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Lizard testis volume measurements: are they always underpinned by the correct assumptions?

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SUMMARY

Two basic assumptions are frequently used in the measurement of testis volume: 1) they are highly conserved and thus measures are frequently restricted to a measure of a single testis/individual, and 2) a prolate spheroid provides an adequate measure of volume. Based on the measurement of museum and road kill *Pogona barbata* (Eastern bearded dragon), we showed these assumptions did not hold and recommend that when considering testis volume, researchers measure both testes and the formula used should be informed by careful consideration of their shape.

Key words: Prolate spheroid formula – Scalene ellipsoid formula – Eastern bearded dragon – *Pogona barbata* – Asymmetry

INTRODUCTION

Ecologists are frequently interested in seasonal variance in reproductive conditions, for which the measurement of testis size is considered informative and is widely used, for example, in birds (Rising, 1996; Merilë and Sheldon, 1999), reptiles (Shanbhag et al., 2000; Aldridge and Arackal, 2005), and mammals (Gorman and Zucker, 1995; Schoeman et al., 2004; Couto and Talamoni, 2005).

The basic structure of the testis is regarded as being highly conserved among vertebrates (Neves et al., 2002). As a consequence, researchers typically rely on the measurement of a single testis/individual (Shea, 1993; Bull et al., 1997; Harlow and Taylor, 2000; Hernandez-Gallegos et al., 2002; Reaney and Whiting, 2002; Taylor, 2004; Aldridge and Arackal, 2005; Couto and Talamoni, 2005; Pyter et al., 2005).

Volume is usually calculated by the prolate spheroid formula [Testis volume = $(4\pi/3)$ $(\text{length}/2)$ $(\text{width}/2)^2$]. This formula is based on a rotated ellipse (Mayhew, 1963; Harlow and Taylor, 2000). The approach assumes that the relationship of depth to width is a constant, and that both testes are equivalent in size (e.g. Mayhew and Wright, 1970). It also assumes that each dimension of the testis is stable and proportionate to the other dimensions (Harlow and Taylor, 2000). An exception to the works cited above is that for a blind snake (Shea, 2001) and a skink (Shea, 1993) for which length, width and depth were used to derive a size index. Greenville and Dickman (2005) showed that in the population of *Lerista labialis* they studied, there was no significant difference between the left and right testes

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using Mayhew's (1963) formula, and consequently published data on only the right testis volume. Beyond herpetology, measurement of scrotal diameter (Taggart et al., 2005) and paired testes mass (Krutzsch et al., 2002) are commonly used for mammals, particularly in animal husbandry.

We were interested in the reproductive condition of the Eastern bearded dragon *Pogona barbata*. A preliminary review of the measurements of testes of a number of individuals indicated that their width and depth were not equivalent, nor in a consistent ratio, between left and right. Further observations indicated that the testes in this species were not a prolate spheroid, but were instead closer to that of a scalene ellipsoid, with the three dimensions length>width>depth (Gullberg, 1997), which was the case in all but one animal. Since width and depth of testes were not found to be equivalent, and not in a consistent ratio, it occurred to us that seasonal variation may not occur consistently in any dimension. If that is the case, the use of only two dimensions may not capture the extent of seasonal variation in a species. While a testis with dimensions length>width>depth may not precisely conform to the shape of scalene ellipsoid, it will be a better approximation of volume than that of a prolate spheroid, and thus capture variation in volume more consistently.

These observations indicated that the Mayhew (1963) formula, used by most researchers since 1963 (e.g., Shea, 1993; Harlow and Taylor, 2000; Taylor, 2004), is not always appropriate for estimation of the relative testis volume of reptiles, and in this case for *P. barbata*. It was hypothesised that there may be variations in length, width and depth between the left and right testes and, therefore, differences in volume.

Methods

Seventy-three preserved *P. barbata* from the Australian Museum (Sydney) with testes of regular shape that did not appear to have been deformed by contact with other internal organs in the preservation or storage were measured together with an additional 13 road-killed animals (total n = 86) that also had undamaged testes. The results were not adjusted for the effect of preservation on tissue since our focus was on a comparison between testes in the same animal.

Snout-vent length was not corrected for because this was also considered irrelevant for the purposes of this exercise. There may be shape changes in the testes with animal size, as an allometric change is seen in volumes. That possibility may provide a good reason for taking all three dimensions for estimation of volume, and would enable analysis of the nature of such changes.

The volume of the testes, using the established approach of Mayhew (1963), was compared with the results of the 3D Volume Method. Testis depth was included in the 3D volume calculation as a third dimension in the formula for a scalene ellipsoid [3D testis volume = $(4\pi/3)(\text{length}/2)(\text{width}/2)(\text{depth}/2)$]. This is a variation on the equation for the volume of a sphere where the cube power is replaced by length, width and depth (Gullberg, 1997).

Seasonal variation was assessed by categorising specimens as breeding season (July to December) and non-breeding season (December to June) and analyzing the distribution of values for each season.

The results of volume calculations were then compared using a Wilcoxon's Test (Sokal and Rohlf, 1998).

Results

Testicular volumes calculated with the 3D method increased through spring to a maximum in November (month 5, Fig.1), and declined to a minimum in January, with a moderate increase in the autumn and this size remained stable throughout winter.

The relative volume of testes increased with relative body mass (Fig. 2) (regression $y = 0.0143x^2 + 0.252x + 0.7112$, $R^2 = 0.4627$), such that larger males had a relatively larger testes volume.

In Figure 3, the calculated volume of the right testis of *P. barbata* using Mayhew (1963) is presented, together with the 3D volume calculation. The difference in these measurements was highly significant ($Z = 7.716$, $n = 79$, $p<0.01$).

When the calculated volume, derived by the Mayhew (1963) method, was multiplied by two (to represent the two testes of the individual) and compared with the results of sep-

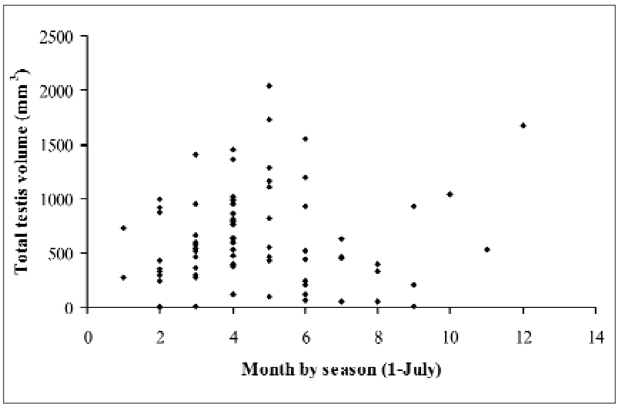

Figure 1. All adult males testis volume by month as seasons (Spring 3 = September). Maximum volume is in month 5 = November, and minimum is in month 7 = January (Summer).

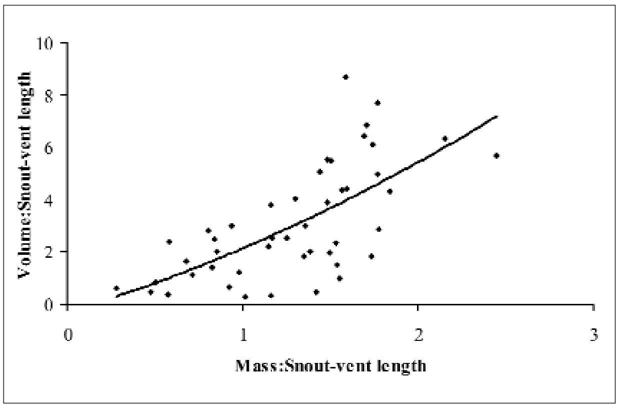

Figure 2. Total testis volume: snout vent length against mass: snout vent length (Regression y= 0.4401x^2 + 1.9708x + 0.2763, R^2 = 0.4425)

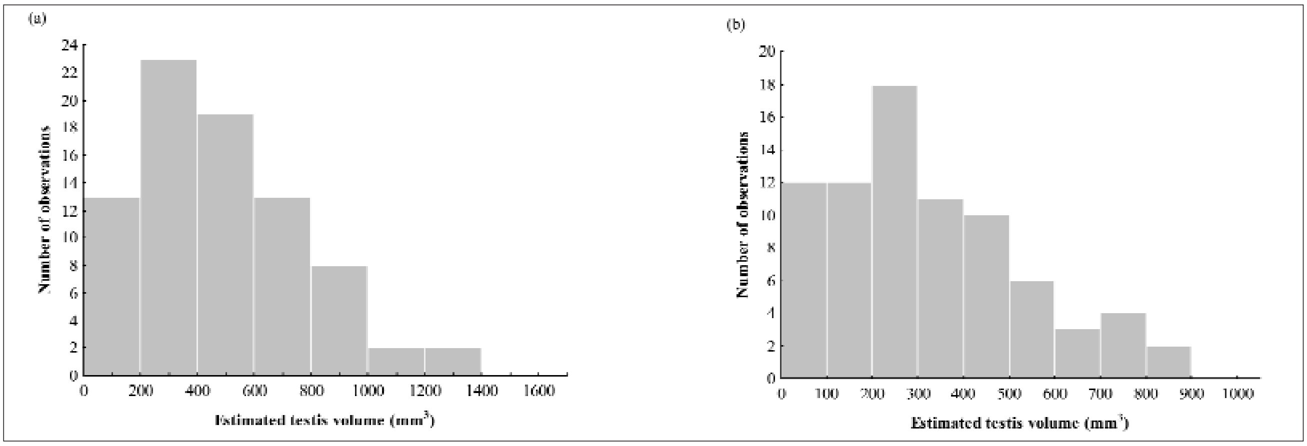

Figure 3. (a) Calculated volume of the right testis using Mayhew's (1963) formula as a basis for calculating volume. (b) Calculated volume of the right testis using the 3D Volume formula as a basis for calculating volume.

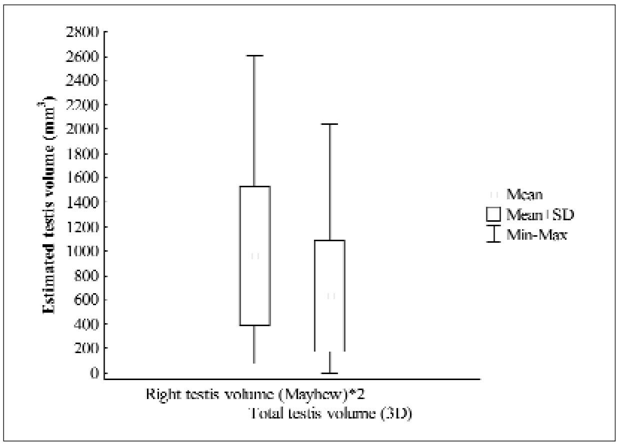

Figure 4. Comparison of the Mayhew (1963) method of calculating testes volume and the 3D Volume method.

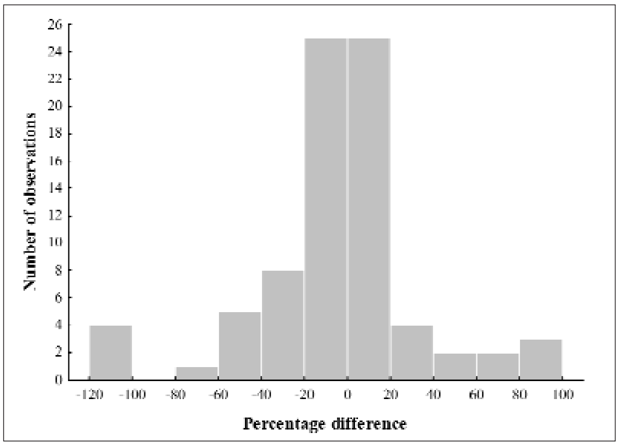

Figure 5. Percent difference between left and right testes in *Pogona barbata* from museum and roadkill specimens.

arate measurements of the two testes summed, calculated using the 3D volume method, the differences were again highly significant (Z = 7.549, p = 0; Fig. 4).

Figure 5 shows the differences in volume between left and right testes (mean = 0.1, se = 4.04). The bilateral asymmetry distribution has both skewness (0.49) and kurtosis (2.46).

Discussion

Estimated testis volumes were systematically over-estimated using the prolate spheroid formula (Mayhew, 1963), as compared to scalene ellipsoid formula. For all investigations where the volume of the testis is of interest, the shape of the testis should therefore be considered in the choice of dimensions measured and, subsequently, in the choice of the equation used as the basis for volume calculation. The advantage of taking all three dimensions is that the 3D volume formula will work as the best approximation whatever the testis shape.

While others have observed no significant difference between left and right testes (e.g., Greenville and Dickman, 2005), variation in humans has been observed (e.g., Thomas and Elder, 2002), and obvious differences in some *P. barbata* clearly require the measurement of both testes.

Hettyey et al. (2005) found that *Rana temporaria* testes showed directional asymmetry in testis weight, with the right testis consistently larger than the left. This phenomenon has also been observed in sparrows (Rising, 1996). Graves (2004) showed that directional asymmetry in Black-throated blue warblers was in the opposite direction, with the left testis consistently larger than the right, and the difference between them was proportional to age.

On the basis of previous reptile data, symmetry was expected (Gribbins and Gist, 2003). However, we found that the differences in testes extended to both left and right (Figure 3) in a leptokurtic asymmetric distribution. This observation, which departed from expectations (Rising, 1996; Merilä and Sheldon, 1999), would not be identified when measurements are restricted to the length and width of the testis, and both testes were not measured. As we have demonstrated, the guidelines of (Mayhew, 1963) which suggest that measurements be restricted to one testis, gave different outcomes to those observed using the more precise ellipsoid-based approach.

These data demonstrate that the measurement of testis volume may not always be underpinned by the correct assumptions. Those that may not be supported include uniformity of shape, volume, bilateral symmetry, and directional asymmetry. In addition, the identification of outliers may also be informative, and this should be considered in the context of the hypotheses being tested. We therefore recommend that when considering testis volume, researchers should always measure both testes and measurements taken, and the formula used should be informed by the general shape of the testis.

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