Oxygen stores and aerobic metabolism in the leatherback sea turtle

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The leatherback sea turtle, *Dermochelys coriacea*, is a large, deep-diving species that has a blood oxygen carrying capacity twice that of smaller, shallow-diving sea turtles. In this study we measured lung volume (by argon dilution) and blood volume (by dilution of Evans' blue dye) in leatherbacks to estimate partitioning of oxygen stores and their potential contribution to aerobic metabolism during diving. Blood volume (77 mL · kg^{-1}) was slightly higher, yet lung volume was considerably smaller (64 mL · kg^{-1}), than in other sea turtles, so that potential oxygen stores were almost equally divided between the lung (12 mL · kg^{-1}) and the blood and tissues (15 mL · kg^{-1}). At a body temperature of 32–34°C and high heart rates (43–48/min), oxygen consumption of beached and netted leatherbacks was 1.1 mL · min^{-1} · kg^{-1}. The respiratory quotient exceeded unity, suggesting that the turtles were repaying an oxygen debt incurred in the netting procedure. Estimates of the probable utilization of oxygen stores and possible maximum and minimum oxygen uptakes were used to obtain a range of dive times (5–70 min) that can be supported aerobically.

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La Tortue luth, *Dermochelys coriacea*, est une espèce de grande taille, capable de plongées profondes, qui a une capacité de transport d'oxygène deux fois plus grande que celle des tortues plus petites qui plongent en eau peu profonde. Dans cette étude, nous avons mesuré le volume pulmonaire (par dilution d'argon) et le volume sanguin (par dilution de Bleu Evans) de Tortues luth afin d'évaluer la répartition des réserves d'oxygène et leur contribution potentielle au métabolisme aérobique au cours de la plongée. Le volume sanguin (77 mL · kg^{-1}) était légèrement plus élevé, mais le volume pulmonaire était beaucoup plus faible (64 mL · kg^{-1}) que chez d'autres tortues marines, et les réserves d'oxygène disponibles étaient alors réparties presque également entre le poumon (12 mL · kg^{-1}) et le sang et les tissus (15 mL · kg^{-1}). À une température corporelle de 32–34°C, à des rythmes cardiaques élevés (43–40/min), la consommation d'oxygène de tortues échouées et capturées dans des filets était de 1,1 mL · min^{-1} · kg^{-1}. Le quotient respiratoire était supérieur à 1, ce qui indique que les tortues compensaient la dette d'oxygène encourue au cours de la période de capture. L'estimation de l'utilisation probable des réserves d'oxygène et des consommations maximale et minimale possibles ont servi à déterminer l'étendue des durées des plongées (5–70 min) qui peuvent être aérobiques.

[Traduit par la rédaction]

Introduction

Among the sea turtles, the leatherback, *Dermochelys coriacea*, is the largest (>300 kg) and the deepest diver, able to dive beyond 1000 m (Eckert *et al.* 1986). Shallow-diving sea turtles are thought to make aerobic dives relying on the lung oxygen store (McCutcheon 1947; Prange and Jackson 1976; Lapennas and Lutz 1982). On the occasions when leatherbacks dive deeply, increased hydrostatic pressure probably collapses their lungs (Berkson 1967). Active metabolic rates of leatherbacks are among the highest in reptiles, equivalent, when scaled allometrically, to those of smaller varanid and iguanid lizards (Lutcavage and Lutz 1986; Paladino *et al.* 1990). Consequently, leatherbacks may have to rely on blood and tissue oxygen stores for deep dives. This is supported by the fact that they have blood O_2 carrying capacities twice those of smaller, shallow-diving sea turtles, and a right-shifted blood O_2 dissociation curve suitable for O_2 delivery to tissues (Lutcavage *et al.* 1990). We measured blood volume by dye dilution to estimate the blood O_2 store available during diving. We also measured lung volume by inert gas dilution to get an estimate of how the total body O_2 store was partitioned. Finally, we measured surface $\dot{V}o_2$ and used this value, along with those

obtained by others, to get an idea of potential aerobic dive limits for leatherback turtles.

Methods

Blood volume determinations and respiratory measurements were performed on female leatherback turtles nesting on Tortuguero Beach, Costa Rica (May 1990). Air temperature on the beach during measurement periods, 24:00 to 05:00, ranged from 22 to 27°C. Upon completion of egg laying, turtles were weighed in a cargo net that restricted limb movement but did not impede respiration (Paladino *et al.* 1990). The turtles remained in the cargo net throughout the blood and lung volume measurement period.

Plasma volume was determined by dilution of Evans' blue dye. Initially, a 30-mL blood sample was taken from the turtle's cervical venous sinus to make a calibration curve. Then two syringes, one containing 5 mL of reptilian saline and the other, 2 mL of preweighed dye solution, were attached to the needle via a three-way stopcock. Blood was withdrawn into the saline-filled syringe to confirm that the needle was in the sinus. The dye syringe was then emptied into the sinus and the syringe needle was rinsed twice with withdrawn blood and then flushed out into the sinus with saline. The empty dye syringe was reweighed to determine the volume of dye injected V_i). The initial dye stock solution ([dye]$_i$) was prepared to give a final concentration of approximately 0.02 mg · mL^{-1} plasma ([dye]$_f$) when mixed in the turtle's circulatory system, assuming the blood volume to be 6% of body weight (Thorson 1968). Three venous blood samples (5 mL) were taken approximately 15, 30, and 50 min after dye injection. When possible, blood was taken from the sinus opposite to the

TABLE 1. Summary of oxygen storage and aerobic metabolism variables of five leatherback sea turtles

	Turtle No.					
	DC401	DC402	DC405	DC407	DC408	Mean ± SD
Body mass (kg)	320	315	370	355	350	342±23
Body temperature (°C)	32	34	33	34	32	33±1
Lung volume (mL · kg^{-1})*	—	—	52.9	70.9	68.2	64.0±9.7
$\dot{V}_{O_2}$ (mL · min^{-1} · kg^{-1})†	—	—	0.97	0.98	1.31	1.09±0.19
$\dot{V}_{CO_2}$ (mL · min^{-1} · kg^{-1})†	—	—	1.49	1.44	1.87	1.60±0.23
RQ	—	—	1.54	1.47	1.37	1.46±0.09
Hematocrit	37	38	32	37	32	35.2±2.9
Plasma volume						
Total (L)	16.0	—	18.8	—	—	17.4
% body mass	5.0	—	5.1	—	—	5.05
Blood volume						
Total	25.4	—	24.7	—	—	25.0
% body mass	7.9	—	7.5	—	—	7.7

*BTPS.
†STPD.

injection site. All samples were capped and refrigerated (2–12 h) until they could be processed.

Repetitive blood sampling, a necessity for completing the dye dilution study, was extremely difficult because the cervical sinus "collapsed" unpredictably. Therefore, we were unable to obtain sufficient samples in three of the five turtles in which the procedure was attempted. In the case of two turtles (DC402, DC407) we could not recover a second blood sample from either sinus in spite of numerous attempts over a 3-h period. In a third turtle (DC408) little of the dye injected into the sinus appeared to have mixed with the general circulation.

Blood for a calibration curve was thoroughly mixed and hematocrit (Hct) was measured following 5 min centrifugation. A 6-point standard dye curve was constructed by serial dilution of the initial dye stock solution into precisely measured aliquots of whole blood. The dye standards and the dye dilution samples were then centrifuged, the plasma was removed, and their optical densities were measured at 540 nm using a Spectronics 20 Spectrophotometer. A calibration curve was constructed by plotting optical density against dye concentration and used to convert the optical density of the turtle blood samples into [dye]$_f$.

Plasma volume (V_p) was calculated using the equation

$$[1] \quad V_p = \frac{V_i \times [\text{dye}]_i}{[\text{dye}]_f}$$

and blood volume (V_b) using

$$[2] \quad V_b = \frac{V_p}{1 - (\text{Hct}/100)}$$

where Hct is hematocrit (percent red blood cells).

Blood and plasma volume measurements were plotted against time and extrapolated by regression analysis to zero time to determine volumes at the time of dye injection.

Breath-hold lung volume (V_L) was determined using an argon rebreathing technique (Glass et al. 1981). Oxygen consumption ($\dot{V}_{O_2}$) was obtained by measuring the difference between initial and final gas concentrations during rebreathing. A helmet was fashioned from a 5-L PVC bottle that had an expandable plastic sleeve secured to the cut edge and a 3.5-cm (i.d.) T-piece fitted to the neck of the bottle (Lutcavage et al. 1990). The expandable sleeve ensured an airtight fit when the helmet was placed over the turtle's head. The helmet allowed free movement of the head and jaws during ventilation and, when placed on the turtle, the T-piece admitting air was located directly in front of and above the turtle's jaws. At the begin-

ning of a nonventilatory period (NVP), a 3-L syringe containing a known volume of argon (Liquid Carbonic Inc., Hollywood, Fla.) and an evacuated 30-L Douglas bag were attached, one to each side of the T-piece. Argon was injected into the rebreathing system and mixed quickly by repeatedly pumping the 3-L syringe. The deadspace volume (V_{ds}) was measured by taking gas samples after mixing but before the turtle breathed again; the turtle then rebreathed the gas mixture 12 times before the final gas samples were collected, in triplicate. Lung volume was calculated on the basis of the change in fractional concentrations of argon before and after the turtle had breathed. V_L was obtained after subtraction of V_{ds}. In all cases, gas was sampled from a septum in the middle of the T-piece, using evacuated, sealed 20-mL glass test tubes (Vacutainer, Inc., Chicago, Ill.).

To test for leaking or contamination, vacutainers were also filled with pure argon or remained unfilled as blanks, and were stored and processed, at random, with turtle gas samples. Two rebreathing trials were completed for each turtle, and the average is reported. Sample concentrations of Ar, O_2, CO_2, N_2, and water vapor were determined in the Chemistry Department at the University of British Columbia with a GC-MS mass spectrometry system (Mermag, model R-1010).

An electrocardiogram was obtained from three turtles by means of skin electrodes. The signal was amplified using a Grass P-5A battery-powered preamplifier and recorded on magnetic tape with a battery-operated tape deck (Aiwa). Cardiac intervals, obtained by measuring the time between beats, were converted to beats per minute (bpm). All values are presented as the mean ± standard deviation.

Results

Although blood volume determinations were attempted in five turtles, all conditions necessary for measurement of plasma and blood volume by dye dilution (Jones 1970) were met in only two turtles. These turtles had mean plasma and blood volumes of 5.1 and 7.7% of body mass, respectively (Table 1). Mean Hct in the five turtles was 35 ± 2.9%.

Breath-hold lung volume (V_L) measured in three turtles was 64.0 ± 9.7 mL · kg^{-1}. Core body temperature during measurements ranged from 32 to 34°C. Heart rate of three turtles sampled intermittently during gas measurements ranged from 43 to 48 bpm. Mean $\dot{V}_{O_2}$ during rebreathing was 1.1 ± 0.2 mL · min^{-1} · kg^{-1}, and ranged from 0.41 to 1.66 mL · min^{-1} · kg^{-1}. Mean CO_2 production (1.6 ± 0.2 mL · min^{-1} · kg^{-1}) was high, probably indicating that the

animal was repaying an oxygen debt incurred during the netting procedure. Turtles took single breaths, between pauses, and respiratory frequency (f) during rebreathing was 4.4 ± 1.38 breaths/min.

Discussion

The blood volumes obtained from the two leatherbacks in this study fall in the range 6.5−7.5% of body mass, which is similar to those of both terrestrial and aquatic chelonians (Thorson 1968). Interestingly, normalized total lung volume (V_L), which scales in direct proportion to body weight (Schmidt-Nielson 1984), was about 20−40% smaller in leatherbacks than in green turtles (Berkson 1966), loggerhead turtles (Lutz and Bentley 1985), and *Pseudemys* (Jackson 1968).

Metabolic rates obtained from restrained turtles were similar to those reported previously by Paladino et al. (1990) but nearly 4 times the mean $\dot{V}O_2$ occurring during egg deposition in unrestrained turtles (0.25 mL · min⁻¹ · kg⁻¹; Lutcavage et al. 1990). It may be that restraint disturbed the turtles and increased $\dot{V}O_2$, since mean breathing frequency was almost twice that of unrestrained turtles depositing eggs (Lutcavage et al. 1990).

Also, the high RQ suggests that the animals were repaying an oxygen debt incurred during the netting procedure that was used to restrain them. Certainly, heart rates of restrained leatherbacks were double those in unrestrained green turtles, *Chelonia mydas* (22−24 bpm; Berkson 1966; Butler et al. 1984). With scaling effects taken into account, heart rates of large leatherback turtles would be expected to be lower than those observed in the much smaller green turtles. Therefore, true resting $\dot{V}O_2$ is probably closer to the value reported in unrestrained turtles. Based on our minimum $\dot{V}O_2$ observed previously (0.25 mL · min⁻¹ · kg⁻¹; Lutcavage et al. 1990) and that measured in active turtles (3.7 mL · min⁻¹ · kg⁻¹; Paladino et al. 1990), the leatherback turtle has an aerobic scope of 15, equal to or surpassing that of some varanid lizards (Gleeson 1981; Bennett 1982) and the green turtle (Prange and Jackson 1976; Butler et al. 1984).

The present data on blood and lung volumes combined with those obtained previously on blood O_2 carrying capacity and tissue myoglobin concentration (Lutcavage et al. 1990) allow us to estimate the partitioning of the total O_2 store available during diving. The leatherback's total myoglobin O_2 store is estimated to be 1.4 mL O_2 · kg⁻¹ by using an assumed muscle mass of 22% of body mass (Lutz and Bentley 1985) and a previously determined myoglobin concentration of 4.9 mg · g⁻¹ muscle tissue (Lutcavage et al. 1990). Total blood O_2 store, 13.8 mL O_2 · kg⁻¹, is the product of blood volume (7.7% body mass) and the blood O_2 carrying capacity (21 mL O_2 · dL⁻¹; Lutcavage et al. 1990). For this estimate we assumed a distribution of one-third arterial and two-thirds venous blood (Lutz and Bentley 1985), and 95 and 80% saturation, respectively, based on previously determined blood O_2 binding curves (Lutcavage et al. 1990). Similarly, the lung O_2 store at the start of a dive is estimated by multiplying lung volume (64 mL · kg) by the fractional concentration of O_2 in the lung (19%; M. Lutcavage, unpublished data). The blood and tissue O_2 store (15.2 mL · kg⁻¹) is slightly larger than that of the lung (12.2 mL · kg⁻¹), whereas in other sea turtles the lung store is larger than the blood and tissue store by at least a factor of 2 (Berkson 1966; Lutz and Bentley

1985). Small lungs, although substantially reducing the lung O_2 store, are advantageous because they will decrease the animal's susceptibility to the bends during deep repetitive diving (Kooyman 1973). Moreover, small lungs might be expected in an animal with a greatly reduced shell compared with other sea turtles, because the role of the lungs as a buoyancy organ will be reduced.

The majority of dives made by leatherbacks tracked in field studies (e.g., Standora et al. 1984; Eckert et al. 1986; Eckert et al. 1989; J. A. Keinath and J. A. Musick, unpublished data) are short, in the range 4−11 min, the longest published record being 37 min (Eckert et al. 1986). The lack of long surface intervals, and dive:pause ratios ranging from 2:1 to 6:1, suggest that routine dives are aerobic. Leatherbacks have a total O_2 store of about 27 mL · kg⁻¹ in the lung, blood, and tissue. If turtles surface from voluntary dives with the arterial blood 50% saturated, as observed for loggerhead turtles (Lutcavage and Lutz 1991), then 75% of the lung and half of the blood−tissue store would be utilized. Based on minimum and maximum mean $\dot{V}O_2$ values obtained on land (Lutcavage et al. 1990; Paladino et al. 1990), the leatherback's aerobic dive limit would range from 5 to 70 min, which encompasses the range seen in nature. Obviously, determination of metabolic rate and diving profiles in free-ranging turtles is essential to refine these estimates.

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