dense than water). Beyond this depth, it will sink because the decreasing air volume is no longer sufficient to keep the animal at a constant depth. Animals engaged in travel may utilise the energy devoted towards swimming to also sustain them at depth. However, submerged animals do not always wish to travel and in these cases, they would need to expend energy specifically to maintain their depth of submergence. Therefore, many marine animals have evolved buoyancy aiding or regulating organs to stabilise their position in the water column without additional expenditure of energy (Schmidt-Nielsen, 1990).

For a diving animal, optimisation of energy utilisation is particularly crucial since it performs with a limited amount of oxygen (Boyd, 1997; Costa and Williams, 1999). The time spent moving against gravity and buoyancy forces should be reduced to a minimum, thus saving energy and using less oxygen. However, the reasons for underwater excursions in diving animals often include the acquisition of food. For pelagic feeders, this involves many activities, including searching the water column for suitable prey, chasing and finally catching it (Wilson et al., 1996). Consequently, such an animal moves frequently up and down and cannot spend much time at the depth of neutral buoyancy. However, when an animal's activity is concentrated at a single depth (see, for example, u-shaped dives in Gentoo penguins, *Pygoscelis papua* (Wilson et al., 1996), and cormorants, *Phalacrocorax carbo* (Grémillet D., 1999), type E dives in northern elephant seals, *Mirounga angustirostris* (Le Boeuf et al., 1988) and square-dives in grey seals, *Halichoerus grypus* (Beck et al., 2000)), a neutrally buoyant state at the modal depth would clearly be advantageous. Sea turtles, for example, often rest for extended periods at the same depth, either on the sea floor (van Dam and Diez, 1996; Hochscheid et al., 1999; Hays et al., 2000; Houghton et al., 2002) or, as recently suggested by Minamikawa et al. (2000), in midwater. Chelonian sea turtles are known to regulate their buoyancy via the volume of air in the lungs (Jacobs, 1940; Milsom, 1975; Milsom and Johansen, 1975), which serve at the same time as the major oxygen store during diving (Lutz and Bentley, 1985). The amount of air inhaled prior to submergence determines, therefore, both the depth at which the turtles become neutrally buoyant and how much oxygen is available for that dive. Moreover, it can be expected that the position of neutral buoyancy will decrease throughout a dive due to the utilisation of the lung oxygen store, thus, reducing the volume of pulmonary gas (Minamikawa et al., 2000).

Only recently has it been recognised that the bifunctional characteristics of the sea turtle lung may have important implications for diving behaviour (Hays et al., 2000; Minamikawa et al., 2000). Numerous studies using time-depth recorders have shown common

dive patterns, such as series of u-shaped dive profiles, in various sea turtles species (e.g. loggerhead turtles (Houghton et al., 2002), green turtles, *Chelonia mydas*, (Hochscheid et al., 1999) and hawksbill turtles, *Eretmochelys imbricata* (van Dam and Diez, 1996)). These “u-dives” have been assigned to resting behaviour, although turtles may also feed during the bottom phase of these dives (Hochscheid et al., 1999). Such resting dives occur in bouts of several regular u-dives to approximately the same depth and of similar dive durations (van Dam and Diez, 1996; Hochscheid et al., 1999; Hays et al., 2000). As the depth of ‘resting’ dives increases so does dive duration. However, only about 15% of the increase in dive duration can be explained by the increased distance and time required to travel to the deeper depth. Hays et al. (2000) suggested that the increase in dive duration may be due to an increase in the oxygen store because a turtle diving deeper has to take more air in their lungs to achieve neutral buoyancy.

With this study, we intended, using loggerhead sea turtles as a model, to elucidate whether a relationship between buoyancy regulation, oxygen store and dive behaviour exists and how it can be characterised. To eliminate confounding factors, such as swimming activity and feeding, we collected data from resting and starved turtles only. The underwater weight (M_{uw}) of the turtles was measured with particular reference to three questions: (1) do resting submerged turtles regulate their buoyancy close to neutral? (2) what is the pattern of change in M_{uw} during a dive and does this reflect the rate of oxygen consumption from the lung? (3) If the specific gravity of a turtle is experimentally increased by attaching weights to the carapace the turtle would be expected to elevate its lung volume to compensate. Does this compensation happen and if so does this result in a greater oxygen store and hence an increase in dive times?

2. Material and methods

Seven juvenile loggerhead turtles with a mean body mass (M_b) of 10.8 kg (total range: 8.2–14.5 kg) were housed in the aquarium of the Stazione Zoologica “Anton Dohrn” (Naples, Italy, 40°50'N 14°15'E) during this study. The turtles were kept in individual indoor tanks supplied with circulating seawater, which was pumped directly from the Gulf of Naples. For this reason and because measurements on each individual took at least 1 week, water temperature increased slightly (due to seasonal warming) during the study period giving a total range of 15.4–18.9 °C. However, maximum water temperature (T_w) variations for each individual while under experimentation were only 0.5–1 °C.

2.1. Underwater weighing

All experiments were conducted in the same circular tank (located in the same room as the other housing tanks) which was 1 m high (water level 0.8 m) with an internal diameter of 1.20 m (total volume: 1000 l). Each turtle was transferred into the experimental tank 2 days prior to the onset of the measurements and was fasted during the experiments. The experimental tank was equipped with a free hanging stainless steel platform, suspended into the water at approximately 10 cm above the bottom of the tank. A digital crane scale

(model MCW60-HD, Tamagnini, Parma, Italy, resolution 0.01 kg, accuracy: ± 0.018 kg) was fixed above the tank and the platform was attached to the scale by nylon strings (thickness: 2 mm). The whole apparatus was centred so that it did not touch the walls of the tank. The platform was enclosed with a 20-cm high plastic mesh fence to prevent turtles touching the tank wall while resting on the platform. The crane scale was linked to a desktop computer and a custom made basic program logged each weight change and corresponding time of day when the weight had been stable for at least 5 s. The balance was tared while the turtle was breathing at the water surface prior to the start of the measurements. In this way, the only weight recorded by the balance was the weight of the turtle when it settled down on the platform. Once the balance-computer set-up had been started, it continued measuring for 48 to 60 h depending on the capacity of the battery of the balance.

2.2. Manipulation of specific gravity

The turtles specific gravity was manipulated by attaching lead weights (1.5–3.2% of M_b) to the carapace. These weights were available in approximately 100- and 200-g pieces and the exact underwater weight of each lead weight was determined using the same crane scale which was used to measure the turtles' underwater weights. Velcro was glued to the lead weights and onto the carapace of the turtle using Araldite® Clear fast setting epoxy adhesive (Ciba Specialty Chemicals). In this way, the weights could simply be attached and detached to the turtle by sticking the two velcro counterparts together. The lead weights remained in place for at least 48 h on the turtles during which the underwater weight (M_{uw}) was recorded. After the weights had been removed, M_{uw} was measured for another 24 h to examine whether there was any residual effect on M_{uw} caused by the weights.

2.3. Data analysis

A resting dive was clearly identifiable from the balance readings because soon after breathing, the turtles settled down on the platform and the balance began registering M_{uw} . Observations confirmed that the turtles remained on the platform during all these resting dives, usually motionless. Shortly, before surfacing, the turtles “woke up” which was characterised by them raising their heads and looking at the surface. These slight movements were recorded by the balance (the balance display light switched on) but they did not cause any weight changes. Shortly, after this, the turtles rose straight to the surface and consequently, the reading on the balance display became zero. The time that passed between two consecutive zero readings, when M_{uw} was measured constantly, was taken as the resting dive time (t_r). The first M_{uw} ($M_{uw,0}$) which was registered for a resting dive was taken as a measure of the buoyancy state of the turtles, since it could be assumed to correspond directly to the last breath that the turtle inhaled, thus, determining the lung volume at the resting depth (ca. 80 cm). The buoyancy force (F_B), given by Archimedes' principle, is

$$F_B = \rho g V \quad (1)$$

where ρ is the density of the water, g is gravitational acceleration (9.81 ms^{-2}) and V is the volume of the displaced water, which equalled the mass of the displaced water divided by its density. The mass of the displaced water was calculated from the apparent weight loss of the turtles in water $M_b - M_{uw}$. Thus, the buoyancy force was

$$F_B = g(M_b - M_{uw}) \quad (2)$$

The force acting in the opposite direction to F_B is F_m due to gravity,

$$F_m = gM_b \quad (3)$$

The resulting force (F_{res}) is

$$F_{res} = F_B - F_m \quad (4)$$

whereby negative and positive signs represent downward and upward, respectively, acting F_{res} . Inserting Eqs. (2) and (3) into Eq. (4) gives the resulting force as

$$F_{res} = -gM_{uw} \quad (5)$$

All statistical analysis were performed using the Minitab 13 (Ryan and Joiner, 2000) software package. Relationships between t_r , T_w and M_b , between F_B and M_b and between ΔM_{uw} and t_r were evaluated with least squares regression analysis, and additionally for t_r a General Linear Model (GLM) was performed to evaluate the influence of T_w , M_b and interactions between $T_w \times t_r$ and $T_w \times M_b$.

A correlation matrix of all intervals between successive weight increments did not reveal any evidence of intercorrelation. Therefore, it was assumed that the data were independent and a GLM was performed to test whether the duration of the intervals between weight increments was different and whether there were different responses from individual turtles.

Finally, a GLM was applied including the weight status (weighted versus unweighted), individual and M_{uw_0} as factors to determine which of these affected t_r . Significance was accepted when p values were < 0.05 .

3. Results

3.1. Resting dive duration

All loggerhead turtles were engaged in periods of long resting dives, performed in a regular behavioural pattern. A total of 294 resting dives were recorded for the turtles before they were weight manipulated. Median dive duration for individual turtles ranged from 20 to 76 min and the maximum-recorded dive time was 97 min (Table 1). Turtles, which were under experimentation at the lower range of water temperatures, made significantly longer resting dives than turtles studied at the higher temperatures. We also noted that turtles with larger body masses tended to stay submerged for longer than the turtles with smaller body masses. There were significant

Table 1

Underwater weights (M_{uw}) and resting times (t_r) of submerged loggerhead turtles. Given are median values and the interquartile range in parenthesis

| Turtle no. | M_b (kg) | M_{uw} (kg) | t_r (min) | V (l) | F_B (N) | F_{res} (N) | M_{pb} (g) |
|------------|------------|------------------|---------------------|---------|-----------|---------------|--------------|
| 1 | 9.51 | 0.03 (0.02–0.06) | 48.03 (28.63–52.62) | 9.2 | 93.0 | –0.2943 | 280 |
| 2 | 13.0 | 0.04 (0.03–0.06) | 76.4 (73.78–89.75) | 12.58 | 127.14 | –0.3924 | 410 |
| 3 | 13.9 | 0.1 (0.08–0.14) | 57.02 (30.87–62.8) | 13.4 | 135.38 | –0.981 | 430 |
| 4 | 14.5 | 0.05 (0.02–0.09) | 30.65 (26.3–33.65) | 14.03 | 141.75 | –0.4905 | 290 |
| 5 | 8.4 | 0.03 (0.02–0.04) | 30.9 (25.17–34.33) | 8.13 | 82.11 | –0.2943 | 180 |
| 6 | 8.2 | 0.04 (0.02–0.06) | 19.87 (14.98–27.7) | 7.92 | 80.05 | –0.3924 | 120 |
| 7 | 8.2 | 0.05 (0.04–0.07) | 20.28 (17.32–23.88) | 7.91 | 79.95 | –0.4905 | 120 |

The body volume of the turtles (V) including the air in their lungs was calculated by solving Eq. (1) for V (= volume of displaced water = volume of turtle). Buoyancy force (F_B) and resulting force (F_{res}) were calculated after Eqs. (2) and (5), respectively. All parameters are given for turtles in the unweighted state. M_b = body mass, M_{pb} = mass of attached lead weight during the specific density manipulation experiment.

effects of T_w , M_b and individual on dive duration ($F_{6,289} = 46.67$, $p < 0.001$). We removed the effect of individual by correcting all of a given individuals estimates to the mean across individuals. The relationship between dive duration, water temperature and body mass, once the effect of individual had been removed, was described by the equation

$$t_r = 122 - 6.61 T_w + 2.7 M_b \quad (6)$$

which explained 67% of the variation in t_r (multiple LSR: $F_{2,289} = 291.37$, $p < 0.001$).

3.2. Buoyancy and underwater weight (M_{uw})

The buoyancy forces acting on the totally submerged turtles at the resting depth of the tank were between 80 and 141.8 N (Table 1), depending on the body mass of the turtle, and the overall relationship was:

$$F_B = 9.7607M_b + 0.0706 \quad (7)$$

with a coefficient of determination $r^2 = 0.9999$. The gradient of Eq. (7) was almost equal the gravitational acceleration g of 9.81 ms^{-2} and the constant was close to zero. This implied that the turtles were very close to neutral buoyancy, in which case F_B would be determined only by $g \times M_b$ (for $M_{uw} = 0$).

The submergence behaviour of all turtles occurred in a characteristic and repeated pattern. During breathing episodes, turtles remained without any obvious swimming effort at the surface. Then, they descended with strong flipper beats in an almost vertical position until they approached the platform where they settled slowly down to rest. Sometimes, they hovered for a while closely above the platform before they finally came to rest in one place. At the end of the resting period, the turtles left the platform with some forceful flipper strokes ascended directly to the surface. The underwater weight measurements

revealed that the turtles were slightly negatively buoyant during resting dives with median M_{uw_0} ranging from 0.03 to 0.1 kg and resulting forces between -0.2943 and -0.981 N (Table 1).

M_{uw} of the turtles was not stable, but increased during a resting dive (Fig. 1). The difference ΔM_{uw} between the final M_{uw} and M_{uw_0} increased significantly with dive duration (Fig. 2). This relationship was best described by the equation:

$$\Delta M_{\text{uw}} = 0.194 + 0.033 \ln(t_r) \quad (8)$$

(LSR: $r^2 = 0.713$, $F_{1,289} = 714.47$, $p < 0.001$). Differences between individual $\Delta M_{\text{uw}} - t_r$ relationships were associated with variations in body mass (General Linear Model: $F_{5,289} = 3.73$, $p < 0.01$), but not water temperature (GLM: $F_{1,289} = 2.22$, $p > 0.05$). ΔM_{uw} was also significantly affected by the interaction between body mass and dive duration ($F_{5,289} = 5.62$, $p < 0.001$) and between dive duration and water temperature ($F_{1,289} = 15.97$, $p < 0.001$).

Fig. 1 indicates that the rate of change of M_{uw} was not constant but that longer intervals passed between two consecutive weight changes at the beginning of the dive. We determined for a subsample of dives for each turtle the time that passed from the beginning of the resting dive until the first 10-g increase in M_{uw} occurred, then the time that passed between the first weight increment and the second increment, etc. On average, the first 1 to 3 weight increments occurred after significantly longer intervals in all turtles, thereafter M_{uw} increased in regular, but shorter intervals (GLM: $F_{1,53} = 11.86$, $p < 0.01$; Fig. 3a–g). In all turtles, M_{uw} increased in the same pattern during a submersion (first longer intervals than shorter, equal intervals; GLM: $F_{6,53} = 1.70$, $p = 0.147$), but intervals between M_{uw} increments were different for individual turtles (GLM: $F_{6,53} = 6.34$, $p < 0.001$).

3.3. Pulmonary gas loss

The changes in M_{uw} during a dive can be converted directly into volume of gas that is lost from the lungs. According to Archimedes' principle, a mass of 10 g in seawater are balanced by $10 \text{ g} / 1.03 \text{ g cm}^{-3} = 9.7 \text{ cm}^{-3}$ or 9.7 ml air and hence, each 10-g increment of M_{uw} was caused by an extraction of 9.7 ml gas from the lung. Consequently, an increase in M_{uw} of 100 g (Fig. 1) would have been caused by a reduction in lung volume of 97 ml. It was therefore calculated how much gas was withdrawn from the lung during the dive and at which rate, taking the average intervals between two weight increments from the data presented in Fig. 3a–g. In correspondence with the observation that M_{uw} increased at a slower rate at the beginning of the dive, also gas loss from the lung was slower, whereas in the second half of the apnoeic period lung volume was reduced much faster and in more regular intervals (Fig. 4).

Based on the assumption that the lung volume decrease during the second half of the submergence corresponds to the rate at which oxygen is taken from the lung, oxygen consumption was calculated as 9.709 ml (the equivalent oxygen volume of a 10 g weight increment) corrected to STP and divided by the average interval length between two consecutive 10-g increments (e.g. from the data for turtle no. 2: $9.905 \text{ ml} / 5.7 \text{ min} = 1.74 \text{ ml O}_2 \text{ min}^{-1}$, Figs. 4 and 5). This calculation was performed for each individual turtle

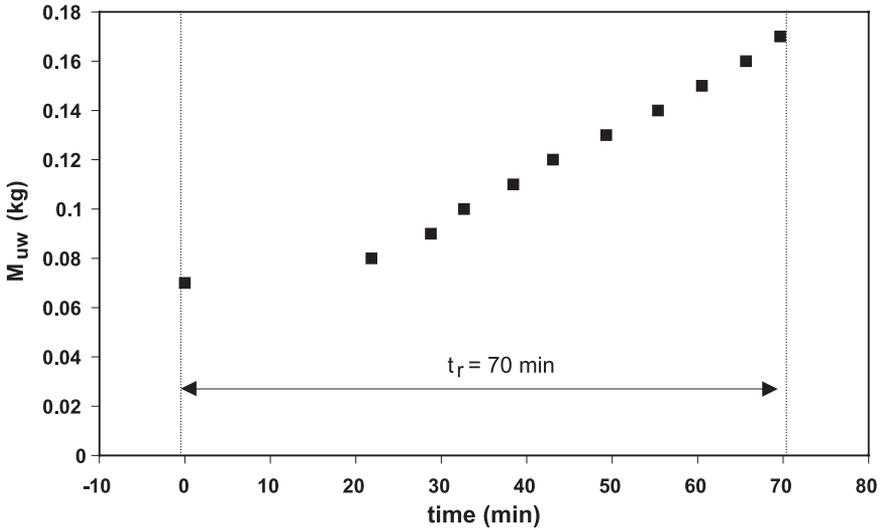


Fig. 1. Changes in the underwater weight (M_{uw}) of a loggerhead turtle while resting on the bottom. Vertical dotted lines indicate beginning and end of the resting dive; t_r =resting time.

and oxygen consumption rates (V_{O_2}) obtained this way were compared to expected V_{O_2} (Fig. 5), which were derived from a known relationship between V_{O_2} , water temperature and body mass (Hochscheid et al., in press).

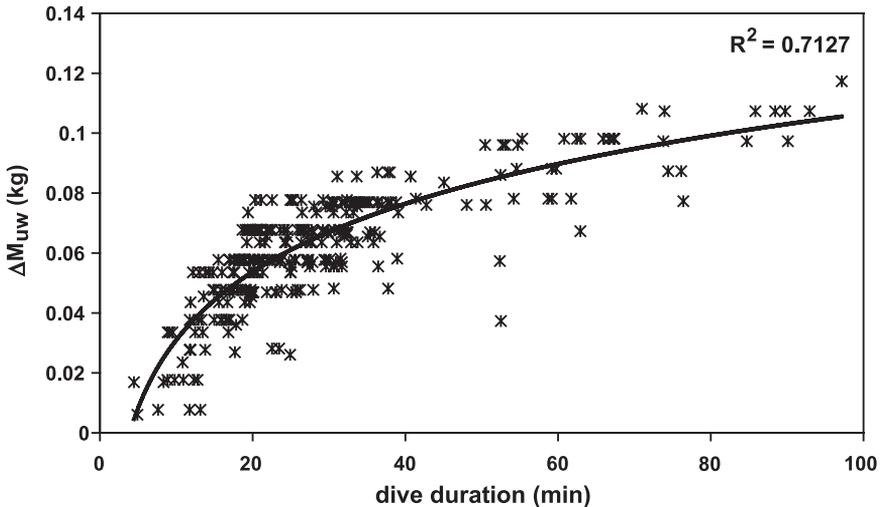


Fig. 2. The relationship between changes in underwater weight (ΔM_{uw} = difference between M_{uw} at the beginning and at the end of the dive) and the resting dive duration (t_r) in loggerhead turtles ($n=7$). The equation of the fitted curve was: $\Delta M_{uw} = 0.194 + 0.033 \ln(t_r)$; see text for more explanations.

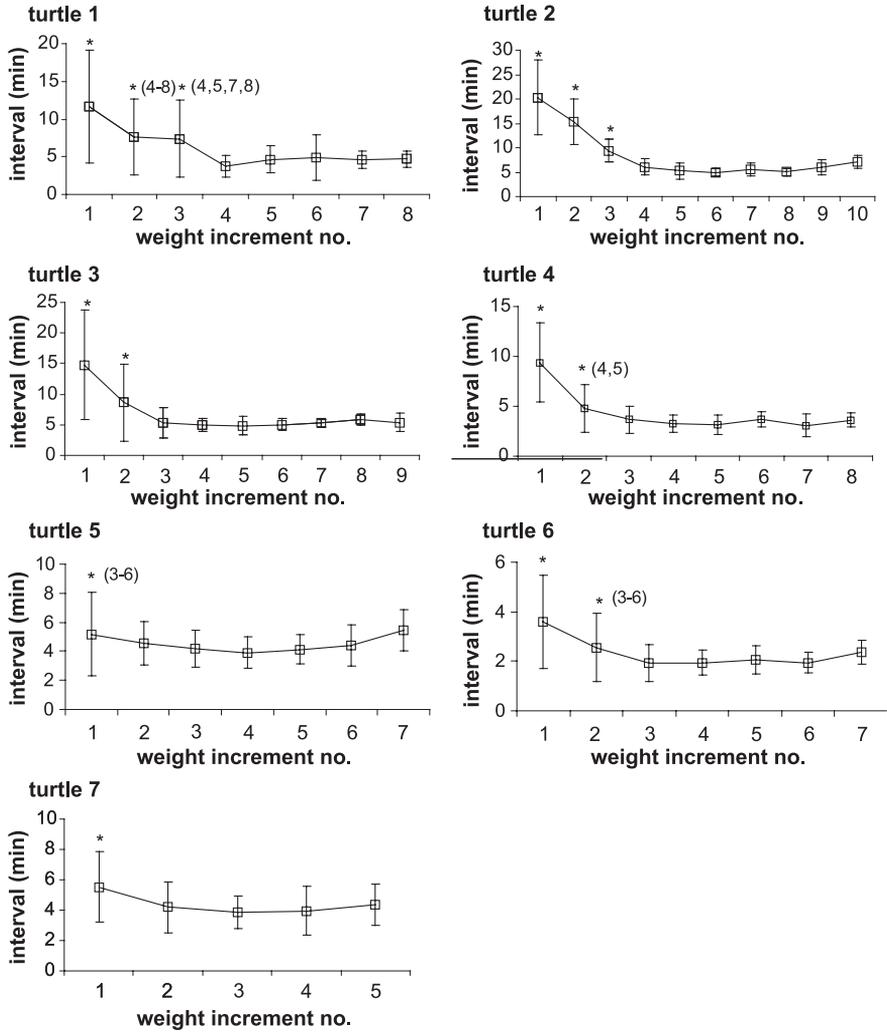


Fig. 3. Resting dives of loggerhead turtles ($n=7$): interval between two successive underwater weight changes in increments of 10 g. Data points are the average interval (open squares) \pm SD (error bars); *= significantly different from all other data points without an asterisk, *(4–6)=significantly different from weight change nos. 4, 5 and 6, etc. Significant differences were detected using Fisher’s pairwise comparison.

3.4. M_{uw} and t_r of weight manipulated turtles

Fig. 6a–f shows the underwater weight for all resting dives in chronological order for the whole experimental period. The dives during which the turtles were carrying additional weights on their carapace are clearly distinguishable since all turtles had higher M_{uw} during this “weighted” period. Some of the turtles showed a tendency of decreasing M_{uw}

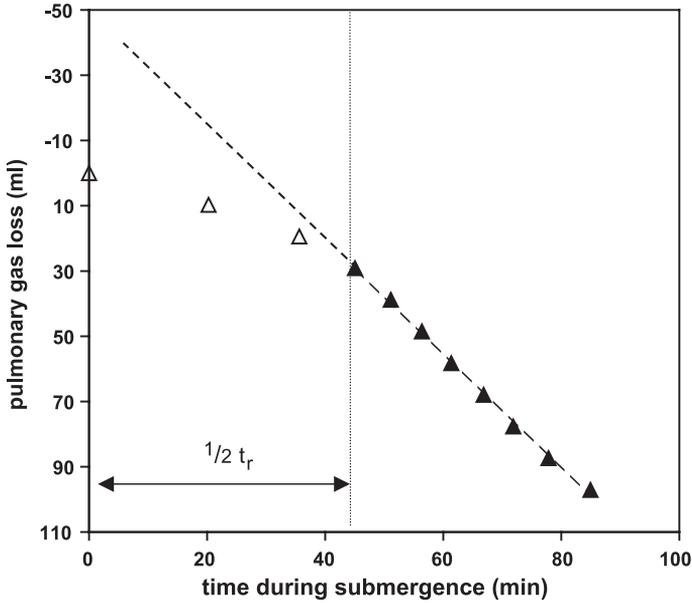


Fig. 4. Net volume of gas withdrawn from the lungs of a loggerhead turtle during a resting dive calculated from the increase in underwater weight and corresponding decrease in lung volume (see text for further explanations on how the calculation was done). During the first half of the submergence ($1/2 t_r$, indicated by vertical dotted line), the lung volume was reduced at a slow rate (open triangles), during the second half of the submergence, lung volume decreased in regular short intervals (filled triangles). A linear regression line (dashed line) was fitted to the data points indicated by the filled triangles and the resulting regression equation was: volume of gas removed = $1.75 \times t_r - 49.69$, $r^2 = 0.9978$.

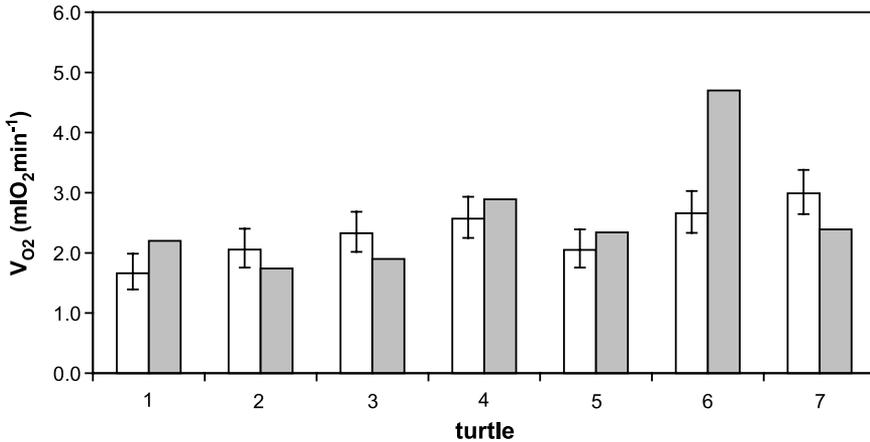


Fig. 5. Comparison between metabolic rates (MR) calculated on the base of lung oxygen consumption (grey columns) and calculated from the equation $\ln MR = -2.87 + 0.168 T_w + 0.353 \times \ln M_b$ established by Hochscheid et al. (in press, white columns). Bars indicate the 95% confidence intervals.

during the weighted period, but average M_{uw} levels remained elevated. Upon weight removal, however, M_{uw} of all turtles returned to pre-weighting values. Resting times for the weighted and the unweighted period were compared for each turtle in those cases

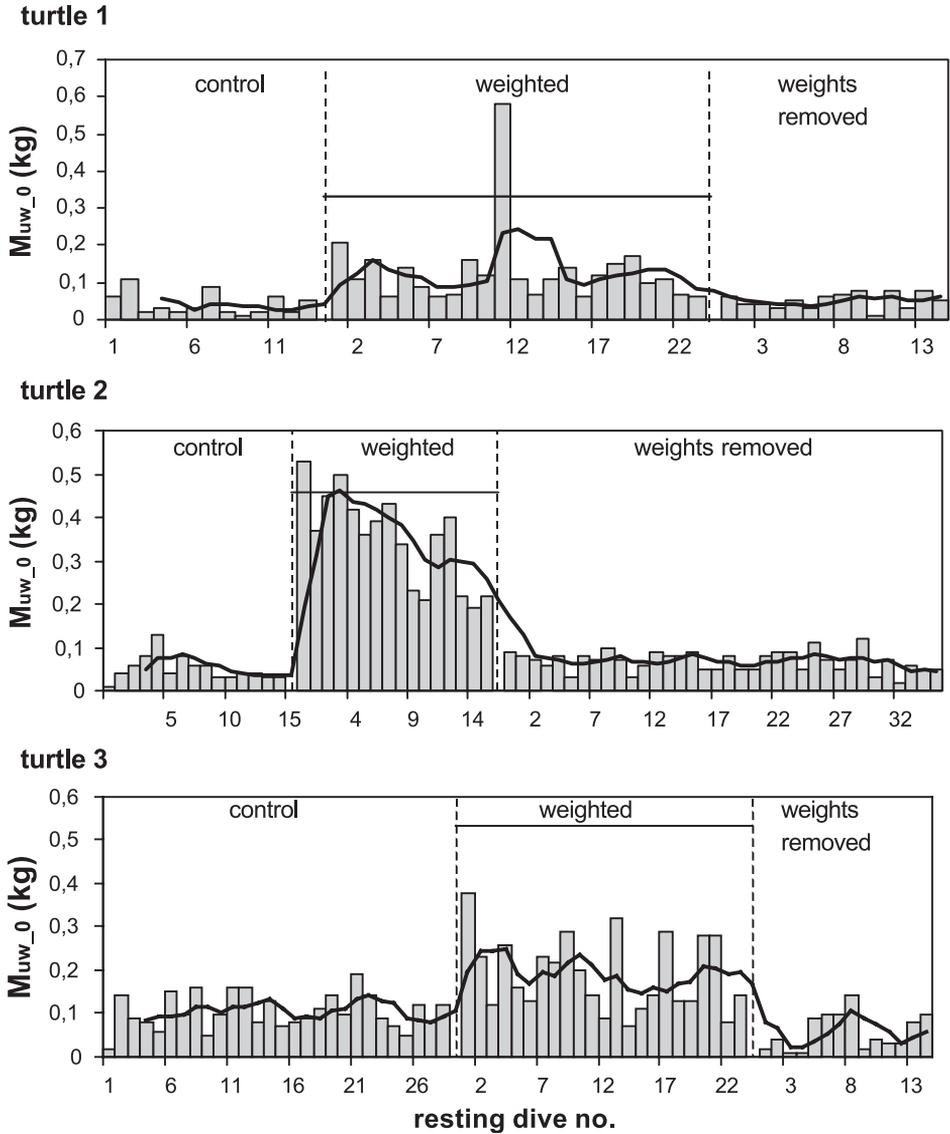
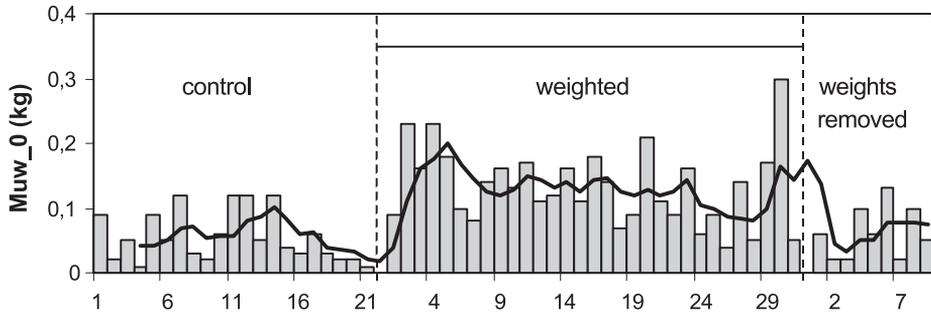
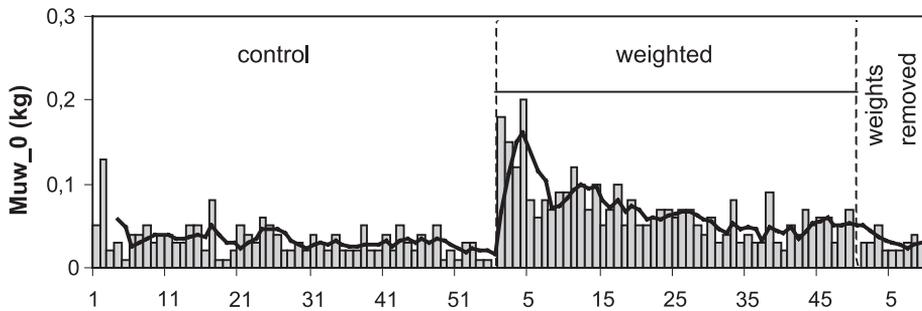


Fig. 6. Underwater weights of single dives (measured at the beginning of a dive = M_{uw_0}) from beginning until end of the experimental period. The vertical dashed lines enclose the period during which the specific gravity of the turtles was manipulated by attached weights, while the horizontal line indicates the theoretical M_{uw} of a turtle which did not compensate for the attached weight. Dives are numbered in chronological order for each period (control, weighted, weights removed). The black solid line represents the moving average (interval = 4).

turtle 4



turtle 5



turtle 6

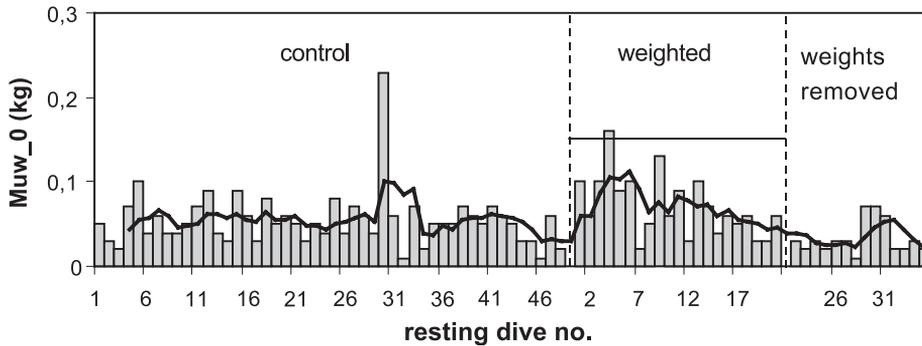


Fig. 6 (continued).

where M_{uw} had the same value (Fig. 7e–f). However, the sample size for this comparison was reduced due to the fact that there were not many overlaps in M_{uw} between the unweighted and the weighted phase. Indeed, for turtle 2, this comparison was not possible at all. Resting time was significantly longer in the weighted condition (GLM: $F_{1,69} = 17.29$, $p < 0.001$) and was significantly different among the individuals (GLM: $F_{5,69} = 4.64$, $p < 0.01$). Although most of the turtles tended to rest for longer, at each M_{uw} , when weight manipulated (Fig. 7) no significance could be established (GLM: $F_{1,69} = 3.5$, $p > 0.05$). In fact, the hypothesis that the resting time would be greater for smaller M_{uw_0}

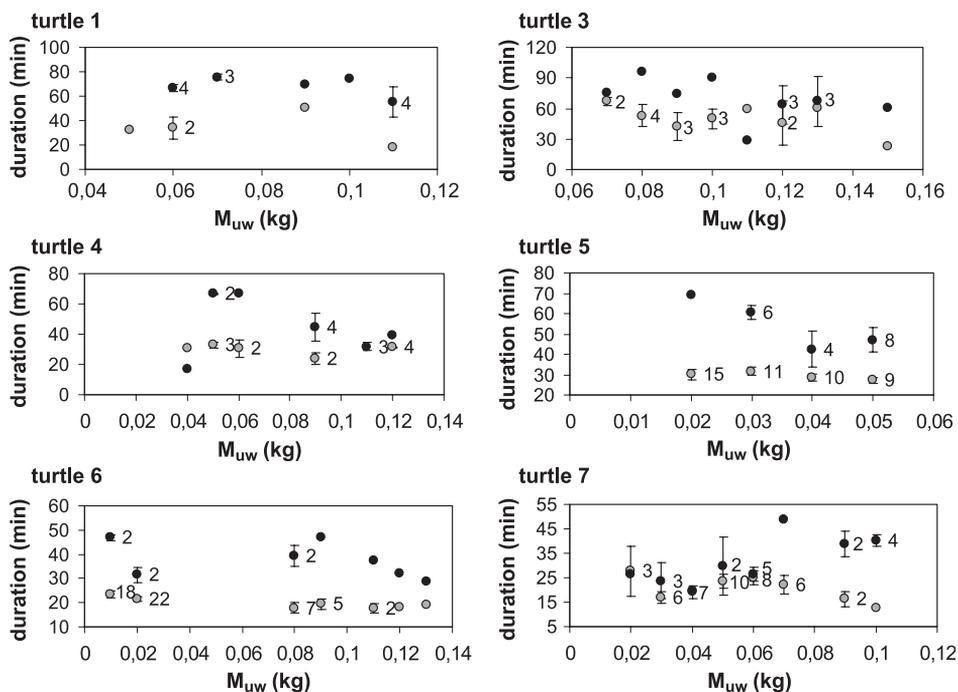


Fig. 7. Comparison of mean dive durations for each underwater weight before (grey-filled circles) and after (black-filled circles) specific weight manipulation of loggerhead turtles. Shown are standard error bars when the number of dives was $n > 2$, but are not shown when the error bars were shorter than the symbol for the mean. Sample sizes for each data point are indicated in numbers next to the symbols when $n > 1$.

(=larger lung volumes or O_2 stores, respectively) was rejected because there was no significant correlation between M_{Uw_0} and t_r . Therefore, weighted turtles dived for longer, thus making more use of their oxygen store, but this increase in dive time could not be explained by the volume of air in their lungs.

4. Discussion

Since all turtles in this study were motionless when submerged and food deprived during resting dives, no major confounding factors were introduced which could have altered the resting metabolic rates. This was important since we attempted to calculate oxygen consumption from the observed changes in buoyancy (see below). Differences in individual M_{Uw} values and resting times were associated with minor differences in body mass and with slight differences in water temperatures during the experimental period. Temperature has a profound effect on the behaviour and physiology of sea turtles (Mrosovsky, 1980; Davenport, 1997), which explains the observed increase in dive duration with decreasing temperature. For example, oxygen is consumed at slower rates when temperature declines thus turtles may dive for longer with the same oxygen store

(Lutz et al., 1989). The longer apnoeic periods of larger turtles were most likely a result of a higher capacity to store oxygen (lung volume scales with an exponent of about 1.0 (Schmidt-Nielsen, 1990)), relative to the allometric scaling of metabolic rate (allometric scaling exponent around 0.85 (Prange and Jackson, 1976)). Allometry of diving capacity has already been shown for numerous groups of air-breathing vertebrates, but data on sea turtles are still too few to allow this type of analysis (Schreer and Kovacs, 1997). Overall, the observed temperature and body mass effects were in the expected directions from this hypothesis. These variables probably acted primarily via their effects on metabolism, which then affected individual M_{uw} .

There are few publications concerning buoyancy of marine air-breathing animals to compare our data with. Moreover, buoyancy changes with depth and with body size, making comparisons even more difficult. Mean \pm SD mass specific buoyancy for turtles in this study was $9.77 \pm 0.02 \text{ N kg}^{-1}$ (calculated from Table 1), whereas aquatic birds had $14.12 \pm 2.19 \text{ N kg}^{-1}$ (Lovvorn et al., 1991), and a 177-kg bottlenose dolphin would have only 0.247 N kg^{-1} at the same depth as the turtles of this study (Skrovan et al., 1999). In contrast, northern elephant seals, *Mirounga angustirostris*, had mean \pm SD mass specific buoyancy of $-0.188 \pm 0.021 \text{ N kg}^{-1}$ (Webb et al., 1998) and grey seals, had -0.45 and -0.137 N kg^{-1} , post-moult and pre-breeding, respectively (Beck et al., 2000), thus they were negatively buoyant. These differences reflect the different diving strategies, with one group diving upon inhalation, taking a volume of air down to depth (aquatic birds, dolphins, sea turtles) and the other group diving upon exhalation (phocid seals). Sea turtles are less buoyant than birds that swim on the surface but they are much more buoyant than dolphins that also inhale prior to diving. This implies that their effort at the surface when they initially start to dive to overcome buoyancy when descending must be comparatively large as well. In line with this explanation, dive angles of free ranging turtles are greater at the beginning of the descent in order to quickly overcome the hindering positive buoyancy (Glen et al., 2001).

The increases in M_{uw} during a resting dive can only be explained by the loss of pulmonary gas, since other possible buoyancy changes are unlikely to occur in such short time intervals. This supports the theory that the oxygen consumption from the lungs directly affects buoyancy. An example on turtle 1 demonstrates that for a resting dive of 48 min, M_{uw_0} was 0.03 kg and the increase ΔM_{uw} in 48 min was 0.08 kg. Therefore, the net-downward force acting on the ascending turtle was -1.079 N (Eq. (5)). Although this means a 3.7-fold increase of the resulting force with respect to the beginning of the dive, it is doubtful that the turtle had to spend a significant amount of energy to fight against such a low force. However, it would cause a turtle, which is resting in midwater (Minamikawa et al., 2000), to sink and once the sinking is initiated the turtle may rapidly descend if it did not swim against it. Thus, the suggestion by Minamikawa et al. (2000) that the turtles move slightly upwards during these midwater resting dives to counteract the buoyancy loss seems plausible.

Tenney et al. (1974) had already estimated a loss of 100 ml gas from the lungs of large turtles due to their metabolism which compares well with values calculated here. There may be two major reasons to explain the observed pattern in lung volume reduction during a dive (Fig. 4). First, only a little oxygen may be removed from the lung during the initial phase of the dive because there is still sufficient oxygen stored in the blood and in the

tissues to fuel basic metabolic processes (Lutz and Bentley, 1985). In favour for this explanation, Wood et al. (1984) observed that oxygen concentration in the blood of a green turtle fell quickly while the turtle dived from the surface to 20 m whereas the lung O_2 store was utilised during progressive dive duration. Presumably, the observed drop in arterial O_2 saturation from 90% to 45% at 20 m in this green turtle occurred during the first 100s of the dive, since the authors reported that the turtle was swimming vigorously during most of the dive and descent velocities are around 0.2 ms^{-1} for sea turtles (Houghton et al., 2002). This relatively short period does not correspond to the shallow period shown in Fig. 4; however, arterial O_2 depletion may have been slower in the resting turtles of this study.

Second, the oxygen that is taken from the lung is replaced by decreasing amounts of CO_2 until no more CO_2 is released into the lung, so that with progressing dive duration volumetric reduction of the lung gas is solely due to oxygen extraction (Glass and Wood, 1983; Wood et al., 1984). This corresponds to the observation by other authors that there is no CO_2 increase in the pulmonary airspaces during the second half of dives lasting 20 min or longer (Burggren and Shelton, 1979; Berkson, 1966). The reason for this is the high solubility of CO_2 in tissue fluids and in blood and the small diffusion gradient from blood to alveolar air, which decreases even more during apnoea due to the decreasing lung volume which concentrates pulmonary CO_2 (Burggren and Shelton, 1979). Burggren and Shelton (1979) conducted an extensive study on the gas exchange and transport during apnoea in Chelonian turtles. In particular, the patterns of gas distribution during longer dives in the freshwater turtle *Pseudemys scripta* correspond well with the changes in M_{uw} observed here: while P_{O_2} in the lungs dropped only slowly during the first third of the dive there was a sharp decline in blood P_{O_2} . The situation changed when more oxygen was drawn from the lung replenishing blood O_2 and consequently reducing lung volume at a higher rate than initially. In summary, the P_{O_2} curve of lung oxygen shown in Fig. 4 of Burggren and Shelton's study matches the curve of pulmonary gas loss in Fig. 4 of this study. According to a calculation by Burggren and Shelton (1979) the gas transfer quotient of the lungs is 0.2 meaning that for each 100 ml O_2 which are withdrawn from the lung 20 ml of CO_2 are added. If this relatively small proportion of CO_2 is released into the lungs predominantly during the first half of a dive then the final linear phase of the pulmonary gas loss probably represented only the V_{O_2} of the turtle. Oxygen consumption rates calculated from increases in M_{uw} ($1.69\text{--}4.86 \text{ ml } O_2 \text{ min}^{-1}$) corresponded well with expected V_{O_2} ($1.66\text{--}2.99 \text{ ml } O_2 \text{ m in}^{-1}$), which were derived from a known relationship between V_{O_2} , water temperature and body mass for loggerhead turtles in the same facility (Hochscheid et al., in press). These values corresponded also to resting V_{O_2} calculated from Lutz et al. (1989) which ranged from 3.1 to $5.5 \text{ ml } O_2 \text{ min}^{-1}$ for the similar sized turtles. We can therefore say with confidence that there are buoyancy changes during a dive which are caused by the oxygen consumption of the turtles.

The general elevated M_{uw} of the weighted animals indicates that turtles often did not increase their lung volume to equilibrate for the additional weight, but rested slightly heavier on the bottom. Minamikawa et al. (2000) reported that free-ranging loggerhead turtles dived shallower with a weight attached to their carapace than without the weight, which, in agreement with the results presented here, indicated that the turtles did not change their lung volume. It appears that, at least in freely diving turtles, depth was altered to maintain lung volume constant rather than the animals responding to the additional

weight by varying their lung volumes to maintain buoyancy constant at a given depth. So far, this applies only to resting dives, either at the bottom or in midwater, but it may have impact on animals feeding pelagically where turtles are forced to dive to the depth where prey is abundant and may regulate their buoyancy accordingly.

Perhaps the most tenuous hypothesis was that dive duration of sea turtles increases with increasing lung volume, i.e. oxygen store. For turtles in this study, which were acclimated to low temperatures and rested most of the time, dive patterns were sufficiently regular to test this prediction. If this hypothesis was correct then dive duration should have correlated with M_{uw} since a turtle would have a greater lung volume, and thus oxygen store, the smaller M_{uw} was. However, there was no such correlation, and moreover, not all of the turtles which equilibrated for the additional weight tended to stay submerged for longer (Fig. 7). Thus, although dive duration increased in all turtles when they were weighted, this was not commonly due to an increase in lung volume. Instead, most of the time, buoyancy regulation was incomplete as shown by the increased M_{uw_0} . This suggests that, while the turtles used only a certain proportion of available O_2 in the control experiments, they made use of a larger proportion of O_2 during the weighted period. The current data set does not allow any explanation of this behaviour, but it implies that turtles dive and surface with variable body oxygen saturations and that the size of the oxygen store may not be a good predictor for the dive duration of resting turtles.

It was possible to demonstrate that diving oxygen consumption affects buoyancy, but not that lung volume changes due to buoyancy regulation consequently affect dive durations. The high buoyancy of sea turtles, which take air down to depth only in their lungs with no possibility to trap air in their body cover, demands further investigations on the energetic costs during the buoyant descent phase. A conflicting outcome of this study was that the lung of loggerhead turtles seemed to have a greater importance for oxygen storage than for buoyancy regulation, but that the available oxygen was never fully consumed.

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