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Comparison of Five Methods for Measuring Nematode Volume[1]

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Measuring nematode volume is important in many investigations with nematodes, in particular, developmental biology and water regulation. Nematode volume has been estimated from body dimensions and from water content determinations. I know of no report on measuring small nematodes by fluid displacement; however, a fluid displacement micromethod for measuring small tissue samples has been described (7). Nematode body dimensions have been measured with ocular micrometers, camera lucidas, and photographs (2). Nematode water content has been measured with interference microscopy (4), visibility in a solution of known refractive index (5), Karl Fischer titration (3), tritiated water content at equilibrium (6), and weights of large numbers of individuals in air (3).

When it is necessary to estimate accurately the volume of a single nematode, morphometric analysis is the most direct approach. Although Andrássy (1) in 1956 rigorously compared morphometric methods for the determination of nematode volume with techniques available at that time, I am aware of no such comparisons since the advent of digital image analyzers. For this reason, I employed a digital analyzer and nematode photographs to compare five methods in terms of precision and bias for 10 nematode species. Three of those methods were used or proposed in previous literature. The other two methods were included because of their adaptability to currently available tablet digitizers.

Method 1: A common method for estimating nematode volume is to assume that a nematode is cylindrical, measure its length and its maximum radius, and calculate the volume of the corresponding cylinder. This approach was used as early as 1949 by Overgaard Nielsen (8). Accuracy is critically dependent upon uniformity of body radius. If it is assumed that the dorsal and ventral margins of a nematode are everywhere parallel or mutually convex, as they frequently are in vermiform nematodes, then the maximum amount of bias that could result from using the cylinder method would occur if the nematode being measured were exactly biconical. This maximum bias would result from the difference between the volumes of a cylinder ($\pi r^2 L$) and a bicone ($\pi r^2 L/3$) with identical lengths (L) and bases (πr^2). The volume of the cylinder would be three times that of the bicone, producing a bias of +200%. Bias is herein defined as $(V_e - V_t)/V_t$, where V_e and V_t are the estimated and true volumes, respectively.

Method 2: An alternate but slightly more difficult method is to calculate an average diameter d for the nematode by dividing the area of the sagittal image by its length and calculate the volume of the corresponding cylinder of diameter d. This technique was used recently by Bird (2) to measure volume changes associated with molting by *Rotylenchulus reniformis* Linford and Oliveira. The area may be measured by any of several planimetric methods, such as weighing the photographic paper or employing a densitometer or an image digitizer. Bias resulting from measuring a perfectly biconical nematode would be much smaller with this technique than with method 1. Where r is the bicone radius and L is its length, the volume calculated from the length and area of the nematode's sag-

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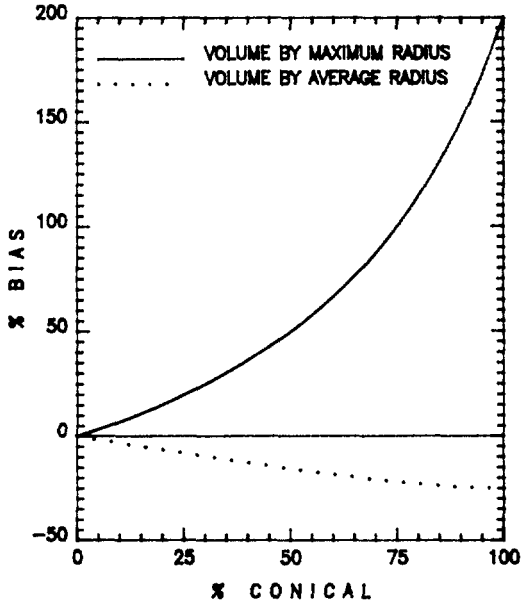

FIG. 1. Bias resulting from estimating the volume of a solid consisting of two cones on either end of a cylinder, such that the percentage of the total length that is conical may be varied, by two methods. In the maximum radius method (method 1), the estimated volume is that of a cylinder with length and maximum radius of the solid. In the average radius method (method 2), the estimated volume is also that of a cylinder with length of the solid, but with diameter equal to the area of the solid's sagittal image divided by its length.

ittal image would be equivalent to $\pi r^2 L/4$, whereas the true volume would be $\pi r^2 L/3$, a bias of -25%.

Few, if any, nematodes are shaped like perfect bicones. Nematodes vary in shape from highly spindleform to highly cylindrical. This variability may be modelled by construction of a solid consisting of two cones on either end of a cylinder, where the percentage of the total length that is conical (P) may be varied. From simple geometric formulae, I derived percentages of bias in measuring such a solid as functions of P for method 1 and method 2. These functions are bias = $(2P)/(3 - 2P)$ and bias = $(3P^2-4P)/(12-8P)$, respectively $(0 \leq P \leq 1.00)$. The bias functions again indicate that the first method is several times more biased than the second (Fig. 1). It is noteworthy that the length/radius ratio does not contribute to the bias in either case. Therefore, a long, slender nematode that is uniformly tapered along most of its length might be measured very inaccurately even though the observer interprets that it is cylindrical.

Method 3: This method is essentially a modification of method 2 and consists of calculating volume for a cylinder with a length ½ the perimeter of the nematode's sagittal image and a diameter twice the area/perimeter ratio.

Method 4: A more accurate but much more complex approach was proposed by Andrássy (1). A nematode may be transected and its diameter measured at several points along the longitudinal axis. The resulting sections may be approximated by truncated cones, and, where appropriate, a complete cone may be substituted for the tail section. The accuracy of this approach depends on the number of sections into which the nematode is divided; the more accurate the estimate, the more prohibitively complex the procedure. Andrássy recognized this problem and therefore regressed volume based on total length and maximum diameter (volume by method 1) against volume based on four cone sections. His regression coefficient (1.7) was applied to 10 species of nematodes to yield two volumes for each species that differed by no more than 5%. I will refer to volume measurement with Andrássy's coefficient as method 4. Method 5 was developed as an extension of Andrássy's multiple section approach.

Method 5: Assuming that the cross section of a nematode is perfectly circular, an entirely unbiased method to calculate volume from its sagittal image would be to integrate volume along the nematode's length using integration by discs. This method does not assume a convex perimeter; consequently, nematodes with attenuated or knobbed tails would present no special problems. Unfortunately, equations for dorsal and ventral margins of the nematode may be difficult to fit statistically, or to integrate, and will change with volume and postural changes in any worm. As an alternative, a FORTRAN program was written which utilizes digitized coordinates of margins of a nematode's sagittal photographic image to calculate rapidly and accurately nematode volume through numerical integration approximation by discs.

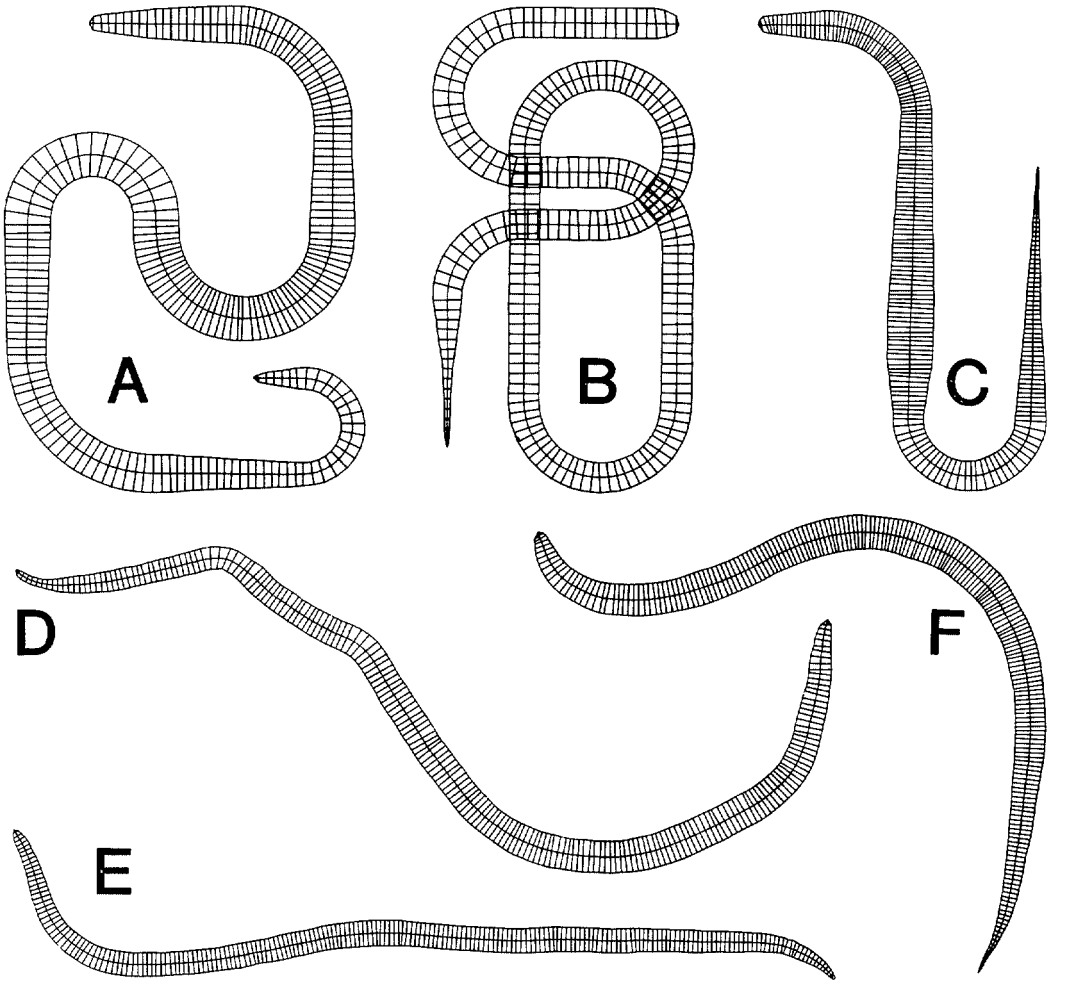

FIG. 2. Images of nematodes perpendicularly transected by a computer program at 200 points along the longitudinal axis of each specimen for volume calculation through numerical integration approximation by discs. A, B, and C are hypothetical nematodes constructed from geometric shapes of known volumes. D, E, and F are *Bursaphelenchus xylophilus* (female), *Ditylenchus dipsaci* (J4), and *Tylenchulus semipenetrans* (juvenile). Nematodes were motile when photographed. Figures are not enlarged to identical scales.

Thus, the program extends Andrássy's multiple section approach in a more detailed fashion.

The nematode image is transected at many points along the longitudinal axis using a digitizer tablet interfaced to a computer. Transections are perpendicular to the longitudinal axis regardless of body curvature, and the number of transections is user selectable. For each quadrangular sector into which the body is divided, the FORTRAN program calculates the volume for a circular disc such that disc diameter equals sector width and disc thickness equals sector thickness along the longitudinal axis. The resulting volumes are summed for the entire nematode. The FORTRAN program was validated by applying it to volume calculations for five hypothetical nematode images constructed from geometrical solids (truncated cones, tori, cylinders, and spheres) with known volumes (Fig. 2). Four hypothetical vermiform nematodes and one hypothetical saccate nematode were measured using 200 transections per image. Hypothetical vermiform nematodes were curved and ranged from highly cylindrical to highly spindle-

TABLE 1. Comparison of volume measurement ($\mu m^3 \times 10^3$) obtained by five methods from digitized margins of sagittal photographic images of nematodes.

	Volume by V_5*	Percentage deviation from V_5			
		V_1	V_2	V_3	V_4
Bursaphelenchus xylophilus (gravid female)	839	+45	−4	−5	+8
Caenorhabditis elegans (juvenile)	42	+59	−11	−12	+20
Caenorhabditis elegans (gravid adult)	420	+62	−10	−11	+22
Ditylenchus dipsaci (J4)	418	+32	−4	−5	0
Meloidodera sp. (J2)	32	+34	−6	−7	0
Meloidogyne incognita (J2)	47	+45	−5	−6	+9
Meloidogyne incognita (saccate female)	74,225	+55	−8	−37	+19
Orrina phyllobia (adult male)	269	+42	−4	−5	+7
Orrina phyllobia (gravid female)	547	+52	−7	−8	+14
Pratylenchus sp. (adult female)	77	+65	−3	−5	+23
Rotylenchulus reniformis (preadult female)	54	+31	−3	−4	−2
Rotylenchulus reniformis (saccate female)	4,021	+80	−13	−22	+35
Tylenchorhynchus sp. (young female)	87	+38	−2	−3	+4
Tylenchulus semipenetrans (juvenile)	42	+50	−7	−8	+11

* V_1 through V_5 are different volume measurements of the same nematode as defined in text. Each datum is from the mean of three measurements per nematode with a mean coefficient of variation of 2.4%. Nematodes were alive and in most cases motile when photographed.

form. The average deviation from predicted volume for the five images was 0.5% with a mean coefficient of variation among three measurements per image of 0.8%. It was concluded that the program yields an essentially unbiased measure of volume from the sagittal image of any shape that is circular in cross section.

To compare bias empirically among the five methods described above, the volumes of various nematodes from 10 species were measured by each method (Table 1). Photographs were printed at a final magnification of ca. 350 × or 700 ×, depending on the size of the nematode, and each photograph was measured three times by each method.

For brevity, volume measurements obtained by methods 1 through 5 are referred to as V_1 through V_5. As described above, the algebraic definitions of these measurements are $V_1 = \pi r^2 L$, $V_2 = \pi A^2/4L$, $V_3 = \pi A^2/2P$, $V_4 = d^2L/1.7$, and V_5 = numerical integration approximation by discs, where r, L, A, P, and d are the maximum radius, length, area, perimeter, and maximum diameter, respectively, of the nematode's sagittal image. Because V_5 was found to be a highly unbiased and precise measure of volume for five hypothetical nematodes of highly variable shapes, bias in the other four methods were calculated by comparing them to V_5. For vermiform nematodes of 10 species, V_1 overestimated volume by 31–65%, as predicted above. For the same nematodes, V_2 and V_3 underestimated volume but by a smaller amount, 3–12%, once again as predicted. Bias in V_4 ranged from −2 to +23%, a much larger range than measured for V_2 or V_3. Percentage deviations from V_5 for V_1, V_2, and V_3 in hypothetical nematodes were similar to those obtained from nematode photographs.

In summary, a FORTRAN program was developed that calculates nematode volume from digitized nematode photographs. The program was tested by measuring a variety of nematode-like objects of known volume and found to be very accurate. It is emphasized that the program assumes nematodes are perfectly circular in cross section, which has not been established conclusively. When volume of a real nematode calculated by the FORTRAN program is used as a standard, volume measurement based only on a cylinder with a nematode's length and maximum radius (method 1) is strongly biased and would seem unsuitable for many applications. The use of Andrássy's coefficient to correct this bias (method 4) may yield an accurate measurement for some nematodes. My measurements, however, did not confirm the level of consistency in bias among nematode species implied by Andrássy's data. Bias depends on the shape of the nematode and occasionally is quite large. Volume measurement based on a cylinder with length that of the nematode's length and diameter that of the nematode's area/length ratio (method 2), or with length half that of the nematode's perimeter and diameter twice that of the nematode's area/perimeter ratio (method 3), are underestimates for vermiform nematodes, but may be suitable for some applications. That the perimeter-based volume measurement approximates the length-based measurement within ca. 1% is of interest because tablet digitizers are easily programmable to output area and perimeter from a single image tracing, often without computer interfacing. My measurements indicate that the methods 2 and 3 are less sensitive than methods 1 and 4 to variation in nematode shape. The FORTRAN program employed to measure nematode volume through numerical integration approximation by discs is available from the author.

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