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## Measuring the volume of prey in anuran trophic ecology studies: accuracy of three methods

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### ABSTRACT

This study aimed to analyze the accuracy of three currently used methods to quantify the volume of the prey of amphibians using the volumetric displacement method as a comparative parameter. The compared methods were the formula for semi-spherical bodies, which measures the height, length, and width of the prey; the formula for ellipsoid bodies, which estimates the height of the prey; and the parallelepiped formula, where the prey is macerated at a height of 1 mm. After the identification of the prey to the taxonomic level of order, the volume of consumed prey was measured using the three methods. The values obtained with the formula for semi-spherical and ellipsoid bodies had a greater difference from the reference values (volumetric displacement method). Regarding the values obtained with the parallelepiped formula, they were similar and had a significant correlation with the reference values. The presented results demonstrate that the parallelepiped formula, using prey maceration, is a more accurate method for estimating prey volume than the semi-spherical and ellipsoid formulas.

### ARTICLE HISTORY

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### KEYWORDS

Volumetric displacement; parallelepiped formula; prey maceration

### Introduction

Studies on trophic ecology are fundamental to understand the natural history of species (Sih & Christensen 2001), besides contributing to several ecological theories (Pianka 1974, 1980; Caldwell 1996). An important factor in trophic ecology studies is to evaluate the relevance of each prey in the diet of a species, such as number, frequency of occurrence, and volume of consumed prey (Parmelee 1999). The number and frequency of occurrence are variables relatively easy to measure but, in terms of volume, there are different methods with varying degrees of complexity and accuracy. An efficient method of quantifying the volume, although hard-working, is the volumetric displacement (Milstead 1957; Itamies & Koskela 1970; Sproston et al. 1999). Still, in anuran diet studies, this method may be difficult to quantify due to the small size of some prey items that are often only fragments in the gastrointestinal content (Magnusson et al. 2003). Due to the limitations in applying volumetric displacement, especially for small amphibians, researchers adopted alternative methods to estimate prey volume from gut contents. The most commonly used method to quantify prey volume in amphibian studies is the formula for

ellipsoid bodies (Colli & Zamboni 1999):  $V = 4\pi/3 * A/2 * (B/2)^2$ , where  $V$  = volume,  $A$  = length, and  $B$  = prey width. A weak point in this formula is that it estimates the height of the prey instead of measuring it, considering it equivalent to the width  $(B/2)^2$ . An expected disadvantage of this method is that the relative error in volume estimation differs between each category of prey, which may result in an overestimated or underestimated volume (Magnusson et al. 2003). An alternative way to eliminate these relative errors is to use the semi-spherical formula:  $V = 4\pi/3 * (A/2) * (B/2) * (C/2)$ , where  $A$  = length,  $B$  = width and  $C$  = height. Although this method is more precise than the first one, it is necessary to measure the height of the prey, making the process more prolonged and laborious. A third alternative is the parallelepiped formula ( $V = A * B * C$ ), using the method of maceration of the prey to homogenize the whole surface at a height of 1 mm (Schoener 1967; Hellawell & Abel 1971). However, there are no studies comparing the accuracy of the prey maceration method with the other formulas presented above. Thus, the objective of this study was to analyze the accuracy of the formula for parallelepiped, semi-spherical and ellipsoid bodies in the volumetric quantification of amphibian prey.

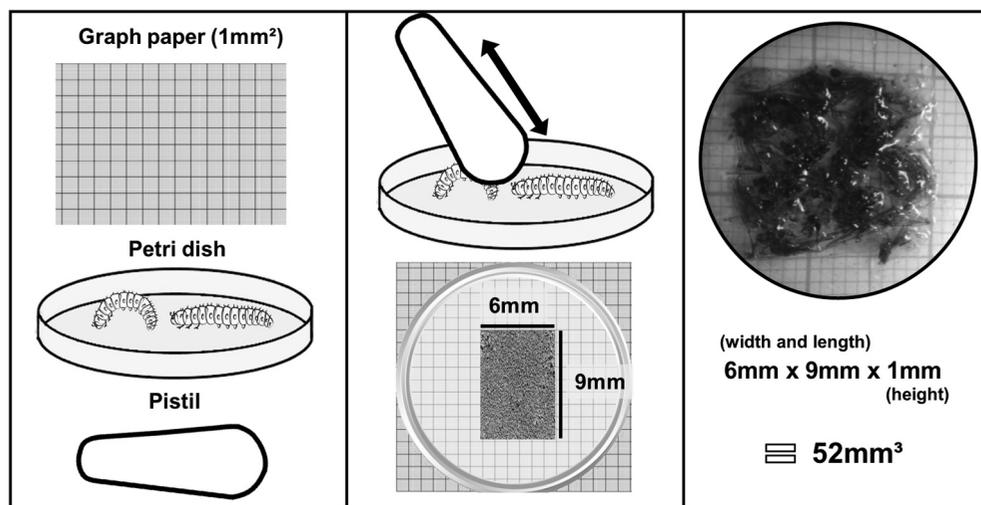


Figure 1. Description of the prey maceration method.

## Materials and methods

Gastrointestinal contents of 31 individuals of three anuran species were used, *Leptodactylus latrans* (Steffen 1815), *Boana pulchella* (Duméril & Bibron 1841), and *Odontophrynus americanus* (Duméril & Bibron 1841). The animals were euthanized with topical anesthetic (xylocaine), fixed in 10% formaldehyde, and preserved in 70% alcohol in the herpetological collection of the Laboratory of Ecology of Terrestrial Vertebrates at the Universidade do Vale do Rio dos Sinos (LEVERT – UNISINOS). For each dissected stomach, the volume of each order of prey was measured separately. The volume of consumed prey was calculated in four different ways: (1) by volumetric displacement (VDL) in a graduated cylinder (method with lower error); (2) by semi-spherical formula (VSE)  $V = 4\pi/3 \cdot (A/2) \cdot (B/2) \cdot (C/2)$ ; (3) by the formula for ellipsoid bodies (VEL)  $V = 4\pi/3 \cdot A/2 \cdot (B/2)^2$ ; and (4) by prey maceration (VM) and volume quantification using the parallelepiped formula  $V = A \cdot B \cdot C$  where A and B are the dimensions of the macerated content distributed on the Petri dish with a graph paper and C the height (standardized in 1 mm). The graph paper must be marked with millimeters and prey maceration must be done so that the height reaches about 1 mm (Figure 1). Prey items were repeatedly pressed with a pestle until the whole macerated layer was homogeneous and uniform with a height of 1 mm.

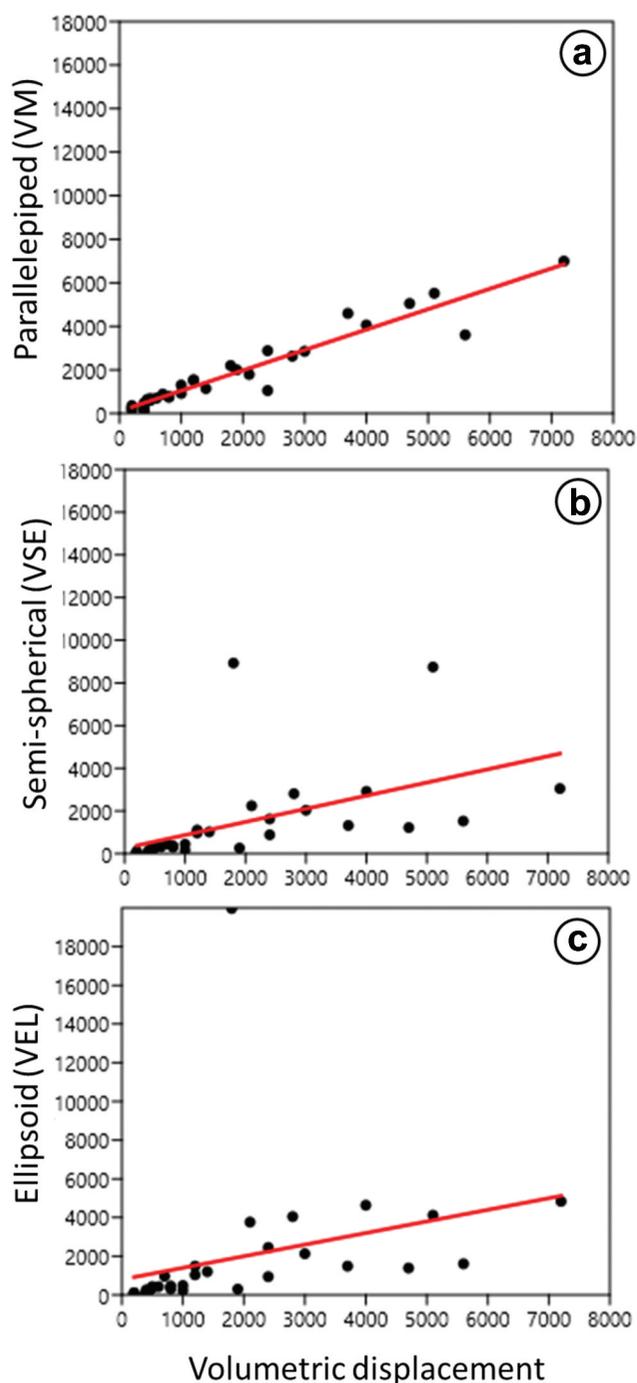
For the analysis of the accuracy, the VDL method was considered the real volume of the prey (the most accurate) and was used in the comparison between the VM, VSE, and VEL methods to find the mean percentage difference. Thus, we consider that the lowest mean percentage would be the most accurate method. In order to verify which of the methods (VM, VSE, and VEL) present a greater correlation with VDL, a linear regression was made.

## Results

Gastrointestinal contents had mean volumes of 1891.9 mm<sup>3</sup> (min = 200; max = 7200) to VDL, 1878.8 mm<sup>3</sup> (min = 137.5; max = 6993) to VM, 1419.8 mm<sup>3</sup> (min = 28.6; max = 8931.3) to VSE and

Table 1. Volume of gastrointestinal contents of the 31 analyzed individuals. VDL – volume by volumetric displacement; VM – volume by maceration and parallelepiped formula; VSE – volume by semi-spherical formula; VEL – volume by formula for ellipsoid bodies.

Individual	VDL (mm <sup>3</sup> )	VM (mm <sup>3</sup> )	VSE (mm <sup>3</sup> )	VEL (mm <sup>3</sup> )
01	700	885	450.3	968,6
02	1400	1145	1018.9	1207,4
03	3000	2856	2036.2	2132,3
04	400	137,5	28.6	33,3
05	3700	4595	1316.3	1487,5
06	1900	2000	257.2	300,8
07	400	220	125.7	128,2
08	1200	1500	967.6	1039,5
09	1000	1300	438	490,2
10	400	480	134.8	265,7
11	2100	1793	2239.9	3765,1
12	2400	1050	879.6	945,0
13	2400	2881	1625.2	2452,8
14	2800	2630	2811.6	4052,5
15	1800	2200	8931.3	19,973,7
16	1200	1550	1106.3	1488,5
17	600	700	317.3	427,2
18	4000	4060	2918.1	4641,8
19	800	747	356.3	464,3
20	7200	6993	3049.9	4837,6
21	1000	919	165	231,0
22	800	775	301.6	315,3
23	5100	5520	8745.9	4121,6
24	5600	3608	1526.2	1613,2
25	4700	5050	1220.4	1390,8
26	500	680	282.7	368,6
27	200	146	74.3	108,4
28	500	600	273.3	433,5
29	200	355	105.8	114,1
30	200	230	37.9	37,9
31	450	640	272.3	217,8
Average	1891.9	1878.8	1419.8	1937.2



**Figure 2.** Graphs showing the correlations found between VDL, VM, VSE and VEL from linear regression. A = positive and significant correlation VDL and VM ( $p < 0.001$ ;  $r^2 = 0.92$ ); B = positive and significant correlation between VDL and VSE ( $p = 0.002$ ;  $r^2 = 0.26$ ); C = positive and non-significant correlation VDL and VEL ( $p = 0.1$ ;  $r^2 = 0.08$ ).

1937.2 mm<sup>3</sup> (min = 33.3; max = 19,973.7) to VEL (Table 1). The mean percentage difference between VDL and VM was 23.2%, VDL and VSE was 62.1% and between VDL and VEL was 76.8%. The regression showed a significant correlation between VDL and VM ( $p < 0.001$ ;  $r^2 = 0.92$ ) and between VDL and VSE

( $p = 0.002$ ;  $r^2 = 0.26$ ), but there was no correlation between VDL and VEL ( $p = 0.1$ ;  $r^2 = 0.08$ ) (Figure 2).

## Discussion

Our results demonstrate that volumetric quantification by maceration (VM) of the prey, using the parallelepiped formula, is more precise than the formula for semi-spherical and ellipsoid bodies. The correlation between the VDL and VM methods demonstrates that there is a strong relationship between them. In other words, VM is the method that most closely matches the real value of prey (VDL). This method is more precise because, with maceration, the height of the prey is homogenized in 1 mm, thus eliminating the relative error of the measurements in a single point (Hellowell & Abel 1971) since they present distinct and multidimensional geometric forms. The prey maceration method has been used in fish trophic ecology studies for decades (Hellowell & Abel 1971; Hyslop 1980; Bastos et al. 2013; Oliveira et al. 2016) but it is still little used in the study of amphibians (Oliveira et al. 2015; Moser et al. 2017, 2019; Farina et al. 2018; Huckembeck et al. 2018). This method, besides relatively practical and fast, provides results that are closer to reality, allowing more precise conclusions about the importance of each prey in the trophic ecology of anurans.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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