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SHORT COMMUNICATION

NON-INVASIVE MEASUREMENT OF RESPIRATORY TIDAL VOLUME IN AQUATIC, AIR-BREATHING ANIMALS

BY G. D. FUNK, C. L. WEBB AND W. K. MILSOM

Department of Zoology, University of British Columbia, Vancouver, BC, Canada, V6T 2A9

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In recent years, a method which allows breathing patterns to be fully quantified in aquatic, air-breathing species while free-ranging and undisturbed, has come into common use (Brett & Shelton, 1979; Glass, Boutilier & Heisler, 1983, 1985; Butler, Milsom & Woakes, 1984; Silver & Jackson, 1985; Milsom & Chan, 1986). Use of this method in our laboratory, however, has revealed several potential sources of error in the normal procedures used for calibrating this system for the measurement of tidal volume (V_T). In the present study we have systematically studied these potential sources of error under a variety of conditions and describe a technique for eliminating these errors in routine measurements.

Our experimental set-up consisted of a thermostatted aquarium filled with water to the level of a Plexiglas cover containing an opening into a ventilation chamber (Fig. 1). The ventilation chamber was equipped with inlet and outlet ports through which it was flushed with air at a constant flow rate. A Fleisch model no. 00 pneumotachograph was connected to the outflow line and the pressure drop across the pneumotachograph was measured using a Validyne DP 103-18 differential pressure transducer and Gould d.c. amplifier. The zero balance of the transducer amplifier was adjusted to cancel the constant signal resulting from the constant gas flow through the system. The ventilation chamber also contained a calibration port through which known volumes of air could be pumped into and out of the chamber using a syringe driven in a sinusoidal fashion by a pump connected to a rheostat. Withdrawal of air from the chamber simulated inspiration of air by an animal in the water below the chamber while injection of air into the chamber simulated expiration into the chamber. The output of the transducer amplifier was also fed through a Gould Integrating Amplifier and thus the pressure deflections resulting from the tidal volumes generated by the pump could be integrated to yield volume directly. Both the differential pressure signal and the integrated volume signal were continuously recorded on a chart recorder.

With this system the correlation between the pump volume and the tidal volume measured at the pneumotachograph could be examined as a function of several variables. Volumes of 2, 4, 6 and 8 ml were pumped at frequencies from 20 to

Key words: methods, tidal volume, non-invasive, aquatic air-breathers.

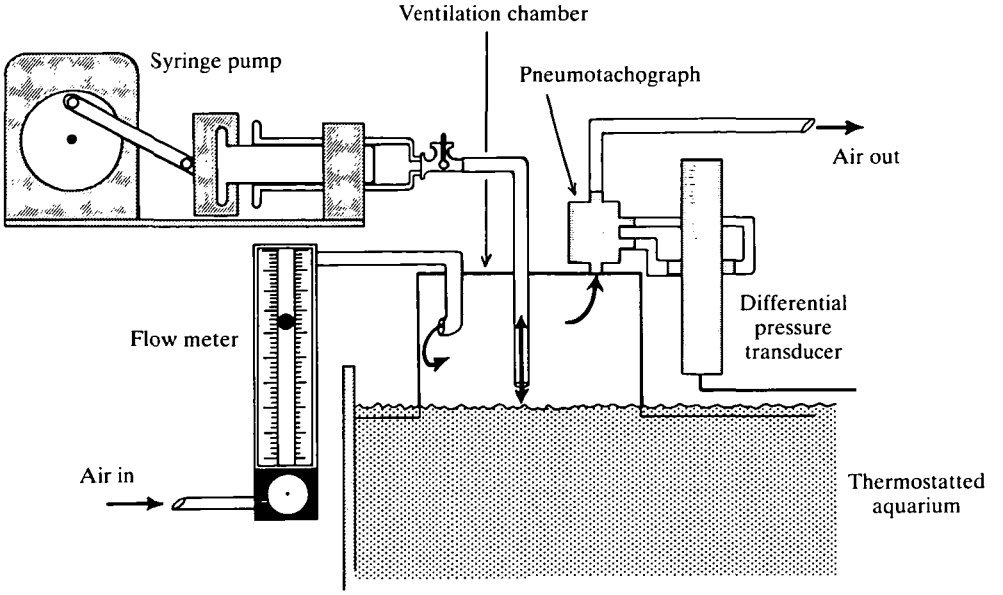

Fig. 1. Schematic diagram of experimental apparatus. See text for details.

80 cycles min^{-1} (i.e. at inspiratory and expiratory intervals from 1·5 to 0·38 s) under a variety of conditions. (1) The effect of changing the constant gas flow through the chamber from 250 to 500 and 1000 ml min^{-1} was examined at 20°C using a chamber volume of 200 ml. (2) The effect of changing the chamber volume from 200 to 400 and 600 ml (by changing the height of the chamber) was studied at 20°C using a constant air flow rate of 500 ml min^{-1}. (3) The effect of changing the water temperature from 10 to 20 and 30°C was examined using a constant air flow rate of 500 ml min^{-1} and a chamber volume of 200 ml. (4) The effect of changing the chamber geometry from a cylinder (cross sectional area = 50 cm^2) to a rectangular box (cross sectional area = 150 cm^2) was examined at 20°C, using a constant air flow rate of 500 ml min^{-1} and a chamber volume of 200 ml.

The initial experiment consisted of examining the effect of changing the pump frequency from 20 to 80 cycles min^{-1} at each pump volume, using a constant flow rate of 500 ml min^{-1} and a chamber of 200 ml. Fig. 2 illustrates the changes seen in the volume output of the integrator (plotted here as pneumotachograph volume) as a function of the pump frequency for each of the pump volumes. The volume of the tidal ventilation measured at the pneumotachograph was always less than the pump volume and this measured volume decreased as the pump frequency increased. This frequency effect on the volume measurement was more pronounced as pump volume increased. No frequency-dependent changes in tidal volume were seen when the pump was directly connected to the pneumotachograph, indicating that such changes were a characteristic of the ventilation chamber and not of the syringe, stopcock and connecting tubing.

Neither the air flow rate through the chamber (Fig. 3A), the ventilation chamber volume (Fig. 3B) nor the water temperature (Fig. 3C) had any significant effect on

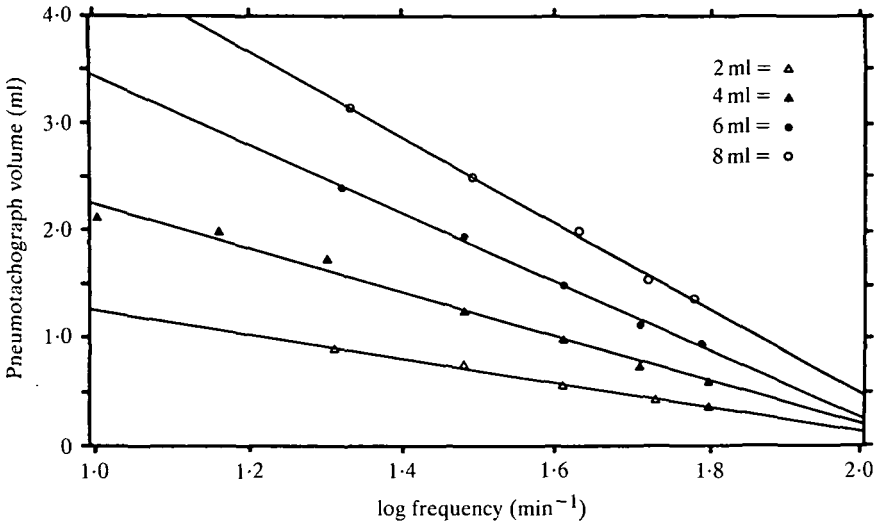

Fig. 2. Effect of changes in pump frequency, at various pump volumes, on the volume measured at the pneumotachograph. Flow through the chamber (200 ml) was 500 ml min^{-1} at 20°C.

the relationships between the integrator output and pump frequency measured for each pump volume. The chamber geometry, however, had a pronounced effect on these relationships (Fig. 3D). For any given pump volume and pump frequency, the integrator output was reduced when the cylindrical chamber was replaced with a rectangular chamber of equal volume. Although data are shown only for a pump volume of 4 ml, similar results were obtained for all pump volumes.

Lines were fitted to all data in Figs 2 and 3 by standard least-squares regression and the coefficients of determination were assessed using an analysis of variance. All regression lines plotted in Figs 2 and 3 have coefficients of determination (r^2) of over 97 %.

These data indicate that, with this method, the tidal volume measured at the pneumotachograph, at least at pump frequencies over 20 min^{-1}, is always less than the true tidal volume. The differences between the pump volume and the volume of gas moving in and out through the pneumotachograph will stem in part from changes in the water level in the chamber due to the pressure changes generated by each breath. Given the cross-sectional area of the chamber, these changes in the water level may not be at all noticeable. The greater the outflow resistance to the chamber (pneumotachograph and tubing), the larger this effect is likely to be. They will also stem in part from frequency-dependent, adiabatic changes within the chamber. Because of the compressibility of the gas in the ventilation chamber, the changes in air flow measured at the pneumotachograph will be smaller than the volume changes produced at the calibration port. The higher the pump frequency, the faster the air flow rates associated with inspiration and expiration and the greater the reduction in the air flow volume measured at the pneumotachograph.

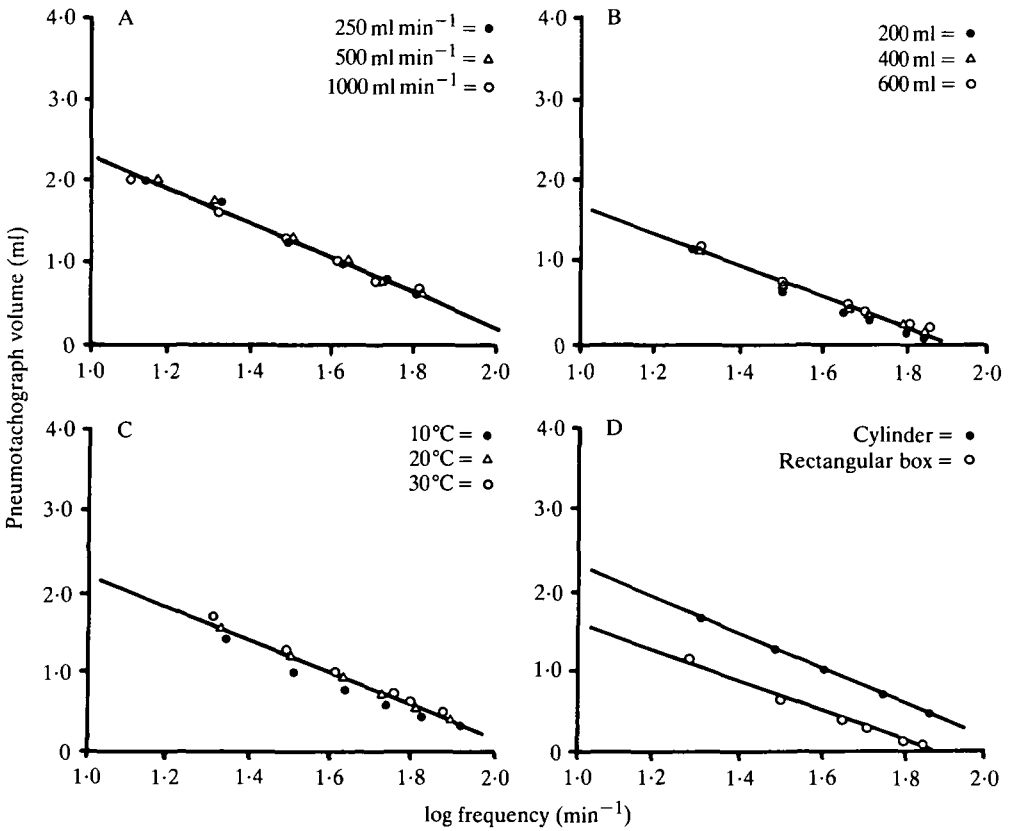

Fig. 3. Effects of changes in (A) air flow rate through the ventilation chamber, (B) chamber volume, (C) water temperature and (D) chamber geometry on the relationship between pneumotachograph volume and pump frequency for a pump volume of 4 ml. See text for details.

While chamber shape also seems to affect the measurement of tidal volume using this method, changes in chamber temperature, volume or background gas flow rate through the chamber do not. The decrease in V_T measured at the pneumotachograph when the cylindrical chamber was replaced with a rectangular chamber of the same volume may reflect the large increase in the air–water contact area due to the increase in surface area. Such an increase may enhance energy dissipation by this route.

Since in most studies the chamber volume and geometry as well as the pneumotachograph and outflow tubing remain constant, the frequency dependence of air flow changes measured at the pneumotachograph for any given tidal volume remain the major potential source of error in the measurement of tidal volume. The time intervals associated with inspiration and expiration can vary widely both within and between individuals in spontaneously breathing animals, particularly in the lower vertebrates (Shelton, Jones & Milsom, 1986). Neglecting to correct for the frequency dependence of the tidal volume measurements made using this experimental set-up can produce large errors. For instance, the data in Fig. 2 indicate that a breath of 2 ml taken in a breath interval of 4 s ($f = 15$, $\log f = 1{\cdot}2$) and a breath of 6 ml taken in a

breath interval of 1 s (f = 60, log f = 1·8) would both be recorded as the same volume at the pneumotachograph.

Calibration curves plotting the logarithm of the instantaneous respiratory frequency (i.e. 60 s min^{-1} divided by the time required to produce an active breath in s) *versus* VT measured at the pneumotachograph, for various tidal volumes in the range anticipated in any particular study, provide good fitting linear regressions which allow true VT to be determined accurately. Presumably, this need only be done once for any given experimental series using the same apparatus.

Using methods for measuring ventilation that involve restraint, anaesthesia or manual or visual disturbance can produce irregularities in the breathing patterns of experimental animals (McDonald, 1976; Jackson, 1978; Cragg, 1978). The non-invasive method for measuring ventilation described by Glass *et al.* (1983) allows an animal to move about freely with minimal disturbance. With proper calibration, this method will produce extremely accurate measurements of all respiratory variables.

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