

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



Tábata E.F. Cordeiro^a, Augusto S. Abe^{b,c}, Wilfried Klein^{c,d,*}

^a Master Program in Animal Diversity, Federal University of Bahia, Brazil

^b State University of São Paulo, Rio Claro, Brazil

^c National Institute of Science and Technology of Comparative Physiology, Brazil

^d School of Philosophy, Sciences and Literature Ribeirão Preto, University of São Paulo, Brazil

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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 crocodylian 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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* Corresponding author at: Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900, Bairro Monte Alegre, CEP: 14040-901 Ribeirão Preto, SP, Brazil.

Tel.: +55 1633150452; fax: +55 1633154886.

E-mail addresses: wklein@usp.br, klein@ffclrp.usp.br (W. Klein).

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 O₂ and CO₂ 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% O₂) or progressively increasing hypercarbic (1.5, 3.0, 4.5, 6.0% CO₂) 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% O₂ 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% CO₂, but *P. unifilis* at 3.0, 4.5, and 6.0% CO₂. 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% O₂ (Fig. 1c).

Compared to normoxia, V_T and \dot{V}_E increased significantly at the lowest O₂ and greatest CO₂ 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% O₂ 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% CO₂ in *P. geoffroanus* and 4.5% CO₂ 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

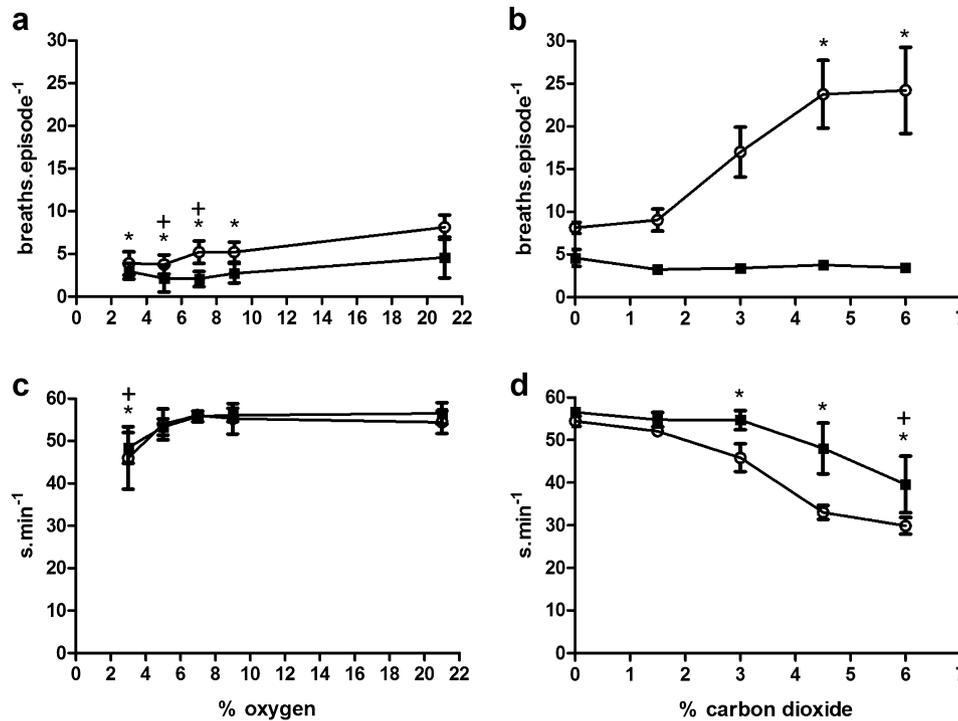


Fig. 1. Breathing frequency during ventilatory episodes (a, b) and duration of non-ventilatory period (c, d) in *Podocnemis unifilis* (open circles) and *Phrynops geoffroanus* (filled square) during hypoxic and hypercarbic exposures. * for *P. unifilis* and + for *P. geoffroanus* denote values that are significantly different from normoxic values.

of *P. unifilis* seem to be lower than previously described for other testudines.

Values for f_R , V_T , \dot{V}_E and \dot{V}_{O_2} of *P. unifilis* fall within the range seen in other species of chelonians (Table 1), with \dot{V}_{O_2} being greater than in other semi-aquatic testudines. This could indicate that animals were not fully resting during the experiments. However, due to the shortage of \dot{V}_{O_2} data from other species, no definite conclusions can be drawn.

Normoxic f_R and V_T in *P. geoffroanus* are at the lower end when compared to other species, but especially \dot{V}_E and \dot{V}_{O_2} are much lower than previously described for testudines (Table 1). These low values could indicate a significant extra-pulmonary gas exchange or metabolic depression during the experiments. It is well known that chelonians possess the ability to exchange gases with surrounding water (Belkin, 1968; Gage and Gage, 1886; Lengler and Winokur, 1983). Aquatic gas exchange may occur through the skin,

Table 1
Respiratory variables of Testudines during normoxia at 20–25 °C. Data are given as mean \pm standard deviation.

Species	f_R (breaths min ⁻¹)	V_T (ml kg ⁻¹)	\dot{V}_E (ml kg ⁻¹ min ⁻¹)	\dot{V}_{O_2} (mlO ₂ kg ⁻¹ min ⁻¹)	Source
<i>Apalone ferox</i>				0.87 \pm 0.6	Bagatto and Henry (1999)
<i>Apalone spinifera</i>				0.36 \pm 0.03	Bagatto and Henry (1999)
<i>Chelonia mydas</i>	0.55 \pm 0.14	4.5 \pm 0.5	17.5 \pm 3.2	1.0 \pm 0.12	Jackson (1985)
<i>Chrysemys picta bellii</i>				0.50 \pm 0.31	Stockard and Gatten (1983)
<i>Chrysemys picta bellii</i>			8.7 \pm 3.6	0.25 \pm 0.04	Jackson et al. (1991)
<i>Chrysemys picta bellii</i>	1.90 \pm 0.27	10.7 \pm 1.2	17.5	0.60	Glass et al. (1985)
<i>Chrysemys picta bellii</i>	1.9 \pm 0.3	9.4 \pm 3.1	16.7 \pm 4.0	0.58 \pm 0.10	Silver and Jackson (1985)
<i>Chrysemys picta bellii</i>	1.8 \pm 0.2	13.5 \pm 0.9	24.8 \pm 3.4		Milsom and Jones (1980)
<i>Pelomedusa subscrofa</i>	1.6 \pm 0.7	33.0 \pm 26.0	39.8 \pm 18.6		Burggren et al. (1977)
<i>Pelomedusa subscrofa</i>	1.4 \pm 0.2	15.5 \pm 2.4	22.4 \pm 5.6		Glass et al. (1978a,b)
<i>Phrynops geoffroanus</i>	0.6 \pm 0.27	3.1 \pm 1.1	1.7 \pm 0.6	0.07 \pm 0.03	This study
<i>Podocnemis unifilis</i>	2.4 \pm 1.0	9.9 \pm 1.4	26.0 \pm 7.6	1.13 \pm 0.7	This study
<i>Pseudemys floridana</i>			28.5	0.9	Kinney and White (1977)
<i>Testudo graeca</i>				1.54 \pm 0.15	Crawford et al. (1976)
<i>Testudo horsfieldi</i>	1.4	8.1	11.5		Benchetrit and Dejours (1980)
<i>Testudo pardalis</i>	2.9 \pm 2.4	11.0 \pm 6.0	25.7 \pm 12.5		Burggren et al. (1977)
<i>Testudo pardalis</i>	2.9 \pm 0.3	10.7 \pm 2.7	13.4 \pm 3.6		Glass et al. (1978a,b)
<i>Trachemys scripta</i>				1.05 \pm 0.13	Bagatto and Henry (1999)
<i>Trachemys scripta</i>				0.90 \pm 0.07	Crawford et al. (1976)
<i>Trachemys scripta</i>			36 \pm 4		Hitzig (1982)
<i>Trachemys scripta</i>	0.82 \pm 0.2	38.0 \pm 8.0	31.0 \pm 4.0		Hitzig and Nattie (1982)
<i>Trachemys scripta</i>	0.95 \pm 0.1	20.0 \pm 3.0	19.0 \pm 4.0		Hitzig and Nattie (1982)
<i>Trachemys scripta</i>	1.03 \pm 0.2	23.0 \pm 5.0	24.0 \pm 6.0		Hitzig and Nattie (1982)
<i>Trachemys scripta</i>	2.0 \pm 0.07	6.9 \pm 1.2	13.8		Vitalis and Milsom (1986a,b)
<i>Trachemys scripta</i>	1.6 \pm 0.8	18.5 \pm 7.0	25.6 \pm 12.9	0.65 \pm 0.28	Jackson (1971)
<i>Trachemys scripta</i>	1.40 \pm 0.15			0.69 \pm 0.08	Jackson (1973)
<i>Trachemys scripta</i>	1.6 \pm 0.2	15.7 \pm 2.1	23.8 \pm 3.4	0.70 \pm 0.08	Jackson et al. (1974)

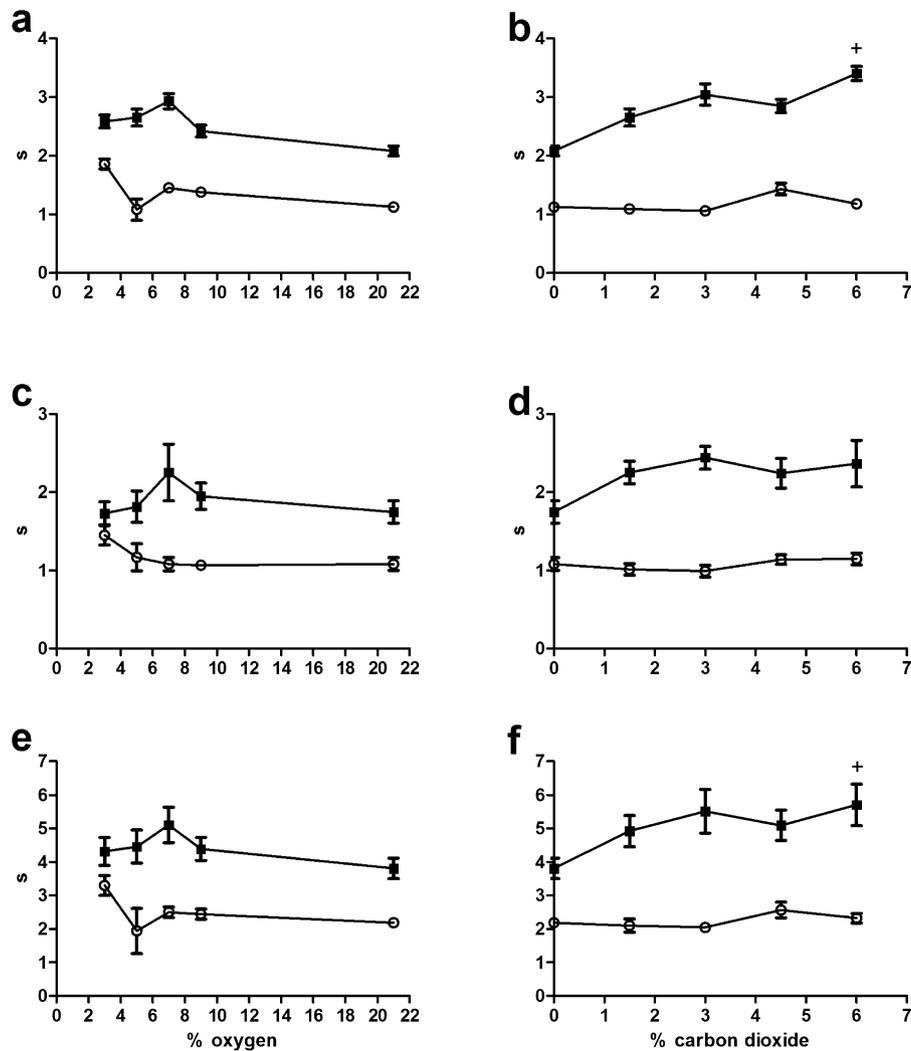


Fig. 2. Duration of inspiration (a, b), duration of expiration (c, d), and total time of a ventilatory cycle (e, f) in *Podocnemis unifilis* (open circle) and *Phrynops geoffroanus* (filled square) during hypoxic and hypercarbic exposures. * for *P. unifilis* and + for *P. geoffroanus* denote values that are significantly different from normoxic values.

buccopharyngeal epithelia, and/or the cloacal bursae (Jackson et al., 2004; King and Heatwole, 1994). Cloacal bursae are appendices to the cloaca that may be highly vascularised and used for aquatic respiration by actively ventilating the cloacal bursae with water (King and Heatwole, 1994). Future studies will be necessary to verify the existence of aquatic gas exchange in *P. geoffroanus* in order to explain the low oxygen consumption observed here.

Both species are occupying similar habitats, frequenting slow flowing rivers, lakes, lagoons or swamps (Ernst and Barbour, 1989). They have been studied regarding reproductive behavior and diet, mostly using captive animals (Malvasio et al., 2003; Souza, 2004), but aspects regarding respiratory behavior are unknown. *P. unifilis*, mostly herbivorous, is known to employ neustophagia, a feeding mechanism to extract food particles from the water surface (Ernst and Barbour, 1989). *P. geoffroanus*, a mostly carnivorous species, has been shown to dwell successfully in urban rivers (Souza and Abe, 2001) even in much polluted waters (Pinã et al., 2009). If these ecological differences are related to differences in respiratory physiology remains to be elucidated.

4.2. Effects of hypoxia and hypercarbia

The ventilatory responses to hypoxic and hypercarbic exposures in both species are similar and comparable to previously

described results. Hypoxia results in slightly increased ventilation at severe levels of hypoxia (about 3% O₂) only, with tidal volume being increased more than breathing frequency to increase ventilation. Similar results regarding hypoxia have been observed as well in other chelonians (Burggren et al., 1977; Glass et al., 1983; Hitzig and Nattie, 1982; Jackson, 1973). In *P. unifilis* hypercarbia acted as a stronger ventilatory stimulus than hypoxia, increasing ventilation significantly at CO₂ levels of 4.5 and 6%, mainly due to increases in f_R and less in V_T . Such responses have been found in other semi-aquatic turtles (Hitzig and Nattie, 1982; Jackson et al., 1974; Milsom and Jones, 1980; Silver and Jackson, 1985; Vitalis and Milsom, 1986b).

Oxygen consumption in *P. unifilis* followed the same pattern as ventilatory parameters, with hypercarbia significantly increasing \dot{V}_{O_2} but not hypoxia. The already very low values of \dot{V}_{O_2} found in *P. geoffroanus* were unaltered by hypercarbic exposure and increased significantly but only slightly at 3 and 5% O₂. Whether this is due to extra-pulmonary gas exchange or metabolic depression remains unknown. Air convection requirement increased significantly in both species during hypoxia and hypercarbia, just as in other chelonians (Jackson, 1973; Jackson et al., 1974).

The increases in ventilation during hypoxia or hypercarbia are achieved in both species by reducing T_{NVP} , but maintaining T_{INSP} and T_{EXP} constant. This pattern has been previously described

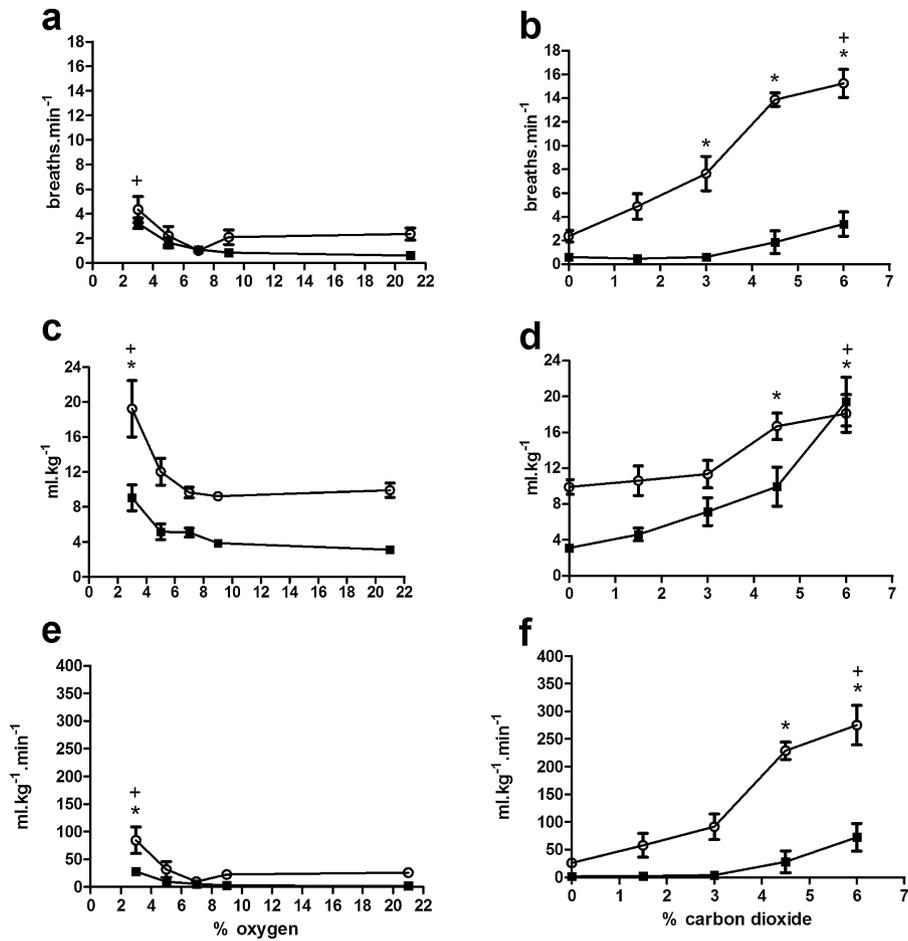


Fig. 3. Breathing frequency (a, b), tidal volume (c, d), and minute ventilation (e, f) in *Podocnemis unifilis* (open circle) and *Phrynosops Geoffroyanus* (filled square) during hypoxic and hypercarbic exposures. * for *P. unifilis* and + for *P. Geoffroyanus* denote values that are significantly different from normoxic values.

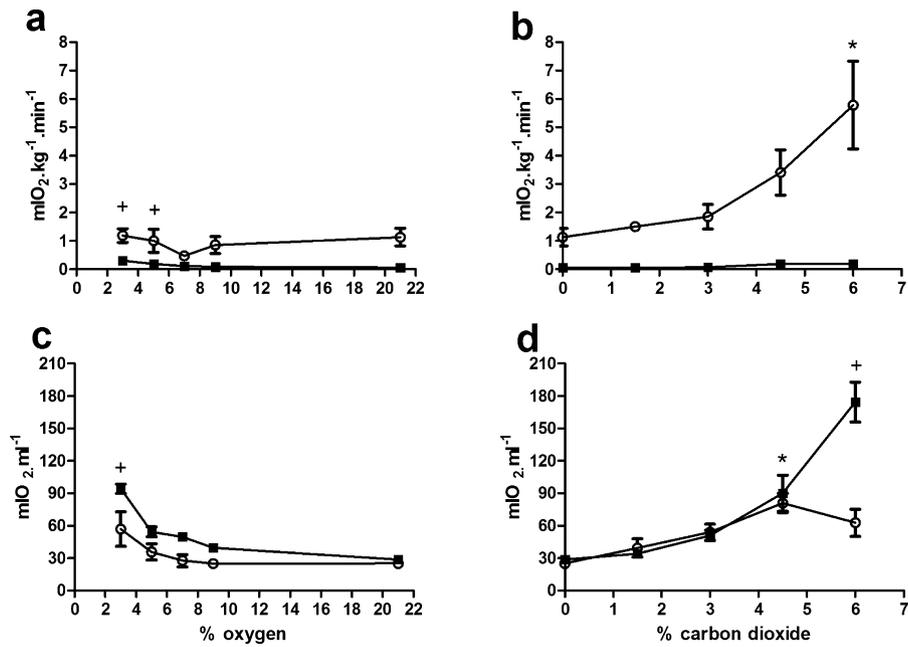


Fig. 4. Oxygen consumption (a, b) and air convection requirement (c, d) in *Podocnemis unifilis* (open circle) and *Phrynosops Geoffroyanus* (filled square) during hypoxic and hypercarbic exposures. * for *P. unifilis* and + for *P. Geoffroyanus* denote values that are significantly different from normoxic values.

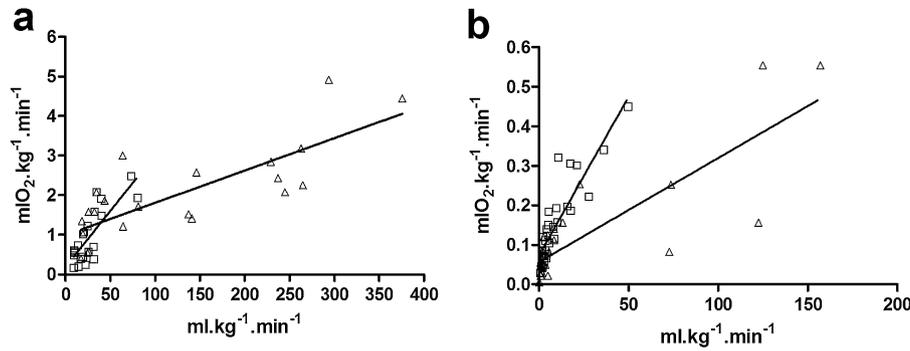


Fig. 5. Ventilation data plotted against oxygen consumption in *Podocnemis unifilis* (a) and *Phrynosops geoffroanus* (b) during hypoxic (square) and hypercarbic (triangle) exposures. Data points represent \dot{V}_E and \dot{V}_{O_2} values obtained from each individual during normoxic, hypoxic and hypercarbic exposures. Regarding hypoxia and hypercarbia in *P. unifilis* two and three outliers, respectively, have been removed to calculate the linear regression lines given for hypoxia and hypercarbia separately.

(Milsom, 1988) and corroborates the conclusion by Vitalis and Milsom (1986b), that increases in \dot{V}_E are most economically achieved by maintaining $T_{I\text{NSP}}$ and T_{EXP} constant while reducing T_{NVP} . The instantaneous breathing frequency ($f' = 60 \cdot T_{\text{TOT}}^{-1}$), a measure for the minimisation of mechanical work of breathing, was found to be $26.0 \pm 3.7 \text{ min}^{-1}$ for *P. unifilis* and $13.3 \pm 1.7 \text{ min}^{-1}$ for *P. geoffroanus* (mean and standard deviation calculated from all treatments). Vitalis and Milsom (1986b) reported f' to be $35 \pm 2 \text{ min}^{-1}$ for *Trachemys scripta*. Whether the lower f' values found in the present study represent different mechanical properties of each species' respiratory system, remains to be elucidated.

4.3. Cost of breathing

To estimate cost of breathing, all \dot{V}_E and \dot{V}_{O_2} values from each individual during normoxia, hypoxia and hypercarbia were plotted (Fig. 5). Some values from *P. unifilis* during hypercarbic (3 data points removed) and hypoxic exposures (2 data points removed) were considered as outliers, and excluded from cost calculations. Linear regressions were calculated for hypercarbic and hypoxic exposures separately and regressions showed a good fit with $r^2 > 0.6$ in all cases. Values obtained from these regressions, however, are amongst the largest ones reported so far for reptiles (Tables 2 and 3), especially the estimate derived from hypoxic exposure in *P. unifilis* which is about 4.5 times larger than the largest reported so far for a resting reptile.

Estimates of oxidative cost of breathing using mainly the regression method have been attempted only in a few species of reptiles and range from $0.006 \text{ mlO}_2 \text{ ml}^{-1}$ down to $-0.0019 \text{ mlO}_2 \text{ ml}^{-1}$ (Table 3). Interestingly, the extremes of oxidative cost of breathing have been found in the same species, *T. merianae*, yielding estimates of relative cost of breathing of 17% and -5% , respectively (Skovgaard and Wang, 2004). In the same species, Andrade and Abe (1999) estimated cost of breathing in dormant tegu lizards

Table 2

Oxidative cost of ventilation of *Podocnemis unifilis* and *Phrynosops geoffroanus* calculated using the regression method pooling individual values for a species and respiratory stimulus.

Species	Stimulus	Oxidative cost ($\text{mlO}_2 \text{ ml}^{-1}$)	r^2
<i>P. unifilis</i>	Hypoxia	0.02826	0.6340
<i>P. unifilis</i>	Hypercarbia	0.008163	0.6316
<i>P. geoffroanus</i>	Hypoxia	0.008018	0.7791
<i>P. geoffroanus</i>	Hypercarbia	0.002628	0.6979

to be around 52% of total metabolic rate. Such large differences in percentages of cost of breathing may be due to methodological limitations of the regression method. This method relies on the assumptions that (1) during hypoxia or hypercarbia only metabolic activity related to lung ventilation changes without metabolic changes in tissues not related to ventilation, and that (2) ventilation and oxygen consumption follow a linear relationship (Skovgaard and Wang, 2004). Since hypoxia or hypercarbia may depress metabolism, the first assumption may be violated, and estimates of oxidative cost of breathing may thus be too high. Currently, we do not know if hypoxia or hypercarbia have any depressive effect on tissue metabolism in the species studied here.

The second assumption may not have been violated in *P. unifilis* where the increase in \dot{V}_E is mainly achieved by increasing f_{RepI} with several ventilatory cycles following one another without any T_{NVP} . Since $T_{I\text{NSP}}$ and T_{EXP} did not change in this species, every ventilatory bout may be considered mechanically isolated from the previous one (Skovgaard and Wang, 2004). However, estimates of oxidative cost of breathing remain to be treated cautiously and different methodological approaches need to be tested. For example, using normoxic, hypoxic and hyperbaric data together suggests that a non-linear curve fit results in a better description for oxidative cost of breathing when compared to the linear regression method.

Table 3

Oxidative cost of ventilation of reptiles calculated using the regression method.

Species	Stimulus	Oxidative cost ($\text{mlO}_2 \text{ ml}^{-1}$)	Source
<i>Alligator mississippiensis</i>	Hypoxia	-0.0008	Wang and Warburton (1995)
<i>Alligator mississippiensis</i>	Hypercarbia	0.005	Wang and Warburton (1995)
<i>Amphisbaena alba</i>	Hypoxia	0.006	Abe and Johansen (1987)
<i>Amphisbaena alba</i>	Hypercarbia	0.004	Abe and Johansen (1987)
<i>Chrysemys picta</i>	Hypercarbia	0.00012	Jackson et al. (1991)
<i>Natrix rhombifera</i>	Hypoxia	-0.001	Gratz (1979)
<i>Natrix rhombifera</i>	Hypercarbia	0.0003	Gratz (1979)
<i>Pseudemys floridana</i>	UDV	0.0047	Kinney and White (1977)
<i>Tupinambis merianae</i>	Hypoxia	0.006	Skovgaard and Wang (2004)
<i>Tupinambis merianae</i>	Hypercarbia	-0.0019	Skovgaard and Wang (2004)

UDV = unidirectional ventilation.

In conclusion, the present study presents the first detailed data on ventilation and gas exchange in *P. unifilis* and *P. geoffroanus* during normoxia, hypoxia and hypercarbia, revealing a significantly lower ventilation and oxygen consumption in the latter species. Future studies are warranted to better understand respiratory physiology in *Phrynops* species.

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