



Ventilation and gas exchange in two turtles: *Podocnemis unifilis* and *Phrynops geoffroanus* (Testudines: Pleurodira)[☆]



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ABSTRACT

Turtles (Testudines) have two major taxa, Pleurodira and Cryptodira. To date, only limited data are available regarding the respiratory physiology of pleurodirans. To begin to address this, we studied ventilation and gas exchange in *Podocnemis unifilis* and *Phrynops geoffroanus*. Breathing pattern in both species could be described as episodic with breathing episodes separated by large non-ventilatory periods. We measured duration of inspiration and expiration, breathing frequency, duration of the non-ventilatory period (time between episodes), tidal volume, and oxygen consumption when breathing normoxia, hypoxia and hypercarbia at 25 °C. In both species hypercarbia caused a greater increase in ventilation compared to hypoxia, increasing both breathing frequency and tidal volume. Minute ventilation and oxygen consumption in *P. geoffroanus* were the lowest described so far in testudines, indicating either extra-pulmonary gas exchange or a significantly lower metabolism. Oxidative costs of breathing, estimated using the regression method, was the highest described so far for any reptile. Further studies are necessary to better understand respiratory physiology in *Phrynops* and *Podocnemis* species.

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1. Introduction

Aspiration breathing occurs in amniotes through passive expansion of the lungs by contraction of skeletal muscles (Brainerd, 1999). These muscles, which may work alone or together to cause inspiratory air flow, are the intercostal muscles, abdominal muscles or some derived form like the mammalian or crocodilian diaphragmatic muscles (Brainerd and Owerkowitz, 2006; Klein and Owerkowitz, 2006). Testudines possess a rigid shell with immovable ribs and face the problem of ventilating their lungs within this encasement. Due to the constraints of a rigid shell on ventilation, functional morphology of the respiratory system and mechanics of breathing in turtles has been of interest to scientists for centuries (Bojanus, 1821; Druzisky and Brainerd, 2001; Gans and Hughes, 1967; Gaunt and Gans, 1969; Hansemann, 1915; Jackson, 1971; Lambert et al., 2010; Landberg et al., 2003; Landberg et al.,

2009; McCutcheon, 1943; Milsom and Johansen, 1975; Mitchell and Morehouse, 1863; Shah, 1962; Shaw and Baldwin, 1935; Townson, 1799; Vitalis and Milsom, 1986a,b). Testudines' solution for their morphological constraint on breathing is to perform ventilatory movements using groups of abdominal muscles associated with their legs (Gans and Hughes, 1967; Gaunt and Gans, 1969). Contractions of these muscles move the viscera and thereby compress or expand the lungs resulting in inspiration or expiration (Gans and Hughes, 1967; Gaunt and Gans, 1969). Ventilatory movements powered by these muscles are carried out at optimal combinations of breathing frequency and tidal volume, thereby keeping the mechanical work of breathing at a minimum (Vitalis and Milsom, 1986a,b).

Estimates of the oxidative cost of ventilation in turtles, however, have yielded significantly different values. Kinney and White (1977) estimated metabolic cost of breathing in *Pseudemys floridana* to be around 10% of resting metabolic rate (RMR) at 37 °C, increasing up to 30% of RMR at 10 °C body temperature. Using a different methodological approach, Jackson et al. (1991) obtained a metabolic cost of breathing of less than 1% of RMR at 25 °C in *Chrysemys picta bellii*. The controversy regarding metabolic cost of breathing is not restricted to turtles, since similar variation has been found in other reptiles. Wang and Warburton (1995), studying *Alligator mississippiensis*, found metabolic cost of breathing to be

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15% of RMR during hypoxic and 1–5% of RMR during hypercarbic exposures. In *Tupinambis merianae*, Skovgaard and Wang (2004) estimated metabolic cost of breathing to be 17% of RMR during hypoxia and less than 1% of RMR during hypercarbia.

The taxon Testudines is composed of Pleurodira (side-neck turtles) and Cryptodira (freshwater turtles, sea turtles, snapping turtles, soft shell turtles, tortoises). Very few data are available regarding lung ventilation in side-neck turtles (Burggren et al., 1977; Glass et al., 1978a) and our ideas about turtle ventilation and gas exchange are mainly based on studies using cryptodires. Since Pleurodira and Cryptodira form separate phylogenetic lineages for about 150 million years (Sterli et al., 2013) it is of interest to study ventilation and gas exchange in pleurodiran species and compare these data with available data from cryptodiran species. In the present study we measured ventilation in the yellow-spotted river turtle (*Podocnemis unifilis*) and in Geoffroy's side-necked turtle (*Phrynops geoffroanus*) during normoxia as well as during hypoxic and hypercarbic exposures with the aim to calculate oxidative cost of breathing in these pleurodiran species.

2. Materials and methods

2.1. Animals

Adult individuals of both sexes of *P. unifilis* ($M_B = 0.875 \pm 0.085$ kg; $N = 5$) and *P. geoffroanus* ($M_B = 1.872 \pm 0.288$ kg; $N = 6$) living under natural conditions were obtained from the Jacarezário, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Rio Claro, SP, Brazil and maintained for 3–5 month before experimentation to acclimate to laboratory conditions at the University of São Paulo in Ribeirão Preto, SP, Brazil. Experiments were performed between September and December and animals were used under licence number 35221-1 (SISBIO, Brazil) and animal care protocol number 12.1.1541.53.0 (CEUA USP/Campus de Ribeirão Preto).

2.2. Setup

Ventilation and gas exchange was measured following Glass et al. (1978b), Wang and Warburton (1995) and Silva et al. (2011). Feeding was suspended seven days before experimentation. Individuals were placed into a chamber filled with water which was sealed with a lid so that animals had to breathe into a funnel. This chamber was placed into an aquarium to maintain water temperature at 25 ± 1 °C. The size of the animal chamber was adapted to the size of each species, restricting the animal's movements. To breathe, an individual only needed to extend its neck and protrude its nostrils into the funnel. The funnel aperture was connected to a pneumotach (Fleisch tube) which was in turn connected to a spirometer (FE141 ADInstruments). Air inside the funnel was sampled, dried, and pushed to a gas analyser (ML206 ADInstruments). Data were recorded and analysed using Powerlab and LabChart 7.0 (ADInstruments). The spirometer and funnel were calibrated by injecting known volumes of gas using syringes; air was used for the spirometer calibration and different volumes of O_2 and CO_2 were used to calibrate the gas exchange measurements. Regression lines resulting from these calibrations showed good fit ($R^2 > 0.9$) and were used to calculate tidal volume (V_T), oxygen consumption (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}).

Gas mixtures were provided using a Pegas gas mixer (COLUMBUS) at a constant flow rate of 2 l/min, directed into the funnel.

2.3. Experimental protocol

Animals were placed into the experimental setup at least 12 h before any measurements. Ventilation and gas exchange were measured initially for 2 h during normoxic conditions, followed by

progressively decreasing hypoxic (9, 7, 5, 3% O_2) or progressively increasing hypercarbic (1.5, 3.0, 4.5, 6.0% CO_2) exposures, with the animals being exposed to each gas mixture for 2 h.

2.4. Data analysis

At each gas mixture, the last 30 min of exposure were used to extract the following data: breathing frequency (f_R), breathing frequency during breathing episodes (f_{Repi}), tidal volume (V_T), duration of expiration (T_{EXP}), duration of inspiration (T_{INSP}), total duration of one ventilatory cycle ($T_{TOT} = T_{EXP} + T_{INSP}$), duration of non-ventilatory period (T_{NVP}), and oxygen consumption (\dot{V}_{O_2}). From these data minute ventilation (\dot{V}_E), oxygen consumption (\dot{V}_{O_2}), and air convection requirement (\dot{V}_E/\dot{V}_{O_2}) were calculated.

Data were analysed using GraphPad Prism 6.0 applying Repeated Measures ANOVA followed by a Tukey's multiple comparison test. A value of $P < 0.05$ was considered significant.

3. Results

Breathing pattern in both species can be described as episodic with large non-ventilatory periods between breathing episodes (Fig. 1). A ventilatory cycle starts with an expiration followed by an inspiration, which are of equal duration in both species, and under normoxia *P. unifilis* spends half the time in inspiration and expiration than *P. geoffroanus* (Fig. 2). Hypoxic exposure elicits small changes in the above pattern, not significantly altering T_{INSP} , T_{EXP} and T_{TOT} in either species (Fig. 2), but significantly reducing f_{Repi} at all hypoxic levels in *P. unifilis* and at 7 and 5% O_2 in *P. geoffroanus* (Fig. 1a). Hypercarbia, on the other hand, significantly increases T_{INS} and T_{TOT} in *P. geoffroanus* (Fig. 2b and f) and significantly increases f_{Repi} in *P. unifilis* (Fig. 1b). Overall f_R changes significantly during 3% hypoxia only in *P. geoffroanus* but during hypercarbia both species increase f_R significantly (Fig. 3a and b), *P. geoffroanus* only at 6% CO_2 , but *P. unifilis* at 3.0, 4.5, and 6.0% CO_2 . The increase in f_R during hypercarbia significantly reduces T_{NVP} in both species (Fig. 1d). During hypoxia, T_{NVP} showed a significant reduction in both species at 3% O_2 (Fig. 1c).

Compared to normoxia, V_T and \dot{V}_E increased significantly at the lowest O_2 and greatest CO_2 concentration in both species, respectively. This increase, however, was more pronounced in *P. unifilis* during hypercarbia (Fig. 3). Oxygen consumption did not change in *P. unifilis* during hypoxia but increased significantly during hypercarbia, whereas in *P. geoffroanus* \dot{V}_{O_2} increased significantly at 3 and 5% oxygen but was unaffected by hypercarbia (Fig. 4a and b). Air convection requirement did increase significantly at 3% O_2 when compared to normoxia in *P. geoffroanus*, but was unaffected in *P. unifilis* (Fig. 4c). Under hypercarbia, \dot{V}_E/\dot{V}_{O_2} increased in both species, being significantly different from normoxic values at 6% CO_2 in *P. geoffroanus* and 4.5% CO_2 in *P. unifilis*, respectively (Fig. 4d).

4. Discussion

4.1. Normoxic conditions

Breathing pattern in both species followed the general chelonian pattern of episodic breathing, where long non-ventilatory periods are separated by short breathing episodes containing several ventilatory cycles. Such a pattern may be an advantage in semi-aquatic and aquatic turtles by reducing the time spend at the water surface to breathe, thereby reducing exposure time to potential predators. The length of inspiration and expiration were similar to the ones observed in other testudines (Benchetrit and Dejours, 1980; Milsom and Jones, 1980), however, the T_{INSP} , T_{EXP} , and T_{TOT} values

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