



Length–dry mass regressions for *Leptonema* (Trichoptera, Hydropsychidae) larvae in a Neotropical headwater stream

Equações de comprimento-massa seca para larvas de *Leptonema*
(Trichoptera, Hydropsychidae) em um riacho de cabeceira Neotropical

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Abstract: Aim: The objectives of this study were to evaluate which allometric measurements of *Leptonema* larvae are most suitable in order to develop mathematical equations to describe biomass relationships for the population of this taxon in a reference condition headwater stream. **Methods:** We measured four body dimensions (body length, interocular distance, horizontal width of cephalic capsule and vertical width of the cephalic capsule) of 65 *Leptonema* larvae, which were collected in February 2022, in the Taboões spring, Serra do Rola Moça State Park, Minas Gerais, using a Surber sampler. For the determination of allometric measurements, each individual was photographed under a dissecting stereomicroscope (Leica M80) equipped with a digital camera. Each photographed specimen's length was measured using the Motic Image Plus 2.0 software. After measuring the linear body dimension and direct measurement of the biomass, we used these values to calculate the length-mass mathematical equations. To the equations use power models: $DM = aL^b$, where a/b are constants, DM is the dry mass, L is the linear body dimension. **Results:** Among body dimensions of *Leptonema* larvae, body length showed the greatest range of variation, with values ranging from 4.03 to 25.28 mm, followed by head capsule vertical width (0.51 - 2.69 mm), head capsule horizontal width (0.55 - 2.22 mm) and interocular distance (0.24 - 1.88 mm). Our results show that body length provided the best-fitting equation for estimating biomass ($R^2 = 0.90$). However, we observed a close fit between the other allometric measures, including high coefficients of determination, head capsule horizontal width ($R^2 = 0.85$), interocular distance ($R^2 = 0.82$), head capsule vertical width ($R^2 = 0.78$). **Conclusions:** These results will be useful in providing the best allometric measurement and equations to estimate the biomass of *Leptonema* larvae from the tropics.

Keywords: allometric measures; biomass; body dimensions; invertebrates.

Resumo: Objetivo: Os objetivos deste estudo foram avaliar quais medidas alométricas do corpo das larvas de *Leptonema* são mais adequadas, a fim de desenvolver equações matemáticas para descrever as relações de biomassa para a população deste táxon em riachos de cabeceira. **Métodos:** Foram medidas quatro dimensões corporais (comprimento corporal, distância interocular, largura horizontal da



cápsula cefálica e largura vertical da cápsula cefálica) de 65 larvas de *Leptonema*, que foram coletadas no mês de fevereiro de 2022, no manancial Taboões, Parque Estadual da Serra do Rola Moça, Minas Gerais, utilizando amostrador Surber. Para a determinação das medidas alométricas, cada indivíduo foi fotografado em um estereomicroscópio dissecante (Leica M80) equipado com câmera digital. O comprimento de cada espécime foi medido usando o software Motic Image Plus 2.0. Após a medição da dimensão corporal linear e medição direta da biomassa, utilizamos esses valores para calcular equações comprimento-massa. Para às equações, usamos modelos de potência: $DM = aL^b$, onde a/b são constantes, DM é a massa seca, L é a dimensão corporal linear. **Resultados:** O comprimento do corpo apresentou a maior variação, com valores variando de 4,03 a 25,28 mm, seguido pela largura vertical da cápsula cefálica (0,51 - 2,69 mm), largura horizontal da cápsula cefálica (0,55 - 2,22 mm) e distância interocular (0,24 - 1,88 mm). Nossos resultados mostram que o comprimento do corpo forneceu a equação de melhor ajuste para estimar a biomassa ($R^2 = 0,90$). No entanto, observamos um ajuste próximo entre as demais medidas alométricas, retornando altos coeficientes de determinação, largura horizontal da cápsula cefálica ($R^2 = 0,85$), distância interocular ($R^2 = 0,82$), largura vertical da cápsula cefálica ($R^2 = 0,78$). **Conclusões:** Esses resultados podem ser úteis para fornecer as melhores medidas alométricas e equações para estimar a biomassa de larvas de *Leptonema* de riachos tropicais.

Palavras-chave: medidas alométricas; biomassa; dimensões corporais; invertebrados.

1. Introduction

The biomass of aquatic macroinvertebrates is an important metric for estimating energetic processes in lotic ecosystems, such as trophic relationships between functional feeding groups, population growth rates, secondary production of communities (Benke et al., 1999; Gjoni et al., 2022) and energy balance of ecosystems (Steele et al., 2007). However, directly weighing each organism is an impractical and often error-prone process, especially in terms of weighing dry mass or ash-free dry mass (Edwards et al., 2009). To avoid such problems, some studies suggest to estimate biomass indirectly, using length-mass equations (González et al., 2002; Horta et al., 2006; Edwards et al., 2009).

Indirect equation-based methods are faster and more efficient and have the advantage of keeping the taxon preserved for future evaluations (e.g., molecular analysis; Towers et al., 1994), without causing loss of the organism in the drying process (Mährlein et al., 2016). Thus, it is usual to establish length-mass relationships of taxa using the available literature (Benke et al., 1999; González et al., 2002; Martins et al., 2014). However, it is important that these data are used with caution, as they may not take into account the environmental and geographic variations of the studied area (Méthot et al., 2012), overestimating the true body mass.

Although there are studies that estimate the biomass of some freshwater invertebrate taxa from tropical regions (Martins et al., 2014; Dekanová et al., 2022), much of the available literature has been compiled for taxa by North American and European literature (e.g., Benke et al., 1999; González et al., 2002; Sabo et al., 2002; Edwards et al., 2009; Méthot et al., 2012;

Mährlein et al., 2016). Furthermore, length-body mass relationships should be taxon-specific, as there may be a different relationship for each taxon (Baumgärtner & Rothhaupt, 2003; Becker et al., 2009). Therefore, studies on the biomass of aquatic insects should consider the specificity of each region.

Aquatic insects of the Hydropsychidae family are among the most diverse and abundant groups of freshwater ecosystems (Pes, 2005), with relevance to ecological processes, such as nutrient cycling and energy flow (Balachandran et al., 2012). Among the genera of this family, *Leptonema* is widely distributed in the tropics, comprising a significant proportion of the invertebrate biomass of tropical streams (Muñoz-Quesada, 1999). *Leptonema* larvae preferentially occur in rocky bottom habitats with strong water currents (Buss et al., 2004), where they filter small particles of organic matter in the water column (Gholizadeh & Heydarzadeh, 2020), forming an important link in the transfer of energy between the trophic chains. Thus, the structure of a *Leptonema* population can be a useful tool for understanding different ecological issues of headwater streams, including thermodynamic indicators (e.g., Linares et al., 2018; 2020), fluvial mesocosm studies assessing drift movements (Calapez et al., 2017).

In this study, the main objectives were to evaluate which allometric measurements of the *Leptonema* body larvae are most suitable in order to develop mathematical equations to describe biomass relationships for the population of this taxon in a headwater stream. We want to identify which allometric measurements (body length, interocular distance, horizontal width of the cephalic capsule and vertical width of the

cephalic capsule) of *Leptonema* larvae show higher correlation to biomass. We expect that body length is a better predictor of biomass, because it has a wide measurement range between allometric measurements.

2. Methods

2.1. Study area

Leptonema larvae were sampled in the Taboões headwater stream (20°03'38" S - 44°03'03" W), located in the Serra do Rola Moça State Park (PESRM), Minas Gerais state, Southeastern Brazil. The PESRM covers an area of 3,942 hectares and is located in a transition area between the Atlantic Forest and Cerrado (Reis & Machado, 2019), in the Rio São Francisco River basin. The Taboões stream is a reference site for human water supply with waters of excellent quality (special quality, Brazilian water classification by Brasil, 2000), 250 L/s discharge. According to the Köppen system, the climate is classified as Cwb (altitude tropical), with rainy summers and dry winters (Brandão et al., 1997). The average annual precipitation varies between 1,300–2,100 mm and the average temperature between 18°–21° C (Meyer et al., 2004).

2.2. Sampling and laboratory procedures

In the Taboões stream, a 50 meter transect was established, and the sediment substrate at 22 sampling points was collected using a Surber sampler (area of 0.9 m² and mesh of 250µm). Each sample was immediately sorted on a white tray, and all *Leptonema* larvae were individually deposited in 2 ml eppendorf-type microtubes, without the addition of preservatives and properly identified. The material was packed in a thermal box with ice and taken to the laboratory.

2.3. Length-mass equation calculations

In the laboratory, *Leptonema* specimens were identified (Pes, 2005) under a stereomicroscope, and then each individual was photographed under a dissecting stereomicroscope (Leica M80 model) equipped with a digital camera (Leica IC 80 HD model). For the determination of allometric measurements (Benke et al., 1999; Mährlein et al., 2016), four measurements of linear body length were chosen as a predictor of biomass: (i) body length; (ii) interocular distance; (iii) horizontal width of the cephalic capsule and; (iv) vertical width of the cephalic capsule (Figure 1). To determine body length, the distance from the anterior section of the head to the posterior section

of the last abdominal segment was measured. Interocular distance was measured as the minimum distance between the eyes. For the horizontal width of the cephalic capsule, we measure the widest section of the head. Vertical width of the cephalic capsule was measured from the anterior part of the head to the beginning of the pronotum. Each photographed specimen's length was measured using the Motic Image Plus 2.0 software. Measurements of length and mass were performed with unbroken individuals, containing all appendages.

The measured individuals were placed individually in pre-weighed porcelain crucibles, dried in an oven at 60°C for 48 h (Becker et al., 2009), allowed to cool in a desiccator and their dry mass measured on a balance with ± 0.001 g accuracy. Subsequently, to estimate the ash weight, the individuals were incinerated in a muffle furnace at 550°C for 4 hours, and their ash mass was measured by the same procedure.

After the direct measurement of biomass, we used these values to calculate the length-mass equations for each of the measurements of linear body length (body length, interocular distance, horizontal width of the cephalic capsule, vertical width of the cephalic capsule). We also calculated a Pearson correlation between each measurement of linear body length and biomass. The power model was calculated for the four body dimensions of *Leptonema* larvae, using the least squares method. The adjustment of the equations was performed by the coefficient of determination (R^2) and the level of significance ($p < 0.01$) obtained by a Generalized Linear Model (GLM) using a Gaussian distribution. All calculations were made using the R software (R Core Team, 2015).

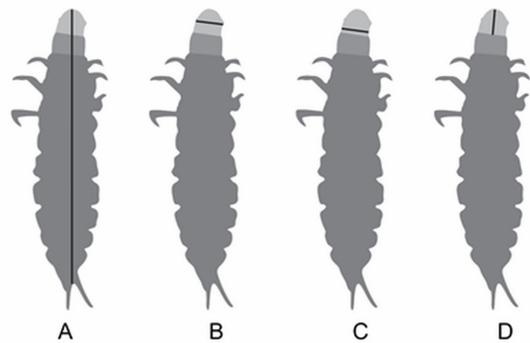


Figure 1. Black lines illustrate the measured body parts for *Leptonema* larvae. (A) body length, (B) interocular distance, (C) horizontal width of the cephalic capsule, and (D) vertical width of the cephalic capsule.

Body length measurements and biomass measurements of the 65 *Leptonema* larvae were used for statistical analysis. To arrive at the equations that determine the length-mass relationship, we used models that predict mass as a power function of a linear dimension.

$$DM = aL^b \quad (1)$$

where a/b are constants, DM is the dry mass, L is the linear body dimension (total body length, interocular distance, horizontal width of the cephalic capsule and vertical width of the cephalic capsule). Equation 1 is often converted to linear form using a logarithmic transformation (Equations 2 and 3):

$$\log_{10}DM = \text{Log}a + b \log L \quad (2)$$

or

$$\text{in linear form: } \ln DM = \ln a + b \cdot \ln L \quad (3)$$

3. Results

Among body dimensions of *Leptonema* larvae, body length showed the greatest range of variation, with values ranging from 4.03 to 25.28 mm, followed by head capsule vertical width (0.51 - 2.69 mm), head capsule horizontal width (0.55 - 2.22 mm) and interocular distance (0.24 - 1.88 mm) (Table 1).

Regression analyses show the length-to-mass relationship for body length, interocular distance, horizontal width of the cephalic capsule and horizontal width of the cephalic capsule of *Leptonema* larvae. Length-mass curves for the *Leptonema* larvae, using linear and logarithmic scales, of the power function (Figure 2).

The equations generated for each linear body length are listed in Table 2. All allometric body dimensions of *Leptonema* larvae showed significance relation for biomass ($p < 0.01$). Body length showed the best fit to estimate biomass, followed by horizontal head size, interocular distance, and vertical head size (Table 2).

4. Discussion

Our results showed that the power models presented a high correlation coefficient, explaining 78% to 90% of the variation in biomass of *Leptonema* larvae as a function of the allometric measurements used (body length, interocular distance, horizontal head size and vertical head size). In fact, our results are in line with other studies in the tropical region (e.g., Becker et al., 2009; Silva et al., 2010). This reinforces that power models for length-mass of aquatic macroinvertebrates provide satisfactory results for the relationship between body dimensions and biomass of freshwater invertebrates, including *Leptonema* larvae.

Although all length-mass relationships were significant across the entire range of allometric dimensions, body length was the best predictor, explaining up to 90% of the biomass variation. The result of this study supports our hypothesis that body length provides a better estimate of biomass for *Leptonema* larvae. Because it has a wider measurement range, body length is a measure often used to estimate insect larvae biomass (e.g., Genkai-Kato & Miyasaka, 2007; Mährlein et al., 2016). Similar results were found by Martins et al. (2014), who found that body length was the best biomass predictor for a population of

Table 1. Range, mean, standard deviation (SD), coefficient of variation (CV = (SD / mean)*100, in 100%) and number of observations (N) for body dimensions and body mass of *Leptonema* larvae from Taboões stream.

Body length	Range	Mean	SD	CV	N
Body length (mm)	4.03 - 25.28	16.6	6.49	39.09	65
Head capsule vertical width (mm)	0.51 - 2.69	1.74	0.61	35.09	65
Head capsule horizontal width (mm)	0.55 - 2.22	1.71	0.54	31.75	65
Interocular distance (mm)	0.24 - 1.88	1.3	0.43	33.3	65

Table 2. Power function equations for length-mass body of *Leptonema* larvae and generalized linear model (GLM) results.

Allometric Measure	Equation	R ²	F	p
Body length	$\ln DM = \ln 0.0005 + 3.50 \cdot \ln L$	0.90	100.840	< 0.001
Horizontal head size	$\ln DM = \ln 0.86 + 4.36 \cdot \ln L$	0.85	75.997	< 0.001
Interocular distance	$\ln DM = \ln 3.21 + 3.95 \cdot \ln L$	0.82	66.169	< 0.001
Vertical head size	$\ln DM = \ln 1.10 + 3.82 \cdot \ln L$	0.78	36.336	< 0.001

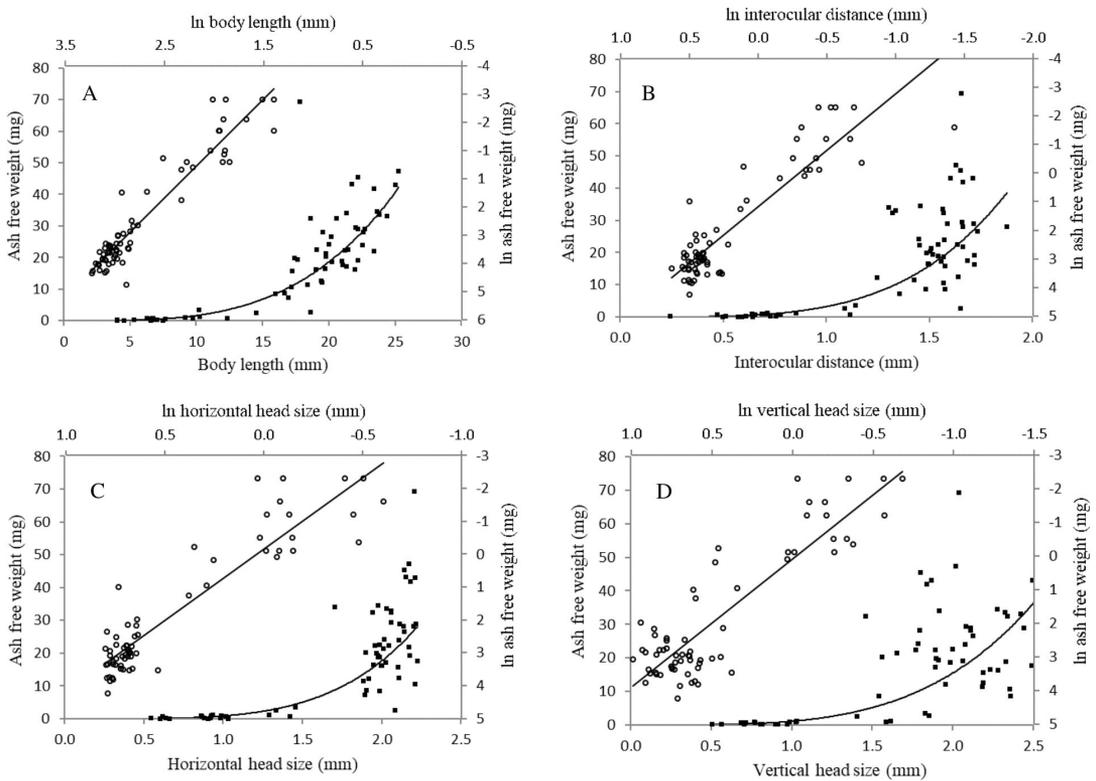


Figure 2. Length-mass curves for the *Leptonema* larvae from the Taboões stream for: body length (A), interocular distance (B), horizontal width of the cephalic capsule (C), and vertical width of the cephalic capsule (D), using both linear (open circles) and logarithmic (filled squares) scales. The power equation is $DM = aL^b$, where DM = dry mass, L = linear length and a/b are constants.

Phylloicus elektoros (Calamoceratidae, Trichoptera) in the Brazilian Central Amazon.

Allometric measurements for intraocular distance, vertical head size, and horizontal head size also showed high correlation with biomass. These body dimensions were also used by Cressa (1999) and Becker et al. (2009), due to sclerotized linear dimensions such as cephalic capsule width and pronotum length being less subject to distortions, breaks and deformations under individual manipulations (Johnston & Cunjak, 1999; Becker et al., 2009), when compared to body length. Becker (2005), studying the life cycle of *Agapetus fuscipes* (Trichoptera) in a stream in Germany, found that pronotum length is a reliable measure for different larval instars of the species. However, in our study, adjusted regression of sclerotized structures provided a lower fit than body length.

Preservation of invertebrates in ethanol or formaldehyde, often indispensable due to the amount and time required to process the collected samples (Nolte, 1990; Dekanová et al., 2022), can lead to the loss of more than 50% of their biomass (Silva et al.,

2010). Preservatives substances can dissolve lipids that are present in the larval body, mainly during the first three weeks after conservation, reducing biomass estimates (Wetzel et al., 2005; Benke & Huryn, 2010). In our study, *Leptonema* larvae were kept at low temperature (-20°C), without preservatives and all measurements were performed on unharmed individuals, which allowed a concise and safe determination of the relation between the biomass and the four body dimensions studied.

5. Conclusions

In conclusion, it was observed that the body length presented the best fit to estimate the *Leptonema* biomass, corroborating our initial hypothesis. However, all other allometric measurements studied provided good estimates for the biomass of this taxon. The power model described the length-mass relationships well and may be a good choice for studies with other aquatic insect species. Likewise, our results also reinforce the need for more length-mass studies of aquatic insects in the tropical region. We believe that, in order to

obtain more reliable results, data on the length-mass relationship should be obtained based on the population of the studied region. Although we may have more than one species for *Leptonema* larvae, we assume that multiple species differences are not relevant for genera biomass equations. As the larvae are so similar that only specialists could identify them if present, we can safely assume that they are similar enough for not have significant differences in the measurements used in this study. Therefore, the results presented here may be useful to determine the biomass of *Leptonema* larvae from the Neotropical Savanna. It is important to highlight that the length-dry mass equations can contribute to approaches with thermodynamic indicators for macroinvertebrate assemblages and will serve as a basis for future experimental studies in a mesocosm system, in order to test the *Leptonema* response to multiple pressures, such as flow change of water and oxygen depletion in streams of the Brazilian neotropical biome.

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