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# The metabolic cost of breathing in red-eared sliders: An attempt to resolve an old controversy

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ABSTRACT

Accurately measuring the metabolic cost of breathing in turtles has been a challenge with cost estimates varying greatly between different studies and/or methods used. To determine the source of discrepancy, we calculated costs using two methods in a single group of red-eared sliders (*Trachemys scripta elegans*). The unidirectional ventilation method yielded an estimate of 3.3 ml $O_2$/L air ventilated while the regression method (using hypoxia as a respiratory stimulus) produced an estimate of 0.8 ml $O_2$/L air ventilated when corrected for hypoxia-induced metabolic suppression. Cost differences may be in part due to the non-linear nature of the relationship between metabolic cost and ventilation. They are also likely due to the challenge of accurately estimating costs from irregular, episodic breathing pattern of turtles and the buffering capacity of their large lungs that lead to inconsistency in the amount of $O_2$ extracted from each breath/breathing episode. Given the difficulty in obtaining consistent measures, the values reported here must be taken cautiously.

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## 1. Introduction

Turtles, tortoises and terrapins possess a rigid shell, a characteristic feature of the Order Chelonia. Although the armor-like shell has its advantages, its stiffness greatly restricts the necessary expansion and contraction of the body wall during ventilation (Gans and Hughes, 1967). Early speculation on how chelonians overcame the rigid shell to fill their lungs gave rise to numerous hypotheses on their mechanism of ventilation (Morgagni, 1719; Mitchell and Morehouse, 1863; Wolf, 1933; Hansen, 1941; McCutcheon, 1943; Root, 1949; Dunson, 1966; Gans and Hughes, 1967; Belkin, 1968; Gaunt and Gans, 1969; Heatwole et al., 1973; Jackson et al., 1979; Jackson and Prange, 1979; Bagatto and Henry, 1999; Druzisky and Brainerd, 2001; Landberg et al., 2003, 2009). It is now well established that contraction of two muscles of the posterior flank cavities, the transverse (expiratory) and the oblique (inspiratory) abdominus, produce the primary forces that drive ventilation (Gans and Hughes, 1967; Gaunt and Gans, 1969; Landberg et al., 2003). In reptiles, both inspiration and expiration are active processes (Gans and Hughes, 1967) during which these respiratory muscles work and consume oxygen to inflate and deflate the lungs. Attempts to calculate this metabolic cost of ventilation in reptiles, and more specifically in turtles, however, has produced discrepant findings.

Two early investigations by Jackson et al. (1991) and Kinney and White (1977) calculated cost of ventilation to be 1 and 20% of total metabolism respectively for animals at 20 °C. These two studies used two different methods to calculate the metabolic cost of breathing and although the two methods varied, they should, in principle, have produced the same results. Kinney and White (1977) first estimated the metabolic cost of breathing in the Florida cooter, *Pseudemys floridana,* at three different temperatures using a method employing unidirectional ventilation (UDV) through the lungs. By calculating the difference in $O_2$ consumption during spontaneous resting ventilation and zero ventilation (spontaneous breathing was suppressed by supplying fresh gas directly to the lungs and producing a unidirectional flow of gas through the respiratory system), they estimated the relative metabolic cost of breathing to be approximately 4.7 ml $O_2$/L air ventilated at 20 °C. This was 20% of resting metabolism and suggested that a significant portion of total metabolism was dedicated to the act of breathing.

A later investigation conducted by Jackson et al. (1991) re-examined the metabolic cost of breathing in the painted turtle, *Chrysemys picta* using a regression method, a method frequently adopted in subsequent studies estimating the metabolic cost of breathing in various reptiles and mammals (see Frappell et al.,

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1992; Wang and Warburton, 1995; Skovgaard and Wang, 2004). With this method, a respiratory stimulus (e.g., hypoxia or hypercapnia) is used to stimulate an increase in breathing. The increase in ventilation and the simultaneous increase in $O_2$ consumption are measured and the relationship between the two variables is subsequently used to calculate the oxygen consumption that should occur when ventilation falls to zero (by regression). Using this method with hypercapnic exposures (5% $CO_2$), Jackson et al. calculated the metabolic cost of breathing to be approximately 0.12 ml $O_2$/L air ventilated at 25 °C. This was less than 1% of resting metabolism, a much smaller value than that estimated by Kinney and White (1977).

More recently the regression method has been used to estimate the metabolic cost of breathing in two side necked turtles, *Podocnemis unifilis* and *Phrynops geoffroanus*) yielding values of 28.3 and 8.0 ml $O_2$/l air ventilated respectively when hypoxia was used as a stimulus and 8.1 and 2.6 ml $O_2$/l air ventilated respectively when hypercapnia was used as a stimulus (Cordeiro et al., 2014).

Both methods operate under two assumptions: all metabolic functions other than ventilation remain constant throughout the trials and, the correlation between $O_2$ consumption and ventilation is linear. Neither, however, may be the case. In several studies in other reptiles the oxygen uptake-ventilation relationship was non-linear (see Wang and Warburton, 1995; Skovgaard and Wang, 2004) with metabolic costs being greater when starting from zero ventilation and falling off as ventilation increased further. It is plausible that it costs less to simply increase ventilation in an already ventilating animal than to start from zero. It is also known that both hypoxia and hypercapnia, as used in the regression method, can suppress metabolism (Busa and Nuccitelli, 1984; Wang et al., 1993). This is particularly true for $CO_2$ and most likely explains why in some instances the cost of breathing decreased as ventilation increased (refer to Table 3). This begs the question of whether the various studies produced different results because they analyzed different stages of a potentially non-linear relationship under different degrees of metabolic suppression.

The aim of this study, therefore, was to examine these possibilities by estimating the metabolic cost of breathing using both methods in a single group of animals and measuring and correcting for any metabolic suppression. Also, using published values of the mechanical cost of ventilation in turtles, and estimates of muscle efficiency, we compare the metabolic costs determined by both methods with theoretical estimates based on the mechanical work required to produce similar breathing patterns.

## 2. Materials and methods

### 2.1. Animals

Adult red-eared sliders of both sexes were obtained from three commercial suppliers (Lemberger Company, Oshkosh, Wisconsin, USA), Sullivan Company Inc. (Nashville, Tennessee, USA) and Niles Biological Inc. (Sacramento, California, USA). They were housed in a rectangular semi-natural outdoor pond with mud and moss available for burrowing. They had access to a dry terrestrial environment for basking and experienced the natural changes in environmental temperature and photoperiod throughout the year. All turtles were fed on a mixture of trout chow (Aquamax Grower 500, 5D05) and dry pellets for freshwater turtles (Mazuri fresh water turtle diet G190) three times a week during the warmer seasons (spring, summer and fall) and only once a week during winter. Animal housing and experimental procedures followed Canadian Council on Animal Care guidelines and were approved by the University of British Columbia Animal Care Committee (animal care certificate A08-0728).

Prior to experiments turtles were brought indoors and held at room temperature (20–23.5 °C) for up to two weeks in a 90 gallon aquarium with access to a dry platform for basking. They were exposed to full spectrum lights that were set by a timer on a 12 h light, 12 h dark photoperiod and were fasted for up to seven days to avoid the confounding effects of digestion on metabolism. All animals were weighed prior to each experimental treatment and experiments were conducted at the same temperatures as the maintenance temperatures (room temperature ranging between 20 and 23.5 °C).

### 2.2. Series 1 and 2

#### 2.2.1. Preparation

Turtles were sedated with 100% $CO_2$ to reduce their resistance to handling. A collar was placed around the neck and attached to the rostral carapace to prevent the head from being retracted and a mould was made of the turtles' head using dental impression material (Jeltrate-Alginate Impression Material (fast set, Dentsply Caulk, Dentsply International Inc., Milford, Delaware)). The sedation lasted for less than a minute and the turtles were allowed to recover from the sedation for 48 h.

Custom-fitted masks were then made for each turtle as described by Glass et al. (1978) and modified by Wang and Warburton (1995). A plaster cast of the head was made from each mould using Plaster of Paris and a custom-fitted mask that would fit perfectly over each turtle's head was made from a sheet of thermo-forming material (clear-mouthguard, 0.040", Henry Schein Inc., Melville, New York). A hole was cut in the mask at the site of the nostrils and a pneumotachograph was mounted on the mask at this site with tubes leading to a differential pressure transducer (Model DP103-18, Validyne Engineering Corp., USA) connected to an amplifier (Model 7P122E, Grass Instruments, USA) for measuring tidal volume ($V_T$) and breathing frequency ($f_R$).

On the day of the experiment, each turtle was instrumented with a mask sealed onto the head with 3 M™ ESPE™ Impregum™ F Impression Material. A gas mixer (Cameron Instrument Company—GF-3/MP) placed upstream of the turtle generated different gas mixtures at a rate of 1 l $min^{-1}$. Gas was drawn from this stream through a t-tube by a suction pump downstream of the turtle at a rate of 350–500 ml $min^{-1}$ (flow rate varied depending on the size of the turtle to ensure sufficient air supply). This air flow travelled past one end of the pneumotachograph connected to the mask through which the turtle could breathe. The expired gas from the turtle was drawn through a drierite drying column and then through $O_2$ (Raytech $O_2$ analyzer) gas analyzer (Fig. 1A).

#### 2.2.2. Series 1 (episodic breathing at normoxia): experimental protocol

In this pilot series, three turtles (0.49 ± 0.03 kg body weight) were first allowed to adjust to the experimental set-up for several hours while breathing room air. Next, baseline respiratory traces (for $O_2$ consumption ($\dot{V}_{O_2}$) and ventilation ($\dot{V}_E$)) were recorded for three hours in normoxia. Based on analysis of these traces (see results below), it was determined that at least two hours of trace recording was necessary to ensure an accurate estimation of total $\dot{V}_E$ and $\dot{V}_{O_2}$ during episodic breathing in these turtles.

#### 2.2.3. Series 2 (regression method): experimental protocol

In this series, six turtles (0.48 ± 0.06 kg body weight) were first allowed to adjust to the experimental set-up for several hours while breathing room air. Next, baseline respiratory traces (for $O_2$ consumption ($\dot{V}_{O_2}$) and ventilation ($\dot{V}_E$)) were recorded for three hours in normoxia. Following this, turtles were exposed to 3% $O_2$ and then to 1% $O_2$. In all cases, traces were recorded for at least two hours

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