Biometric relationships in earthworms (Oligochaeta)

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ABSTRACT

Digging and hand-sorting of soil blocks is a very widespread method in the study of earthworm communities. One disadvantage of this method is that it is very time consuming and often many earthworms are incomplete because they were cut by the digging tools. When authors report earthworm biomass, no mention is made of the assessment of any relationship between the mass of those cut earthworms and their overall weight. In such cases, biomass is generally underestimated. In this paper, our objective was to propose a new method to estimate the weight of incomplete earthworms on the basis of preclitellar diameter and its usefulness for studying the dynamics of earthworm populations. Complete earthworms were collected from samplings performed in native savannahs and man-made pastures of the eastern plains of Colombia and from a poplar grove (Populus sp.) in Central Spain. A strong correlation between the preserved fresh weight and the maximum preclitellar diameter was found for all the species studied. Three types of models have provided a convenient method to estimate earthworm biomass: (i) linear for almost all the species; (ii) exponential for a large Neotropical anecic species, Martiodrilus carimaguensis (Glossoscolecidae); and (iii) second degree polynomic equation.

Key words: Earthworms, Oligochaeta, Biomass, Regression, Population Ecology

1. **INTRODUCTION**

Differences in size of animals imply ecological differences [10] and the choice of a body part is complicated by allometric relationships [8]. Generally, the power equation $y = ax^b$ has been used to describe the majority of allometric relationships [19]. Hand-sorting and washing-sieving of soil samples are some of the most used methods in the study of earthworm communities. These are very time consuming, tedious and often many earthworms are incomplete, either due to cutting during collection or to the fragility of some species. When authors report earthworm biomass, it is unusual to find that those cut specimens have been estimated according to their overall weight [2, 4].

Fernández (op. cit. in [14]) is the first author who gives a valid estimation of the earthworm weight when it is cut for the species Dichogaster terrrae-nigrae Saussey (Octochaetidae) in the African savannas of Lamto (Ivory Coast). He plotted a regression of the live weight against a value equals to the product of the preclitelar diameter by its length until segment XIII. Collins [5] calculated a regression model that related earthworm length to dry weight for some lumbricids from northern Wisconsin forests.

Some ecological processes are dependent on the size of the animals at several scales of time and space. The size of larger species may be a handicap for living in the soil environment, as they have to make bigger efforts to dig into the soil than smaller ones. Besides, large species create functional domains that affect in nested spatio-temporal scales other taxa of soil biota [16].

Bouché and Gardner [4] established an estimation of losses of the caudal parts by natural factors, i.e. predation. They calculated a percentage of cut postembryos versus all postembryos, being the larger species that had the greater frequency of amputation. In some ecological studies where demography of a given species is performed, those fragmented individuals are included within the more abundant weight classes of the sample. This leads to a bias that generally is hard to avoid (see [4] for details). A precise knowledge of the earthworm's full weight is fundamental to study the demography across time.

In this study our objective is to propose a new method to estimate the weight of incomplete earthworms and its usefulness for studying the dynamics of earthworm populations.

2. MATERIALS AND METHODS

2.1. Site description

The species employed were extracted from two different sites, tropical and temperate sites. The tropical site is Carimagua research station, in the Eastern Plains of Colombia (4° 37' N, 71° 19' W and 175 meters altitude). Respective average annual rainfall and temperature are 2280 mm and 26 °C, with a dry season fo four months from December to March. This site is settled on the well-drained isohyperthermic savannas where soils of two types occured: low-fertility Oxisols and Ultisols. The former are characterized by their acidity (4.5, H_2O) and high Al saturation (> 90%) [13] .

The temperate site is located in Central Spain in the province of Segovia. Sampling was performed in a poplar grove (Populus sp.) 7 km west of Sepúlveda village settled on brown soils with high humus contents. Climate is defined as semiarid Mediterranean with a yearly average rainfall about 600 mm.

2.2. Earthworm sampling

Earthworms were hand-sorted during the rainy season in the tropical site, from July to September 1993 and during all of 1997 in the temperate site. They were carried to the laboratory, weighed (i.e. live weight) and killed in a solution that contained 4 % formalin in 96° alcohol. After a few minutes, they were stored in a 4 % formalin permanent solution and weighed again (i.e. fresh weight) 48 h later, when the weight of the earthworm was stabilized. Only complete specimens, either adults (sexual marks and clitellum present), sub-adults (only sexual marks present) or immatures (no sexual marks) were used to plot the regression, so fragmented specimens were not used and, for instance, no relationship was sought for their weight losses. They were separated in the laboratory according to species, each individual being weighed separately after the maximum preclitellar diameter was measured using a Vernier Caliper with 130-mm scale in 0.05-mm subdivisions. The preclitellar zone in earthworms refers to a zone situated before a tegumental glandular tumescence, the clitellum. This organ is developed by earthworms when they are adults near to reproduction and it is responsible for cocoon formation.

The fresh weight of earthworms in formol was 15 % lower than their live weight on average (table I). Madge [18] reported weight losses of worms in tropical grassland of Nigeria of about 20 % of their live weight. Since earthworm biomass is normally expressed in several ways, i.e. dry or formalin weight, we have employed the latter since many other authors have used it [7, 17]. The weight loss in preservative solution has no consequence in the preclitellar diameter. What it is first necessary is to find out the percentage of live weight the worm loses when it is fixed, whatever the preservative solution used.

2.3. Statistical analysis

A regression analysis was employed to assess the best equation to fit the data. The type of regression, equation parameters and correlation coefficients were calculated using Sigmaplot 4.0 Jandel Scientific software.

3. RESULTS AND DISCUSSION

A strong correlation between the fresh weight and the maximum preclitellar diameter appeared for all the species studied. Three types of relationships were found, linear, second degree polynomic and exponential. Mainly all species were adjusted to a linear regression and only *Martiodrilus carimaguensis*, from the tropical site, to a non-linear regression (*figures 1, 2*). In the case of both lumbricids *Allolobophora caliginosa* and *Lumbricus friendi*, data were best fitted to a second degree polynomial equation. All regressions were significant at $P < 0.001$.

One of the disadvantages of hand-sorting is that many earthworms are cut into pieces, making rather difficult the evaluation of their own individual weight. This is an important task when the population dynamics of the whole earthworm community is being assessed. Portions of the anterior region of the earthworm are counted as individuals in density values calculations [4].

Not only accurate biomass estimation must be made on the basis of lost of weight in preservative solutions but also the assessment of the whole body weight from portions of worm, especially when the hand-sorting method is employed.

Edwards [6], Madge [18] and Reynolds [21] all employed the length of the earthworm to estimate its weight. We also employed this variable but as some species showed a strong variation in body length this led us to use the preclitellar diameter. The variation of this part of the body is minimum since the gizzard, normally located in segment VI, is an inner structure of thick muscles that is slightly affected by formalin preservation, although no data are available but some authors agree with this assumption (Bouché, pers. comm.; Lavelle, pers. comm.).

In this study, we sought a clear relationship that could be employed for a large number of cut specimens, either due to the use of a spade or to the fragility of the earthworm, and its usefulness in long-term studies concerning the demography of earthworm populations. Moreover, estimation of earthworm weight can be used to relate efficiency of the handsorting method to washing-sieving techniques since hand-sorting mainly misses the smaller worms ([20]; Jiménez, unpubl.).

We agree with Madge [18] who also obtained a non-linear relationship between the fresh weight of *Hyperiodrilus africanus* Beddard (Eudrilidae) against its length. The ecological significance of this feature could be an increasing efficiency of energy assimilation by the earthworm as larger species should increase their length but are limited by an hydrostatic skeleton. Our results showed an image of the validity of the relationship that exists between morphology and ecology within any animal taxon [9].

A change in size may lead for example to a change in respiratory efficiency. The amount of oxygen required depends on the volume of the organism concerned. Therefore changes in area:volume ratios are more likely to lead to changes in the respiratory efficiency [1]. And if, respiratory efficiency is to be maintained, this must be done by allometric alterations.

The non-linear relationship found for *M. carimaguensis*, and probably for other still undefined species, may be the reflection of allometric differences between adults and juveniles (i.e. they are not isometric) or maybe it defines two distinct periods in the life cycle of this species: growth and development (maturity). And this species presents the largest life cycle within all the species studied with particularities in the aestivating period, where diapause improves the chances of survival when environmental conditions are not suitable [11].

Hence, a new width-weight model was provided to give very satisfactory results to accurately estimate the weight of worms, either fresh or in preservative solutions, in those studies of earthworm communities that apply physical methods of extraction. A detailed study of earthworm communities in a native savannah and a selected pasture from Carimagua, in the Colombian Orinoco basin, was carried out with this procedure [12]. The global efficiency of hand-sorting is about 60 % for *Glossodrilus* n. sp. when compared to the washing-sieving method, and less than 40 % for the Ocnerodrilid worm (Jiménez, unpubl.). An assessment of the efficiency of these physical methods will be compared in a next paper.

Studies on determination of indirect biomass in other groups of macro-invertebrates should be considered (i) in those population dynamics and demography studies of any organism and (ii) because of the scientific rigor. We are concerned about this tedious and back-breaking work, but it needs doing.

Discussion

One of the disadvantages of hand-sorting method is that many earthworms are cut into pieces, making rather difficult the evaluation of their own individual weight. This is an important task when the population dynamics of the whole earthworm community is being assessed. Portions of the anterior region of the earthworm are counted as individuals to give density values (Bouché & Gardner, 1984)

Not only accurate biomass estimation must be made on the basis of lost of weight in preservative solutions but the assessment of the whole body weight from portions, especially when hand-sorting method is employed.

Edwards (1967), Madge (1969) and Reynolds (1972) employed the length of the earthworm to estimate its weight. We employed this variable too but as some species showed a strong variation in body length this lead us to use the preclitelar diameter. The variation of this part of the body is minimum since the gizzard is a thick wall muscle organ hard structure that is slightly affected by formaline preservation.

Therefore, relationships have been sought to relate the weight of one complete specimen to one biometric variable. The maximum preclitelar diameter has been an useful variable and used to estimate the total weight of those incomplete individuals taken from soil samples.

In this study we sought for a clear relationship that could be employed for a large number of cut specimens, either by the use of a spade or by the fragility of the eathworm and its usefulness in long-term studies concerning the demography of earthworm populations. Besides, estimated weights of earthworms can be used to relate efficiency of hand-sorting method to washing-sieving techniques since hand-sorting mainly misses the smaller worms (Raw, 1960).

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A change in size may lead to a change in efficiency, i.e. respiratory. The amount of oxygen required depends on the volume of the organism concerned. Therefore changes in area:volume ratios are more likely to deserve changes in the respiratory efficiency (Begon et al., 1996). And if, respiratory efficiency is to be maintained, this must be done by allometric alterations.

The non-linear relationship appeared for M. carimaguensis, and probably for others still unknown, may be the reflect of allometric differences between adults and juveniles (that is, they are not isometric) or maybe it defines two distinct periods in the life cycle of this species: growth and development (maturity). And this species presents the largest life cycle within all the species studied with particularities in the aestivating period, where diapause improves the chances of survival when environmental conditions are not suitable (Jiménez et al. 1998).

Hence, a new width-weight model was provided to give very satisfactory results to accurately estimate the weight of worms in those studies of earthworm communities that apply physical methods of extraction. In a next paper, an assessment of the efficiency of these physical methods will be compared.

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REFERENCES

[1] Begon M., Harper J.L., Townsend C.R., Ecology: Individuals, Populations and Communities, 3rd ed., Blackwell Science Ltd., Oxford, UK, 1996, 1080 p.

[2] Bouché M.B., Comparaison critique de méthodes d'évaluation des populations de Lombricidés, Pedobiologia 9 (1969) 26–34.
[3] Bouché M.B., Lombriciens de France, Écologie et Systématique, Inra, Paris, 1972, 671 p.
[4] Bouché M.B., Gardner R.H., Earthworm functions VIII. Population estimation techniques, Rev. Écol. Biol. Sol 21 (1984) 37–63.
[5] Collins P.T., Length-biomass relationships for terrestrial gastropoda and oligochaeta, Am. Midl. Nat. 128 (1992) 404–406.
[6] Edwards C.A., Relationships between weights, volumes and numbers of soil animals, in: Graff O., Satchell J.E. (Eds.), Progress in Soil Biology, North Holland Publishing Company, Amsterdam, 1967, pp. 585–594.
[7] Edwards C.A., Bohlen P.J., Biology of Earthworms, 3rd ed., Chapman and Hall, London, UK, 1996, 426 p.
[8] Grant P.R., Convergent and divergent character displacement, Biol. J. Linn. Soc 4 (1972) 39–68.
[9] Hespenheide H.A., Ecological inferences from morphological data, Ann. Rev. Ecol. Syst. 4 (1973) 213–229.
[10] Huxley J., Evolution: The Modern Synthesis, New York, Harper, 1942, 645 p.
[11] Jiménez J.J., Moreno A.G., Lavelle P., Decaëns T., Population dynamics and adaptive strategies of *Martiodrilus carimaguensis* (Oligochaeta, Glossoscolecidae), a native species from the well-drained savannas of Colombia, Appl. Soil Eco. 9 (1998) 153–160.
[12] Jiménez J.J., Moreno A.G., Decaëns T., Lavelle P., Fisher M.J., Thomas R.J., Earthworm communities in native savannas and man-made pastures of the eastern plains of Colombia, Biol. Fert. Soils 28 (1998) 101–110.
[13] Lascano C.L., Estrada J., Long-term productivity of legume-based and pure grass pastures in the Eastern Plains of Colombia, in: Proceedings of the XVI International Grassland Congress, Nice, 1989, pp. 1179–1180.
[14] Lavelle P., Les vers de terre de la savane de Lamto (Côte d'Ivoire) : peuplements, populations et fonctions dans l'écosystème, Ph.D. thesis, Paris-VI, 1978, 301 p.
[15] Lavelle P., Stratégies de reproduction chez les vers de terre, Acta Oecol 2 (1981) 117–133.
[16] Lavelle P., Beare M.H., Ecosystem engineers, functional domains and processes in soil. Looking for integrative concepts, in: Proceedings of the 16th World Congress of Soil Science, Montpellier, 20–26 August 1998.
[17] Lee K., Earthworms: Their Ecology and Relationships with Soils and Land Use, Academic Press, New York, 1985, 411 p.
[18] Madge D.S., Field and laboratory studies on the activities of two species of tropical earthworms, Pedobiologia 9 (1969) 188–214.
[19] Peters R.H., The Ecological Implications of Body Size, Cambridge University Press, Cambridge, UK, 1983.
[20] Raw F., Earthworm population studies: a comparison of sampling methods, Nature 183 (1960) 257.

[21] Reynolds J.W., The relationship of earthworm (Oligochaeta: Acanthodrilidae and Lumbricidae) distribution and biomass in six heterogeneous woodlot sites in Tippecanoe County, Indiana, J. Tenn. Acad. Sci. 47 (1972) 63–67.

Tables

Species	Ecological Category[1]	Average adult fresh weight (g)	Loss of weight[2] (%)	Number of obsevations
Andiodrilus n. sp.	Mesohumic	1.30	18.8 ± 3.2	11
Andiorrhinus n. sp.	Epi-anecic?	7.10	15.4 ± 2.6	13
Epigeic n. sp.	Epigeic	0.06	17.4 ± 4.8	10
Glossodrilus n. sp.	Polyhumic	0.09	16.9 ± 5.3	10
M. carimaguensis	Anecic	11.2	12.1 ± 4.1	15
Ocnerodrilidae n. sp.	Oligohumic	0.006	15.8 ± 3.9	19

[1] Defined by Bouché (1972) and Lavelle (1981)

[2] Mean ± standard deviation

Figure caption

Figure 1. Plot of regression obtained for six species from the eastern plains of Colombia: a) Andiodrilus n. sp.; b) Andiorrhinus n. sp.; c) Epigeic n. sp.; d) Glossodrilus n. sp.; e) M. carimaguensis; f) Ocnerodrilidae n. sp.

Figure 2. Plot of regression obtained for five species from the European temperate region: a) A. caliginosa; b) A. chlorotica; c) A. rosea; d) L. friendi;

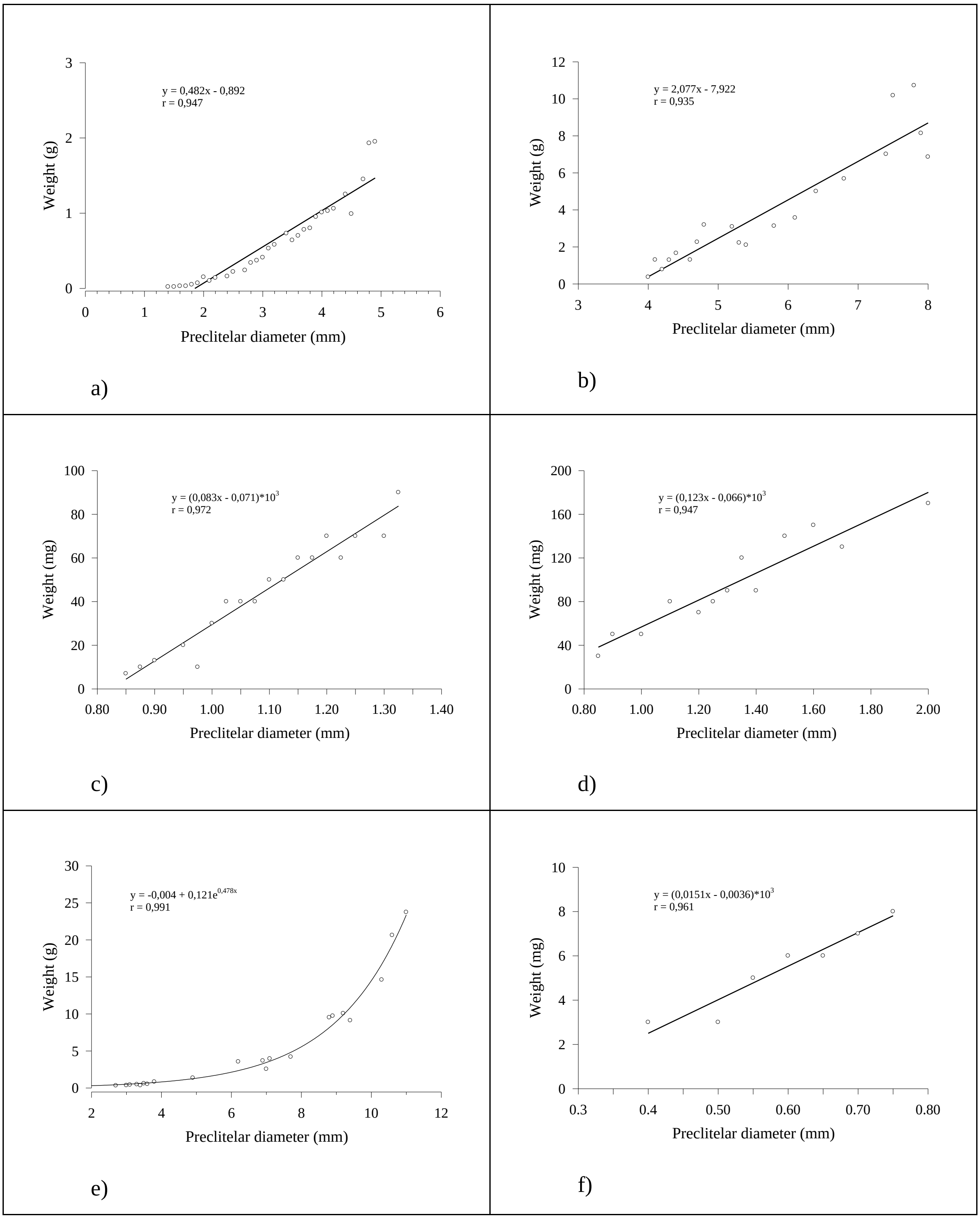
y = 0,482x - 0,892
r = 0,947
Weight (g)
Preclitelar diameter (mm)
a)
y = 2,077x - 7,922
r = 0,935
Weight (g)
Preclitelar diameter (mm)
b)
y = (0,083x - 0,071)*10^3
r = 0,972
Weight (mg)
Preclitelar diameter (mm)
c)
y = (0,123x - 0,066)*10^3
r = 0,947
Weight (mg)
Preclitelar diameter (mm)
d)
y = -0,004 + 0,121e^0,478x
r = 0,991
Weight (g)
Preclitelar diameter (mm)
e)
y = (0,0151x - 0,0036)*10^3
r = 0,961
Weight (mg)
Preclitelar diameter (mm)
f)

Figure 1

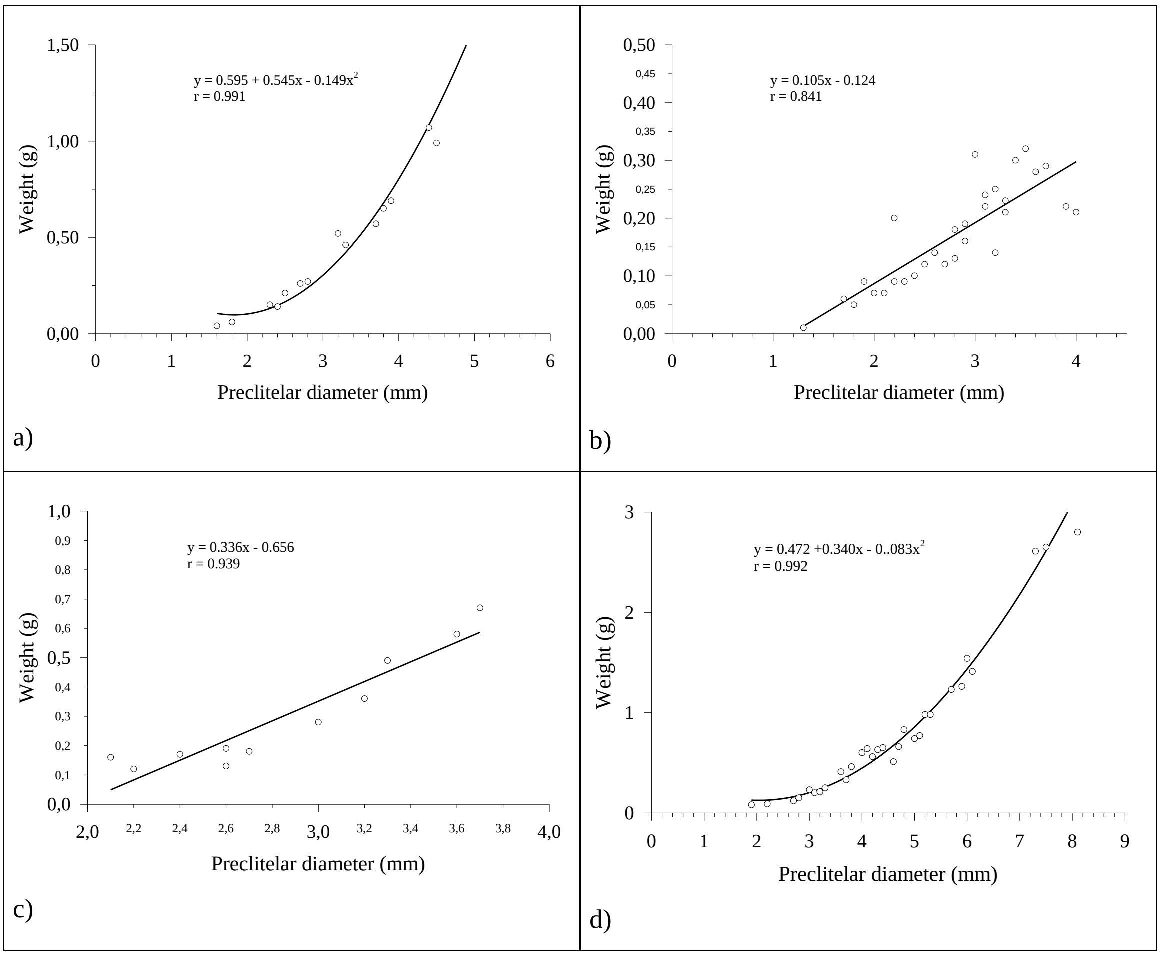
y = 0.595 + 0.545x - 0.149x2
r = 0.991
Weight (g)
Preclitelar diameter (mm)
a)
y = 0.105x - 0.124
r = 0.841
Weight (g)
Preclitelar diameter (mm)
b)
y = 0.336x - 0.656
r = 0.939
Weight (g)
Preclitelar diameter (mm)
c)
y = 0.472 +0.340x - 0..083x2
r = 0.992
Weight (g)
Preclitelar diameter (mm)
d)

Figure 2