

GAS EXCHANGE IN THE LOGGERHEAD SEA TURTLE *CARETTA CARETTA*

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SUMMARY

Pulmonary CO diffusion capacity (DL_{CO}), lung volume (V_L), oxygen uptake (\dot{V}_{O_2}) and pulmonary blood flow (\dot{Q}_L) were measured simultaneously in the loggerhead sea turtle *Caretta caretta* (Linnaeus) (8–11 kg) using a gas rebreathing technique (syringe pump ventilation).

Calculated DL_{CO} and \dot{V}_{O_2} values in the loggerhead turtle are approximately twice those of non-varanid reptiles and about 25% of values for resting mammals. Evidence based on an estimated lung–capillary O_2 gradient, ΔP_{O_2} , shows that only a small driving gradient is required for O_2 conductance across the loggerhead turtle lung. Pulmonary blood flow values are high compared to those for other reptiles; on the basis of the Fick principle, this implies a substantial blood convection requirement.

Differences in oxygen transport mechanisms between the loggerhead sea turtle and other highly aerobic but terrestrial reptiles may result from divergent lung structures and breathing patterns. In sea turtles, the coupling of respiration with locomotory behaviour is one factor that may limit aerobic performance. Since sea turtles have only intermittent access to air, they are required to load both arterial and venous blood with O_2 before submerging.

INTRODUCTION

The sea turtle is a large, active, aquatic reptile that spends less than 2% of its time at the surface (Lutz & Bentley, 1985). Several species of sea turtles are known to make long migrations at speeds of up to 9 km h^{-1} to nesting and foraging grounds, and their diving capacities exceed those of most marine mammalian divers (Berkson, 1966). It is reasonable to expect that the sea turtle lung is adapted to this active aquatic lifestyle and, furthermore, that striking differences might be found in lung structure and function compared to those of smaller, less active, nonaquatic reptiles.

Sea turtles, varanid lizards and crocodiles are the only groups of reptiles with extensively subdivided, multicameral lungs (Tenney & Tenney, 1970). It is generally believed that this lung structure provides a large surface for gas exchange and accounts for the higher rates of oxygen delivery and uptake attained by these groups.

Key words: diffusion, lung, sea turtle.

However, although the sea turtle has perhaps the most finely subdivided lung of any reptile, it is also extensively infiltrated with smooth muscle and structural fibres (Tenney, Bartlett, Farber & Remmers, 1974). While muscle and connective tissue would enhance the mechanical stability of the lung and are a characteristic feature of the lungs of some diving mammals, any thickening of lung respiratory parenchyma should also increase resistance to gas transfer.

It should be noted that if sea turtle lungs are to function well, their structure must accommodate not only mechanical strength for rapid transfer of gas while at the surface, but the interior must provide an effective means for oxygen storage and transport during submergence (Lutz & Bentley, 1985).

In previous studies, oxygen uptake rates recorded for active adult green turtles (Jackson & Prange, 1979) and for juveniles (Butler, Milsom & Woakes, 1984) are much higher than those of most other reptiles. Not surprisingly, these studies have shown that the sea turtle can increase its resting oxygen uptake rate by 8- to 10-fold, attaining maximal rates comparable to those of varanid lizards and to the resting rates of most mammals.

The currently held concept of optimal design in respiratory systems (Weibel, 1984) is that diffusion capacity should be set by the functional demand for oxygen required during exercise. Many studies have shown that morphometric diffusion capacity is a direct function of the surface area of the lung (Tenney & Tenney, 1970; Weibel, 1979). Together, these observations led to the conclusion that reptiles with the highest oxygen demands would be expected to have finely partitioned lungs and, consequently, a high diffusion capacity. It is somewhat surprising, then, that the diffusion capacity recorded for the green sea turtle lung by Gatz, Glass & Wood (1979) is similar to values determined for some other non-varanid reptiles that have much lower maximal metabolic rates.

In view of the very limited information on sea turtle lungs and gas exchange, and the intriguing problems posed by this specialized lung structure, we have investigated in some detail the oxygen transport capacity of the loggerhead sea turtle (*Caretta caretta*), using a non-invasive rebreathing technique. Since the animal's habitat is in temperate seas and it does not encounter the wide diurnal temperature range experienced by some terrestrial reptiles, we examined these aspects at one temperature within its selected activity range and we related these findings to the structure of the loggerhead sea turtle lung.

MATERIALS AND METHODS

Measurements were made on eight juvenile loggerhead turtles weighing 8–11 kg. The turtles had been raised in captivity and housed outdoors in large tanks supplied with a flow-through seawater system. Tank seawater temperatures ranged from 22 to 27°C during the course of the study. Turtles were removed from the tanks and held at room temperature ($25 \pm 1^\circ\text{C}$) for at least 2 h before measurements were taken.

Pulmonary function

Lung volume (V_L), oxygen uptake (\dot{V}_{O_2}), carbon monoxide diffusing capacity (DL_{CO}) and pulmonary perfusion (\dot{Q}_L) were measured simultaneously using a gas rebreathing technique. Measurements were performed with a Perkin Elmer 1100 respiratory mass spectrometer using a gas sampling rate of 10 ml min^{-1} . The rebreathing gas mixture (Liquid Carbonic) consisted of 10% helium, 0.1% $C^{18}O$, 0.5% acetylene, 21.5% oxygen and balance nitrogen.

We used the rebreathing procedure developed in mammalian studies by Butler (1965), which was subsequently modified for use on reptiles by Crawford *et al.* (1976), with further modifications using helium-corrected volumes according to Glass, Johansen & Abe (1981).

Turtles were given a light dose of anaesthetic (Ketamine hydrochloride, $18\text{--}23 \text{ mg kg}^{-1}$), which prevented struggling but did not alter spontaneous respiration. They were then placed on their plastrons so that the lung was not compressed by the weight of other body organs, but they were not otherwise restrained.

Tracheal tubes (Mallinckrodt) were inserted into the open glottis during inspiration and secured to the lower jaw with surgical tape. A T-section with a three-way valve was joined to the tracheal tube and the sample inlet of the mass spectrometer was attached to one arm of the T-section.

At the beginning of a breathhold, a gas-tight syringe containing the test gas was attached to the third arm of the T-section and approximately one tidal volume was repeatedly withdrawn and re-injected into the turtle's lung at a rate of 30 min^{-1} for at least 1 min. During rebreathing the mass spectrometer output was continuously recorded on a five-channel (Electronics for Medicine) recorder. Data were discarded if the turtle struggled during the rebreathing procedure.

The helium dilution method was used to calculate lung volume (V_L). Since lung volume in this study was measured during breathholding with an open glottis, the closest mammalian analogue of this value is functional residual capacity. Oxygen uptake (\dot{V}_{O_2}) was calculated from the linear rate of decrease in fractional concentration of oxygen during the rebreathing period.

Carbon monoxide diffusing capacity ($DL_{C^{18}O}$) was calculated from the exponential clearance of $C^{18}O$ relative to helium from the lung-syringe system.

Since the concentration of acetylene in the lungs decreases at a rate proportional to pulmonary blood flow, \dot{Q}_L was calculated using the slope of the disappearance curve of acetylene relative to helium, where time zero and lung tissue volume were determined according to Butler (1965).

During rebreathing, the $C^{18}O$ disappearance rate was constant and independent of rebreathing duration. However, 15–20 s after introduction of the test gas, a change in the slope of the exponential clearance rate of acetylene in the lung-syringe system indicated recirculation to the lungs. Consequently, data used for calculating \dot{Q}_L were based on the first 15 s of rebreathing.

Three trials were conducted on each turtle and the mean values for V_L , \dot{V}_{O_2} , DL_{CO} and \dot{Q}_L were calculated. From these, group means were obtained.

RESULTS

A summary of functional pulmonary parameters determined during rebreathing is given in Table 1. Lung volume (V_L) ranged from 24 to 75 ml kg⁻¹. Resting tidal volume for loggerhead turtles in this size range is about 45 ml kg⁻¹ (Lutz & Bentley, 1985); when added to V_L , this gives a breathhold lung volume of about 83 ml kg⁻¹. Oxygen uptake values measured here ranged from 1.5 to 3.6 ml min⁻¹ kg⁻¹ and DL_{CO} ranged from 0.067 to 0.136 ml min⁻¹ Torr⁻¹ kg⁻¹ (1 Torr = 133.3 Pa).

DISCUSSION

The gas dilution techniques used here and in several other reptilian pulmonary studies (Glass *et al.* 1981; Hlastala, Standaert, Pierson & Luchtel, 1985) assume that pulmonary blood flow does not change during rebreathing. We were not permitted to use invasive techniques that would directly determine pulmonary blood flow because the loggerhead turtle is a protected species. We recorded heart rate in two animals using skin electrodes before and during rebreathing and we found that heart rate did not change significantly during rebreathing. Although pulmonary arterial blood flow in the lizard *Varanus exanthematicus* increased by only 10% or less during rebreathing (Glass *et al.* 1981), we do not have sufficient evidence to rule out an increase in cardiac output during our experimental conditions.

In loggerhead sea turtles the calculated breathhold lung volume is close to the value predicted for neutral buoyancy based on the relationship of biomass to lung volume determined for juvenile loggerhead turtles by Milsom & Johansen (1975). Mean lung volume obtained by Wood, Gatz & Glass (1984) using rebreathing techniques on *Chelonia mydas* is somewhat higher but well within the range reported here for the loggerhead turtle. However, it should be noted that breathhold lung volume following inspiration in spontaneously breathing loggerhead turtles cycles above the relaxed system volume and is highly variable.

Oxygen uptake values measured here on inactive *Caretta caretta* are only slightly higher than a mean \dot{V}_{O_2} reported by Lutz & Bentley (1985) for resting loggerhead turtles; when scaled for mass, they compare reasonably well with the values of \dot{V}_{O_2} in resting *Chelonia mydas* reported by Gatz *et al.* (1979).

Table 1. Summary of pulmonary functions in the loggerhead sea turtle *Caretta caretta*

	V_L (ml kg ⁻¹)	\dot{V}_{O_2} (ml min ⁻¹ kg ⁻¹)	DL_{CO} (ml min ⁻¹ kg ⁻¹ Torr ⁻¹)	\dot{Q}_L (ml min ⁻¹ kg ⁻¹)	a-v difference (vol%)	\dot{Q}_L/\dot{V}_{O_2}
Mean	35.7	1.4	0.11	86*	2.1*	59.3*
±S.D.	14.9	0.50	0.03	29.7	1.7	33.1

Body mass = 9.5 ± 1.5 kg, N = 8; at 25°C. *N = 5.

Values were recorded simultaneously on prone animals with a multiple gas mixture using the rebreathing method.

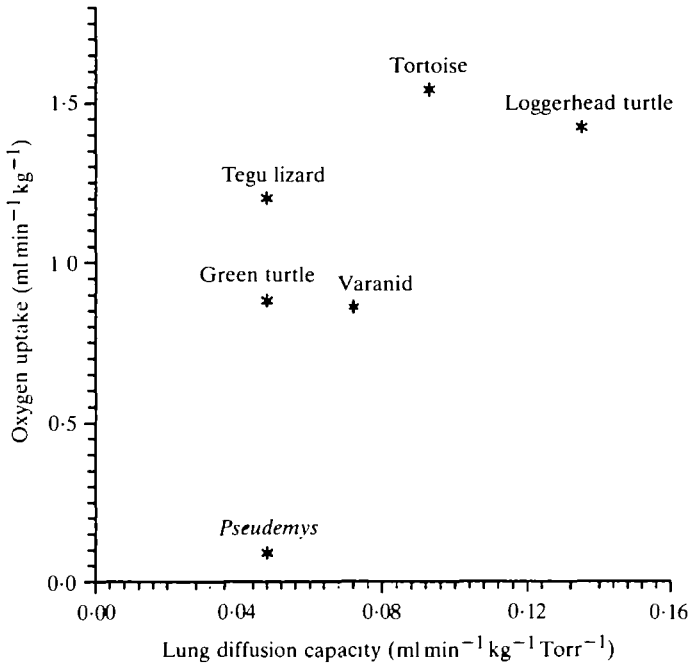


Fig. 1. Relationship between lung diffusion capacity (DL_{O_2}) and oxygen consumption (\dot{V}_{O_2}) of selected reptiles at 25°C. DL_{O_2} was estimated using DL_{CO} and the conversion factor 1.2 (Forster, 1964). Loggerhead turtle, this study; tortoise, *Pseudemys*, Crawford *et al.* 1976; varanid, Glass, Johansen & Abe, 1981; green turtle, Gatz, Glass & Wood, 1979.

The diffusion capacity of the loggerhead lung found in this study is the highest value reported for any reptile at 25°C. Gatz *et al.* (1979), however, using a rebreathing method found a significantly lower value (0.04 ml min⁻¹ kg⁻¹ Torr⁻¹) for large green turtles *Chelonia mydas* (mass = 70 kg), which is less than half of the value we measured in *Caretta*. Since we have found that the lungs of green and loggerhead turtles are structurally similar (M. E. Lutcavage, P. L. Lutz & H. Baier, in preparation), differences in measured diffusion capacity are probably related to variation in experimental protocols such as the duration of rebreathing or gas composition (Crawford *et al.* 1976).

Fig. 1 illustrates the plot of resting \dot{V}_{O_2} vs DL for the loggerhead turtle and other reptiles, using the solubility ratio conversion value 1.2 and DL_{CO} to estimate DL_{O_2} (Forster, 1964). The ratio (\dot{V}_{O_2}/DL_{O_2}) relates the oxygen partial pressure gradient ΔP_{O_2} between alveolar air and pulmonary capillary blood and is a function of the total O_2 transfer rate. In the loggerhead sea turtle, this pressure difference is 10.4 Torr, which is considerably lower than the values estimated for the varanid lizard and other reptiles. Crawford *et al.* (1976) argue that the large driving gradient required for O_2 conductance in some reptiles is the result of a low lung O_2 diffusion capacity in relation to uptake. This finding clearly does not apply in large varanid lizards and sea turtles, which have large respiratory surface areas (Tenney & Tenney, 1970).

Table 2. *Comparative oxygen transport properties of reptiles and man*

Species	D_{LCO} (ml min^{-1} $\text{kg}^{-1} \text{Torr}^{-1}$)	a-v difference (vol%)	\dot{Q}_L/\dot{V}_{O_2}	P_{50} (Torr)
Loggerhead sea turtle ¹	0.11	2.1	59.3	47
Green sea turtle ^{1,2}	0.04	4.0	24.4	29
Varanid lizard ³	0.06	4.0	25.4	32
<i>Pseudemys</i> ⁴	0.04	1.7	57.8	21
Human ^{5,*}	0.31	4.1	23.2	27

Values at 25 or *37°C recorded using rebreathing method, pH 7.4 or 7.5.

1, this study and Lapennas & Lutz, 1982; 2, Wood, Gatz & Glass, 1984; 3, Wood, Johansen & Gatz, 1977; 4, Burggren, Glass & Johansen, 1977; 5, Dejours, 1981.

The values for pulmonary perfusion (\dot{Q}_L) of *C. caretta* at 25°C are higher than those reported by Glass *et al.* (1981) for *Varanus* and *Tupinambis* under resting conditions (34.3 and 30.8 $\text{ml min}^{-1} \text{kg}^{-1}$, respectively) and are significantly higher than values given by Wood *et al.* (1984) for *Chelonia mydas* (24 $\text{ml min}^{-1} \text{kg}^{-1}$). Nonetheless, similar perfusion rates were found in the semiaquatic turtle *Pseudemys scripta* (57.8 $\text{ml min}^{-1} \text{kg}^{-1}$, Crawford *et al.* 1976).

Oxygen uptake of the loggerhead turtles was determined simultaneously with pulmonary blood flow. Under these circumstances, the Fick principle can be used to estimate the O_2 content difference between pulmonary venous and arterial blood (a-v difference) as well as a mean blood convection requirement, \dot{Q}_L/\dot{V}_{O_2} . These data and other aspects of the oxygen transport system of *C. caretta* are compared with other reptiles and man in Table 2.

It is interesting that compared to values reported by Wood *et al.* (1984) and Crawford *et al.* (1976) for some large green turtles and varanid lizards, the loggerhead turtle has a small a-v difference and a markedly higher blood convection requirement. Yet both sea turtles and large varanid lizards are highly active, aerobic reptiles. Differences in O_2 transport mechanisms in two reptilian groups having comparable levels of aerobic metabolism may be related to breathing patterns.

To retain a steady transfer of oxygen in several species of large varanid lizards, the lungs are ventilated in a rhythmic, almost continuous pattern (Shelton, Jones & Milsom, 1985). In consequence, a relationship can be seen between varanids and the oxygen delivery patterns in terrestrial birds and mammals that normally sustain a high alveolar oxygen content and a large, constant a-v difference (Piiper, 1982). The loggerhead turtle, in contrast, has an episodic, breathhold ventilatory pattern and inspires before diving. Under these conditions, Lapennas & Lutz (1982) have shown that the lung must be used both for oxygen storage and for gas transfer if submergence is prolonged.

We have measured an arterial P_{O_2} of approximately 100–110 Torr and a venous P_{O_2} of 60 Torr before voluntary dives in loggerhead turtles, which, based on the oxygen dissociation curves (Lapennas & Lutz, 1982), point to predive arterial and venous

blood being about 95 % and 88 % saturated, respectively. In the loggerhead sea turtle the blood is almost fully saturated at the beginning of a dive, and may serve as an additional O₂ store to the lung. However, a consequence of high venous loading is that pulmonary perfusion must remain high in order to sustain an elevated \dot{V}_{O_2} with a minimal a-v difference.

During prolonged submergence, there is evidence that diving sea turtles deplete lung, arterial and venous O₂ stores (Lutz & Bentley, 1985). Under these circumstances, a high pulmonary diffusion capacity may be of significance since the driving gradients for O₂ transport in the lung-blood circuit are progressively reduced. This contrasts with the mammalian-like breathing pattern and nearly constant oxygen delivery characteristics of the aforementioned varanids that have unrestricted access to air.

Finally, since it appears, on the basis of high pre-dive arterial saturation and the lack of a substantial R-L shunt (Wood *et al.* 1984), that gas exchange in the sea turtle lung is not diffusion-limited, it is interesting to consider what factors limit its aerobic performance. In sea turtles ventilatory frequency increases with exercise load (Prange, 1976; Butler *et al.* 1984). Yet tidal exchange in chelonians is coupled to locomotor muscles (George & Shah, 1959) and sea turtles usually change their power swimming stroke in order to breathe (M. E. Lutcavage, personal observation). An increase in swimming speed during a breathhold dive would lead to an increased rate of depletion of lung P_{O₂} and an increased breathing frequency. However, lifting the head to breathe increases the energetic cost of swimming (Prange, 1976) and necessitates a break in swimming speed. It is likely that the sea turtle's maximal rate of activity is ultimately restricted by how often it must ventilate its lung. This also applies to activity on land, since laborious nesting manoeuvres are likewise coupled to locomotory movements and nesting is interrupted while the female stops to breathe.

In conclusion, respiratory patterns in the sea turtle contrast with those of mammals and terrestrial reptiles, where lung gas is continuously exchanged during exercise and alveolar P_{O₂} is maintained at a much higher partial pressure than in arterial blood. In the loggerhead turtle lung, the low resistance to gas transfer conferred by a high diffusion capacity enables the lung to be used as an oxygen store, even though alveolar and venous P_{O₂} decline to low values. This would allow the turtle to minimize trips to the surface to top up its lung, thereby reducing its energetic costs.

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