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Size-mass relationships of aquatic insects in forest streams: addressing a knowledge gap in Neotropical communities

Relações tamanho-massa de insetos aquáticos em riachos florestados: abordando uma lacuna de conhecimento em comunidades neotropicais

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ABSTRACT

Determining individuals' dry mass is crucial for better understanding community structure in terrestrial and aquatic ecosystems. The establishment of size–mass relationships for different populations constitutes a helpful tool for indirectly determining the total biomass of freshwater communities. In this study, we determined the size–mass relationships of 14 genera from the orders Ephemeroptera, Plecoptera, Trichoptera, and Odonata commonly found in leaf patches in Atlantic Forest streams (SE Brazil). We used individual body length as a size measure and three mathematical models (linear, exponential, and power) to establish the best-fitting equations. The equations described by the power model showed the best fit (coefficient of determination [r^2]≥0.80) for the genera *Anacroneuria*, *Macrogynoplax* (Plecoptera), *Smicridea* (Trichoptera), *Archaegomphus*, *Idiataphe*, *Hetaerina*, *Heteragrion*, and *Neocordulia* (Odonata); the exponential model presented the best fit for *Phylloicus* (r^2=0.74), *Triplectides* (Trichoptera, r^2=0.60), *Enderleina* (r^2=0.96), and *Tupiperla* (Plecoptera, r^2=0.60); and the linear model exhibited the best fit for *Elasmothemis* (Odonata, r^2=0.85) and *Massartella* (Ephemeroptera, r^2=0.63). Our findings demonstrated that body length is a strong predictor of dry mass for most of the studied genera but some exceptions suggest that it may not fully capture biomass variation. The power models performed better in general, whereas the exponential and linear models were optimal for specific genera, indicating diverse influences on taxa allometry. This study reinforces the need to evaluate different approaches for estimating the dry mass of aquatic insects and underscores the caution required when using indirect methods for biomass determination.

Keywords: body length; dry mass; biomass determination; freshwater invertebrates; Atlantic Forest streams.

RESUMO

A determinação da massa seca dos indivíduos é crucial para o melhor entendimento da estrutura das comunidades em ecossistemas terrestres e aquáticos. O estabelecimento das relações tamanho–massa para diferentes populações constitui uma ferramenta útil para determinar indiretamente a biomassa total de comunidades de aquáticas. Neste estudo, determinamos as relações tamanho–massa de 14 gêneros das ordens Ephemeroptera, Plecoptera, Trichoptera e Odonata comumente encontradas em acúmulos de folhas em riachos da Mata Atlântica (SE Brasil). Utilizamos o comprimento do corpo dos indivíduos como medida de tamanho e três modelos matemáticos (linear, exponencial e *power*) para estabelecer as equações de melhor ajuste. As equações descritas pelo modelo *power* apresentaram o melhor ajuste (coeficiente de determinação [r^2]≥0,80) para os gêneros *Anacroneuria*, *Macrogynoplax* (Plecoptera), *Smicridea* (Trichoptera), *Archaegomphus*, *Idiataphe*, *Hetaerina*, *Heteragrion* e *Neocordulia* (Odonata); o modelo exponencial mostrou o melhor ajuste para *Phylloicus* (r^2=0,74), *Triplectides* (Trichoptera, r^2=0,60), *Enderleina* (r^2=0,96) e *Tupiperla* (Plecoptera, r^2=0,60); enquanto o modelo linear apresentou o melhor ajuste para *Elasmothemis* (Odonata, r^2=0,85) e *Massartella* (Ephemeroptera, r^2=0,63). Os resultados obtidos demonstraram que o comprimento do corpo é um forte preditor da massa seca para a maioria dos gêneros estudados, mas algumas exceções sugerem que ele pode não capturar totalmente a variação da biomassa. O modelo *power* exibiu o melhor desempenho em geral, enquanto os modelos exponencial e linear foram ótimos para gêneros específicos, indicando diversas influências na alometria dos táxons. Este estudo reforça a necessidade de avaliar diferentes abordagens para estimar a massa seca de insetos aquáticos e ressalta o cuidado necessário ao utilizar métodos indiretos para determinação de biomassa.

Palavras-chave: comprimento do corpo; massa seca; determinação de biomassa; invertebrados aquáticos; riachos de Mata Atlântica.

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Introduction

Determining the dry mass of individuals in a community allows a better understanding of natural ecosystem functioning (Benke et al., 1999; Jacquet et al., 2022). Individuals' mass can be used to estimate standing stock biomass in different compartments of an ecosystem (Miserendino, 2001; Méthot et al., 2012). For example, estimating standing stock biomass at diverse trophic levels is essential to determining primary and secondary production, providing valuable information about energy flow in green and brown food webs (Benke et al., 1999; Kamburska et al., 2023). Variations in biomass proportions of different taxa can also be used to evaluate the effects of environmental changes on ecosystems and the resilience of communities in altered environments (Krynak and Yates, 2020; Loomer et al., 2023). For instance, a higher proportion of the biomass of generalist taxa than of specialist taxa may indicate anthropogenic impacts (Brito et al., 2021; Liebmann et al., 2022). Therefore, determining the dry mass of individuals is crucial for better understanding the structure of communities in terrestrial and aquatic ecosystems.

Establishing mathematical models between the body size and dry mass of individuals in the same population constitutes a helpful tool for indirectly determining total biomass values in freshwater communities (Smock, 1980). Because they allow individuals' dry mass determination quickly and accurately, size–mass relationships have been widely used in studies involving fish and aquatic invertebrates, especially when samples contain many individuals (Burgherr and Meyer, 1997; Azrita et al., 2024). In addition to size–mass relationships, direct measurements of dry mass and indirect determination by individuals' biovolume are also used in ecological studies (Benke et al., 1999). However, directly measuring dry mass results in the loss of individuals, preventing their subsequent use in molecular analyses or maintenance in scientific collections (Mährlein et al., 2016). Moreover, indirect determination of dry mass through individuals' biovolume is less accurate, mainly because it underestimates the mass of individuals with larger body sizes (Burgherr and Meyer, 1997). Despite the advantages of using size–mass relationships, they must be employed cautiously (Dekanová et al., 2022; Kamburska et al., 2023), as individuals of the same taxon found in different ecosystems may present morphological variations (Johnston and Cunjak, 1999; Martins et al., 2014). Furthermore, resource availability and water temperature can affect individuals' development and, consequently, size–mass relationships (Wonglersak et al., 2021; Collyer et al., 2023; Zamora-Camacho, 2025).

Size–mass relationships are often used in studies on aquatic insects (Smock, 1980; Burgherr and Meyer, 1997), as they are responsible for a significant part of secondary production in freshwater ecosystems (Benke et al., 1999). Investigating aquatic insects is essential for studying trophic relationships and energy transfer in food webs due to their considerable diversity and abundance (Miserendino, 2001; Wahl et al., 2021). Special attention should be given to the orders Ephemeroptera, Plecoptera, Trichoptera, and Odonata, as taxa of these groups directly and indirectly affect critical ecological processes (Arnaud et al., 2022; Oester et al., 2023). Moreover, many species from these orders are particularly susceptible to changes in the physical and chemical characteristics of water and resource availability, making them valuable indicators of aquatic ecosystem health (Akamagwuna and Odume, 2020; Lima et al., 2022; El Yaagoubi et al., 2024). Therefore, the size–mass relationships determined for Ephemeroptera, Plecoptera, Trichoptera, and Odonata taxa are key tools for studies assessing stream ecosystem responses to environmental changes.

In a global meta-analysis of insect size–mass power coefficients, Martin et al. (2014) found that allometric coefficients were positively related to latitude, with aquatic insects presenting higher coefficients than terrestrial ones. Despite the recognized relevance of biomass values in studies on aquatic insect communities, only a few taxa have had their size–mass relationships determined in neotropical streams (Zilli et al., 2017). This lack of knowledge contrasts with the high abundance and taxonomic richness of tropical aquatic communities, which makes direct measurement of individuals' dry mass exhaustive in ecological studies. Aquatic insects are a diverse group, leading to significant variation in size–mass relationships among different families or genera within the same order, as well as among different populations of the same species (Johnston and Cunjak, 1999; Méthot et al., 2012; Mocq et al., 2024). While size–mass relationships can vary among species, differences in maximum size between species within the same genus are generally smaller than variations between different genera (Mocq et al., 2024). Therefore, size–mass relationships at the genus level are valuable for capturing general trends that are useful for large-scale ecological modeling (Benke et al., 1999). To increase the availability of information for aquatic insects in neotropical streams, we determined the size–mass relationships of the most abundant genera from the orders Ephemeroptera, Plecoptera, Trichoptera, and Odonata found in leaf patches in Atlantic Forest streams (SE Brazil). We used linear, exponential, and power mathematical models to establish the best-fitting equations.

Methods

Study area

We collected all individuals used in this study from a preserved forest stream (20°27'30" S, 40°50'06" W) located in the municipality of Marechal Floriano in the mountainous region of Espírito Santo state (SE Brazil). According to the Köppen climate classification, the region's climate is considered as "Aw" (hot temperate), with no dry season in winter (Alvares et al., 2013). Throughout the year, the monthly average precipitation and air temperature range from 53.8 to 251 mm and from 16.8 to 22.9°C, respectively. Despite agricultural activities being the base of its economy, the municipality is surrounded by remnants of the Atlantic Forest. The studied stream was located within a forest fragment of 5,848 m^2, at an altitude of 780 m. Samples were collected in Oc-

tober and November 2022 and in January, February, and March 2023. During this period, the stream water exhibited temperatures between 15.6 and 22.2°C, a slightly acidic pH (6.3 to 7.2), good oxygenation (7.1 to 10.4 mg/L), and low electrical conductivity (0.017 to 0.030 mS/cm).

Aquatic insects

The aquatic insects were collected from leaf patches formed on the streambed via a Surber sampler (0.09 m^2, 500 µm). During the study period, we collected ten samples every 30 days, five from pools and five from riffles, for a total of 50 samples. We could not collect samples in December, because the stream was inaccessible for some weeks due to intense rains. We stored the samples individually in plastic bags and transported them to the laboratory on the same day. In the laboratory, we washed the samples with running water through 500- and 250-µm sieves and preserved the retained material in 70% ethanol for later sorting and identification. We sorted the samples under light boxes and counted and identified individuals under a stereoscopic microscope (ZEISS Stemi 305, 40x magnification). We identified the individuals of the orders Ephemeroptera, Plecoptera, Trichoptera, and Odonata at the genus level using specific taxonomic keys (Hamada et al., 2014; 2019).

To determine the size–mass relationships, we selected the most abundant genera found in the samples. Table 1 presents the selected genera. We used body length to predict dry mass because several previous studies described size–mass equations with good predictive power for aquatic insect taxa sampled in different world regions (see Smock, 1980; Meyer, 1989; Benke et al., 1999; Becker et al., 2009; Dekanová et al., 2024). We photographed each individual under a stereoscopic microscope (ZEISS Stemi 305, 32x magnification) with an HD digital camera (ZEISS Axiocam). We determined the body size by measuring the length of each individual via ImageJ software (US National Institutes of Health, Bethesda, Maryland, USA; accuracy of 0.1 mm) (Schneider et al., 2012). The body length of each individual was measured as the distance from the anterior end of the head capsule to the posterior end of the last abdominal segment, excluding the anal prolegs and cerci (Benke et al., 1999). For dry mass determination, we placed the photographed individuals separately in pre-weighed aluminum crucibles and dried them in an oven (60°C for 48 hours) (Becker et al., 2009). The crucibles were then cooled in a desiccator (1 hour) and weighed on an analytical balance (accuracy of 0.01 mg). Because storing aquatic invertebrates in preservative solutions can lead to the leaching of body fluids and loss of body mass (Dekanová et al., 2023), we measured and determined the dry mass of all individuals within one month after sampling.

Data analysis

We used the linear (Equation 1), exponential (Equation 2), and power (Equation 3) mathematical models, or their logarithmic equivalents, to determine the best-fitting equation between body length and dry mass for each genus, as follows:

$$DM = a + b.\,L \quad (1)$$

$$DM = a.\,e^{bL} \text{ (linear format: } \ln DM = \ln a + b.\,L) \quad (2)$$

Table 1 – Number of individuals (n), ranges and coefficient of variation (in percentage) of body length (mm) and dry mass (mg) values for the most abundant genera of Ephemeroptera, Plecoptera, Trichoptera, and Odonata found in leaf patches in an Atlantic Forest stream (SE Brazil).

Order	Family	Genus	n	Body length		Dry mass	
				Range (mm)	CV (%)	Range (mg)	CV (%)
Ephemeroptera	Leptophlebiidae	*Massartella*	15	8.20–18.29	62.16	0.71–7.07	50.77
Plecoptera	Perlidae	*Anacroneuria*	31	2.90–10.40	23.31	0.03–4.37	65.42
	Perlidae	*Enderleina*	9	3.20–20.00	67.64	0.09–38.90	182.03
	Perlidae	*Macrogynoplax*	37	2.57–14.13	40.26	0.10–9.32	183.99
	Gripoptegygidae	*Tupiperla*	31	2.15–7.65	28.62	0.04–0.49	62.15
Trichoptera	Calamoceratidae	*Phylloicus*	241	1.19–17.73	43.48	1.10–66.50	135.32
	Leptoceridae	*Triplectides*	134	3.35–18.30	40.64	0.10–71.30	126.55
	Hydropsychidae	*Smicridea*	35	2.67–20.54	55.26	0.03–7.73	175.22
Odonata	Gomphidae	*Archaegomphus*	32	4.38–30.00	61.16	0.15–56.18	189.98
	Libellulidae	*Elasmothemis*	12	3.64–9.58	32.92	0.27–5.7	82.14
	Libellulidae	*Idiataphe*	13	3.85–17.43	41.16	0.18–11.86	96.88
	Calopterygidae	*Hetaerina*	23	2.64–20.66	40.91	0.06–8.67	83.71
	Megapodagrionidae	*Heteragrion*	41	2.67–14.37	43.32	0.04–4.58	108.14
	Corduliidae	*Neocordulia*	17	3.25–19.38	37.18	0.11–16.46	95.83

CV: coefficient of variation.

$$DM = a.\ L^b \text{ (linear format: } \ln DM = \ln a + b.\ \ln L) \quad (3)$$

Where:
DM=dry body mass (mg);
L=body length (mm);
a and *b*=regression constants; and
e=mathematical constant (2.718, Euler's number).

We assessed the fit of the regression equations through the coefficient of determination (r^2), the significance level (*p* obtained from regression analysis of variance [ANOVA]), and residual analysis. We classified the equations as having good predictive power (r^2>0.80), moderate predictive power (0.60<r^2<0.80), or low predictive power (r^2<0.60). We performed all analyses via R software (R Core Team, 2024).

Results

We measured 671 individuals and determined the size–mass relationships for 14 of aquatic insect genera, including one Ephemeroptera, four Plecoptera, three Trichoptera, and six Odonata (Table 1). Except for the genus *Enderleina* (Perlidae; 9 individuals), all the studied genera had at least 12 individuals sampled and measured. The coefficients of variation (CVs) of body length ranged from 23.31% in *Anacroneuria* (Perlidae) to 67.64% in *Enderleina*. The dry mass values had greater variation, and the CVs ranged from 50.77% for *Massartella* (Leptophlebiidae) to 189.98% for *Archaegomphus* (Gomphidae; Table 1). We observed a wide range of body length and dry mass values for each genus, suggesting that we used individuals in different developmental stages to determine the size–mass relationships.

Body length determined size–mass relationships at a high significance level in the three mathematical models (p<0.001; Table 2). The coefficients of determination (r^2) for the significant equations ranged from 0.51 (*Tupiperla*) to 0.85 (*Elasmothemis*) in the linear model, from 0.60 (*Tupiperla*) to 0.96 (*Enderleina*) in the exponential model, and from 0.58 (*Tupiperla*) to 0.94 (*Neocordulia*) in the power model. The slope values (*b*), indicating the effect of size on the dry mass of individuals, ranged from 0.06 (*Tupiperla*) to 2.65 (*Phylloicus*) in the linear model, from 0.15 (*Massartella*) to 0.55 (*Anacroneuria*) in the exponential model, and from 1.57 (*Tupiperla*) to 3.59 (*Archaegomphus*) in the power model (Table 2).

The equations described by the power model showed good predictive power (r^2≥0.80) for the genera *Anacroneuria*, *Enderleina*, *Macrogynoplax* (Plecoptera), *Smicridea* (Trichoptera), *Archaegomphus*, *Elasmothemis*, *Idiataphe*, *Hetaerina*, *Heteragrion*, and *Neocordulia* (Odonata). The power model also described equations with moderate predictive power (0.60<r^2<0.80) for the genera *Massartella* (Ephemeroptera), *Triplectides*, and *Phylloicus* (Trichoptera) and low predictive power (r^2<0.60) for the genus *Tupiperla* (Plecoptera; Table 2). The size–mass equations determined for the genera *Phylloicus* (r^2=0.74), *Enderleina* (r^2=0.96), and *Tupiperla* (r^2=0.60) presented the best fit with the exponential model, whereas those for the genera *Elasmothemis* (r^2=0.85) and *Massartella* (r^2=0.63) had the best fit with the linear models (Table 2). The equations determined for the genera *Tupiperla* and *Triplectides* had the lowest coefficients of determination across all the studied taxa, with the best fits observed in the exponential and power models (r^2=0.60, Table 2). Figure 1 shows the relationships of the biomass of the studied genera as a function of body length.

Discussion

Our results indicated that body length is a robust predictor of dry mass for most of the insect genera studied, with a high predictive power (r^2>0.80) in most cases. However, the size–mass relationships determined for *Massartella* (Ephemeroptera), *Tupiperla* (Plecoptera), *Triplectides*, and *Phylloicus* (Trichoptera) presented a moderate predictive power (0.60<r^2<0.80), suggesting that body length may not fully capture the variation in dry mass for these taxa. These findings are consistent with previous research, which has established body length as a reliable proxy for estimating the dry mass of aquatic invertebrates (Smock, 1980; Meyer, 1989; Benke et al., 1999; Becker et al., 2009), particularly regarding its broader measurement range than other morphological metrics, such as head capsule width or tarsus length. Body length usually presents the highest coefficient of variation among other metrics, representing effectively the size variation among specimens of a population. Notably, the power models provided the best fit for the size–mass relationships across most genera, outperforming both the exponential and linear models in terms of predictive accuracy.

The power models accounted for 75 to 94% of the variation in dry mass across most genera, including *Anacroneuria*, *Macrogynoplax*, *Archaegomphus*, *Idiataphe*, *Neocordulia*, *Heteragrion*, *Hetaerina*, and *Smicridea*. These results corroborate previous studies in aquatic ecosystems, which have consistently demonstrated that power models are effective in describing the relationship between the body length and dry mass of freshwater invertebrates (Burgherr and Meyer, 1997; Benke et al., 1999; Becker et al., 2009), suggesting a typical nonlinear relationship between size and mass. This high level of predictive power reinforces the utility of the power model as a reliable tool in ecological research, particularly for taxa in which body length is a strong indicator of biomass (Meyer, 1989; Miserendino, 2001). However, the exponential model showed the highest predictive power for some genera, such as *Enderleina*, *Phylloicus*, and *Tupiperla*, suggesting that different ecological and biological factors may influence size–mass relationships (Smock, 1980; Kiffer et al., 2016). While the power model is broadly applicable across many taxa, incorporating alternative models may provide a more nuanced understanding of biomass estimation in particular ecological contexts (Wenzel et al., 1990; González, 2002).

As indicated by the slope values, the effect of size on dry mass varied considerably among taxa and models. Body dimensions increase proportionately when the coefficient *b* is close to 3, indicating isometric growth, where body dimensions, i.e., length, width, and height, scale

Table 2 – Parameters (± SE) of the linear, exponential, and power models for the relationship between body length (L, mm) and dry mass (mg) of the most abundant genera of Ephemeroptera, Plecoptera, Trichoptera, and Odonata found in leaf patches in an Atlantic Forest stream (SE Brazil).

Taxon	Function	A	ln a	b	r^2
Massartella (Leptophlebiidae)	Linear	-2.74±1.28		0.45±0.09	0.63*
	Exponential		-1.05±0.45	0.15±0.03	0.61*
	Power		-4.21±1.06	2.04±0.40	0.63*
Anacroneuria (Perlidae)	Linear	-2.19±0.40		0.49±0.05	0.74*
	Exponential		-4.01±0.32	0.55±0.04	0.84*
	Power		-6.99±0.37	3.59±0.18	0.92*
Enderleina (Perlidae)	Linear	-9.26±4.37		1.75±0.39	0.69*
	Exponential		-3.63±0.27	0.37±0.02	0.96*
	Power		-6.86±0.76	3.32±0.35	0.91*
Macrogynoplax (Perlidae)	Linear	-2.68±0.29		0.68±0.05	0.82*
	Exponential		-2.93±0.17	0.41±0.03	0.82*
	Power		-4.99±0.30	2.67±0.18	0.85*
Tupiperla (Gripoptegygidae)	Linear	-0.11±0.05		0.06±0.01	0.51*
	Exponential		-3.71±0.28	0.36±0.05	0.60*
	Power		-4.31±0.38	1.57±0.23	0.58*
Phylloicus (Calamoceratidae)	Linear	-11.54±1.14		2.65±0.14	0.59*
	Exponential		-0.54±0.08	0.27±0.01	0.74*
	Power		-1.89±0.16	1.78±0.08	0.64*
Smicridea (Hydropsychidae)	Linear	-1.64±0.41		0.33±0.04	0.62*
	Exponential		-3.79±0.23	0.32±0.02	0.83*
	Power		-6.36±0.35	2.80±0.17	0.88*
Triplectides (Leptoceridae)	Linear	-11.26±1.80		2.39±0.19	0.52*
	Exponential		-1.04±0.19	0.29±0.02	0.60*
	Power		-3.52±0.35	2.43±0.17	0.60*
Archaegomphus (Gomphidae)	Linear	-13.18±2.28		1.70±0.16	0.78*
	Exponential		-2.15±0.18	0.22±0.01	0.90*
	Power		-6.57±0.33	3.05±0.13	0.93*
Elasmothemis (Libellulidae)	Linear	-2.63±0.60		0.73±0.09	0.85*
	Exponential		-1.98±0.33	0.37±0.05	0.82*
	Power		-3.82±0.60	2.35±0.33	0.81*
Idiataphe (Libellulidae)	Linear	-4.11±1.11		0.73±0.09	0.81*
	Exponential		-2.14±0.26	0.27±0.02	0.91*
	Power		-4.96±0.47	2.52±0.20	0.92*
Hetaerina (Calopterygidae)	Linear	-2.45±0.69		0.43±0.04	0.77*
	Exponential		-2.53±0.30	0.24±0.02	0.85*
	Power		-5.62±0.39	2.51±0.15	0.92*
Heteragrion (Megapodagrionidae)	Linear	-1.33±0.23		0.33±0.02	0.76*
	Exponential		-3.00±0.19	0.34±0.02	0.82*
	Power		-5.19±0.26	2.48±0.13	0.89*
Neocordulia (Corduliidae)	Linear	-6.11±1.52		1.02±0.13	0.77*
	Exponential		-2.11±0.32	0.29±0.02	0.86*
	Power		-5.39±0.39	2.81±0.17	0.94*

a/*b*: regression coefficients; r^2: coefficient of determination (*p<0.001).

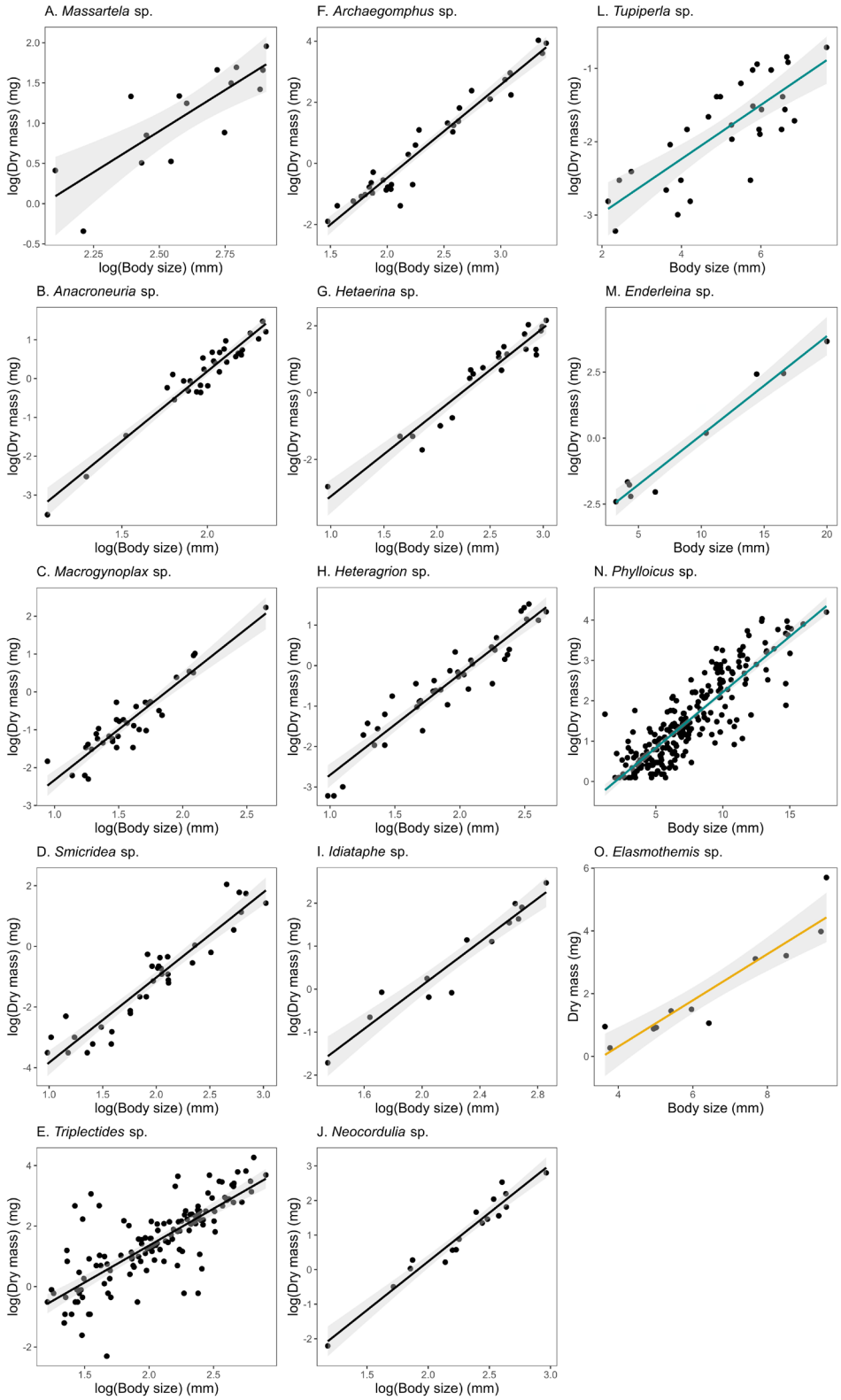

Figure 1 – Scatter diagrams of dry mass versus body length for the most abundant genera of Ephemeroptera, Plecoptera, Trichoptera, and Odonata found in leaf patches in an Atlantic Forest stream (SE Brazil). The regression equation is power dry mass=a. L^b (in linear format: ln dry mass=ln a+b. ln L; Panels A to J, black regression lines), exponential dry mass=a. e^{bL} (in linear format: ln dry mass=ln a+b. L; Panels L to N, cyan regression lines), and linear dry mass=a+b. L (Panel O, orange regression line). The confidence intervals are represented in light gray.

proportionally (Benke et al., 1999). However, *b* values smaller than 3 (negative allometry) are frequently observed in aquatic invertebrate populations (Smock, 1980; Burgherr and Meyer, 1997; Cressa, 1999), suggesting that mass does not scale directly with body length (Costa et al., 2018). On the other hand, *b* values closer to 2 indicate that the body surface, which scales with the square of length, is more correlated with mass than body length (Wenzel et al., 1990; Towers et al., 1994). In this study, we identified seven genera with powerful size–biomass relationships and *b* coefficients ranging from 2.65 (*Phylloicus*) to 3.59 (*Anacroneuria*). However, the other half of the genera presented *b* coefficients ranging from 0.06 (*Tupiperla*) to 2.52 (*Idiataphe*). These findings highlight the diverse growth patterns among aquatic invertebrates (McCarty et al., 2022), with some taxa showing a closer relationship to isometric growth and others demonstrating significant deviations that suggest a greater influence of other morphological variables, such as body surface area (Costa et al., 2018). This variability underscores the complexity of determining size–mass relationships in aquatic ecosystems and emphasizes the need for specific approaches when these relationships are used to estimate dry mass across different taxa (Martins et al., 2014; Nakagawa and Takemon, 2014).

Interestingly, we observed a notable variation in the values of coefficient *b* within the same family, specifically in Libellulidae and Perlidae. This pattern aligns with that reported by Mocq et al. (2024), where allometric slopes in larvae of Culicidae varied significantly among species. Such variability within families suggests that relying on size–mass relationships at the family level may introduce substantial errors (Dekanová et al., 2024). The diversity observed in aquatic insects within and across families and even within genera underscores the importance of describing size–mass equations at the most specific taxonomic levels possible (Benke et al., 1999). This approach is crucial because aquatic insects exhibit significant changes in size–mass relationships not only within various families or genera (Hurtado-Borrero et al., 2020) but also among different populations of the same species (Johnston and Cunjak, 1999; Méthot et al., 2012; Mocq et al., 2024).

Conclusion

Our results emphasize the importance of body length as a reliable predictor of dry mass for most aquatic insects found in leaf patches. However, the moderate to low predictive power of the equations determined for *Tupiperla*, *Triplectides*, and *Phylloicus* suggests that body length may not fully account for variations in the dry mass of some shredders in Neotropical streams. These findings highlight the need to evaluate different approaches to estimating the dry mass of aquatic insects. While power models generally performed better for most genera, exponential and linear models were more effective for specific genera, possibly reflecting the diverse ecological and biological factors influencing insect growth patterns. Additionally, the wide variation in coefficient *b* of the described equations suggests that other morphological variables may better predict dry mass than body length for some genera. This fact underscores the complexity of determining size–mass relationships for aquatic insects and the importance of using indirect methods for biomass determination with caution.

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Authors' contributions

Chagas, F.S.: conceptualization, formal analysis, investigation, writing – original draft, visualization. **Omena**, P.M.: investigation, writing – original draft, formal analysis, data curation. **Costa**, L.C.: investigation, writing – original draft, visualization. **Serpa**, K.V.: investigation, writing – review & editing. **Moretti**, M.S.: conceptualization, writing – original draft, supervision, project administration.

References

Akamagwuna, F.C.; Odume, O.N., 2020. Ephemeroptera, Plecoptera and Trichoptera (EPT) functional feeding group responses to fine grain sediment stress in a river in the Eastern Cape, South Africa. Environmental Monitoring and Assessment, v. 192 (4), 214. https://doi.org/10.1007/s10661-020-8187-4.

Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.M.; Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift, v. 22 (6), 711-728. https://doi.org/10.1127/0941-2948/2013/0507.

Arnaud, S.; Kari, K.; Lenin, C.; André, M.; Olga, P.; Justin, P.; David, B.; Nedim, T.; Robby, S., 2022. Odonata trophic ecology: from hunting behavior to cross-ecosystem impact. In: Cordoba-Aguilar, A.; Beatty, C.; Bried, J. (Eds.), Dragonflies and Damselflies: Model Organisms for Ecological and Evolutionary Research. Oxford University Press, Oxford, pp. 219-232. https://doi.org/10.1093/oso/9780192898623.003.0016.

Azrita, A.; Syandri, H.; Aryani, N., 2024. Length and weight relationship, condition factor, and morphometric characteristics of eleven freshwater fish species in Koto Panjang reservoir, Indonesia. International Journal of Zoology, v. 2024, 9927705. https://doi.org/10.1155/2024/9927705.

Becker, B.; Moretti, M.S.; Callisto, M., 2009. Length–dry mass relationships for a typical shredder in Brazilian streams (Trichoptera: Calamoceratidae). Aquatic Insects, v. 31 (3), 227-234. https://doi.org/10.1080/01650420902787549.

Benke, A.C.; Huryn, A.D.; Smock, L.A.; Wallace, J.B., 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the Southeastern United States. Journal of the North American Benthological Society, v. 18 (3), 308-343. https://doi.org/10.2307/1468447.

Brito, J.P.; Carvalho, F.G.; Juen, L., 2021. Response of the Zygopteran community (Odonata: Insecta) to change in environmental integrity driven by urbanization in Eastern Amazonian streams. Ecologies, v. 2, 150-163. https://doi.org/10.3390/ecologies2010008.

Burgherr, P.; Meyer, E.I., 1997. Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. Archiv für Hydrobiologie, v. 139 (1), 101-112. https://doi.org/10.1127/archiv-hydrobiol/139/1997/101.

Collyer, G.; Perkins, D.M.; Petsch, D.K.; Siqueira, T.; Saito, V., 2023. Land-use intensification systematically alters the size structure of aquatic communities in the Neotropics. Global Change Biology, v. 29 (14), 4094-4106. https://doi.org/10.1111/gcb.16720.

Costa, L.C.; Kiffer, W.P.; Casotti, C.G.; Moretti, M.S., 2018. Size-mass relationships in *Trichodactylus fluviatilis* Latreille, 1828 (Decapoda: Brachyura: Trichodactylidae), a macroconsumer in coastal streams of the Atlantic Forest, southeastern Brazil. Journal of Crustacean Biology, v. 38 (5), 539-546. https://doi.org/10.1093/jcbiol/ruy065.

Cressa, C., 1999. Dry mass estimates of some tropical aquatic insects. Revista de Biología Tropical, v. 47 (1-2), 133-141. https://doi.org/10.15517/rbt.v47i1-2.19062.

Dekanová, V.; Streberová, Z.; Novikmec, M.; Svitok, M., 2023. The effect of preservation on biomass and length estimates and its variation within and between two mayfly species. Limnology, v. 24 (3), 181-191. https://doi.org/10.1007/s10201-023-00715-8.

Dekanová, V.; Svitok, M.; Bento, S.; Caramelo, J.; Peixe e Sousa, P.; Carreira, B.M., 2024. Length-mass relationships of pond macroinvertebrates do not hold between Southern and Northern Europe. PeerJ, v. 12, e18576. https://doi.org/10.7717/peerj.18576.

Dekanová, V.; Venarsky, M.P.; Bunn, S.E., 2022. Length–mass relationships of Australian aquatic invertebrates. Austral Ecology, v. 47 (1), 120-126. https://doi.org/10.1111/aec.13077.

El Yaagoubi, S.; Edegbene, A.O.; El Alami, M.; Errochdi, S.; Harrak, R., 2024. Ephemeroptera, Plecoptera and Trichoptera (EPT) trait-based biomonitoring of rivers within the northwestern Rif of Morocco: implications for determining riverine ecosystems ecological health in Africa. Aquatic Sciences, v. 86 (2), 54. https://doi.org/10.1007/s00027-024-01070-1.

González, J.M., 2002. Size-mass relationships of stream invertebrates in a northern Spain stream. Hydrobiologia, v. 489 (1/3), 131-137. https://doi.org/10.1023/a:1023220501921.

Hamada, N.; Nessimian, J.L.; Querino, R.B., 2014. Insetos aquáticos na Amazônia brasileira: taxonomia, biologia e ecologia. Editora do INPA, Manaus, 724 p.

Hamada, N.; Thorp, J.H.; Rogers, D.C., 2019. Thorp and Covich's Freshwater Invertebrates, Volume Three: Keys to Neotropical Hexapoda. Academic Press, [S.l.].

Hurtado-Borrero, Y.; Pinilla-A, G.; Tamaris-Turizo, C.E., 2020. Relations length-dry weight of nymphs *Anacroneuria caraca* Stark, 1995 and *A. marta* Zúñiga & Stark, 2002 (Plecoptera: Perlidae) from a neotropical mountain river. Hidrobiológica, v. 30 (3), 203-209. https://doi.org/10.24275/uam/izt/dcbs/hidro/2020v30n3/hurtado.

Jacquet, C.; Carraro, L.; Altermatt, F., 2022. Meta-ecosystem dynamics drive the spatial distribution of functional groups in river networks. Oikos, v. 2022 (11), e09372. https://doi.org/10.1111/oik.09372.

Johnston, T.A.; Cunjak, R.A., 1999. Dry mass–length relationships for benthic insects: a review with new data from Catamaran Brook, New Brunswick, Canada. Freshwater Biology, v. 41 (4), 653-674. https://doi.org/10.1046/j.1365-2427.1999.00400.x.

Kamburska, L.; Zaupa, S.; Boggero, A., 2023. Size-pattern and larval-length–mass relationships for the most common chironomid taxa in the deep subalpine Lake Maggiore. Water, v. 15 (15), 2730. https://doi.org/10.3390/w15152730.

Kiffer, W.P.; Mendes, F.; Rangel, J.V.; Barbosa, P.; Serpa, K.; Moretti, M.d.S., 2016. Size-mass relationships and the influence of larval and case size on the consumption rates of *Triplectides* sp. (Trichoptera, Leptoceridae). Fundamental and Applied Limnology, v. 188 (1), 73-81. https://doi.org/10.1127/fal/2016/0855.

Krynak, E.M.; Yates, A.G., 2020. Intensive agriculture alters the biomass size spectrum and body-mass of benthic insects: evidence from a reciprocal transfer experiment. Hydrobiologia, v. 847, 1221-1235. https://doi.org/10.1007/s10750-020-04178-1.

Liebmann, L.; Vormeier, P.; Weisner, O.; Liess, M., 2022. Balancing effort and benefit – How taxonomic and quantitative resolution influence the pesticide indicator system SPEARpesticides. Science of The Total Environment, v. 848, 157642. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.157642.

Lima, M.; Firmino, V.C.; De Paiva, C.K.S.; Juen, L.; Brasil, L.S., 2022. Land use changes disrupt streams and affect the functional feeding groups of aquatic insects in the Amazon. Journal of Insect Conservation, v. 26 (2), 137-148. https://doi.org/10.1007/s10841-022-00375-6.

Loomer, H.A.; Kidd, K.A.; Erdozain, M.; Benoy, G.A., Chambers, P.A.; Culp, J.M., 2023. Stream macroinvertebrate community responses to an agricultural gradient alter consumer-driven nutrient dynamics. Hydrobiologia, v. 850, 315-334. https://doi.org/10.1007/s10750-022-05070-w.

Mährlein, M.; Pätzig, M.; Brauns, M.; Dolman, A.M., 2016. Length–mass relationships for lake macroinvertebrates corrected for back-transformation and preservation effects. Hydrobiologia, v. 768 (1), 37-50. https://doi.org/10.1007/s10750-015-2526-4.

Martin, C.A; Proulx, R.; Magnan, P., 2014. The biogeography of insects' length–dry mass relationships. Insect Conservation and Diversity, v. 7 (5), 413-419. https://doi.org/10.1111/icad.12063.

Martins, R.T.; Melo, A.S.; Gonçalves Jr, J.F.; Hamada, N., 2014. Estimation of dry mass of caddisflies *Phylloicus elektoros* (Trichoptera: Calamoceratidae) in a Central Amazon stream. Zoologia, v. 31 (4), 337-342. https://doi.org/10.1590/s1984-46702014000400005.

McCarty, J.D.; Cross, W.F.; Albertson, L.K.; Tumolo, B.B.; Sklar, L.S., 2022. Life histories and production of three Rocky Mountain aquatic insects along an elevation-driven temperature gradient. Hydrobiologia, v. 849, 3633-3652. https://doi.org/10.1007/s10750-022-04978-7.

Méthot, G.; Hudon, C.; Gagnon, P.; Pinel-Alloul, B.; Armellin, A.; Poirier, A.M.T., 2012. Macroinvertebrate size–mass relationships: how specific should they be? Freshwater Science, v. 31 (3), 750-764. https://doi.org/10.1899/11-120.1.

Meyer, E.I., 1989. The relationship between body length parameters and dry mass in running water invertebrates. Archiv für Hydrobiologie, v. 117 (2), 191-203. https://doi.org/10.1127/archiv-hydrobiol/117/1989/191.

Miserendino, M.L., 2001. Length-mass relationships for macroinvertebrates in freshwater environments of Patagonia (Argentina). Ecología Austral, v. 11 (1), 3-8. ISSN 1667-782X.

Mocq, J.; Dekanová, V.; Boukal, D.S., 2024. Length–mass allometries of the larvae of aquatic dipterans: differences between taxa, morphological traits, and methods. Journal of Insect Science, v. 24 (1), 10. https://doi.org/10.1093/jisesa/ieae012.

Nakagawa, H.; Takemon, Y., 2014. Length-mass relationships of macroinvertebrates in a freshwater stream in Japan. Aquatic Insects, v. 36 (1), 53-61. https://doi.org/10.1080/01650424.2014.1001400.

Oester, R.; dos Reis Oliveira P.C.; Moretti, M.S.; Altermatt, F.; Bruder, A., 2023. Leaf-associated macroinvertebrate assemblage and leaf litter breakdown in headwater streams depend on local riparian vegetation. Hydrobiologia, v. 850, 3359-3374. https://doi.org/10.1007/s10750-022-05049-7.

R Core Team, 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods, v. 9 (7), 671-675. https://doi.org/10.1038/nmeth.2089.

Smock, L.A., 1980. Relationships between body size and biomass of aquatic insects. Freshwater Biology, v. 10 (4), 375-383. https://doi.org/https://doi.org/10.1111/j.1365-2427.1980.tb01211.x.

Towers, D.J.; Henderson, I.M.; Veltman, C.J., 1994. Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. New Zealand Journal of Marine and Freshwater Research, v. 28 (2), 159-166. https://doi.org/10.1080/00288330.1994.9516604.

Wahl, C.; Diaz, R; Kaller, M., 2021. Invasive floating fern limits aerial colonization and alters community structure of aquatic insects. Wetlands, v. 41, 60. https://doi.org/10.1007/s13157-021-01457-y.

Wenzel, F.; Meyer, E.; Schwoerbel, J., 1990. Morphometry and biomass determination of dominant mayfly larvae (Ephemeroptera) in running waters. Archiv für Hydrobiologie, v. 118 (1), 31-46. https://doi.org/10.1127/archiv-hydrobiol/118/1990/31.

Wonglersak, R.; Fenberg, P.B.; Langdon, P.G.; Brooks, S.J.; Price, B.W., 2021. Insect body size changes under future warming projections: a case study of Chironomidae (Insecta: Diptera). Hydrobiologia, v. 848 (11), 2785-2796. https://doi.org/10.1007/s10750-021-04597-8.

Zamora-Camacho, F.J., 2025. We are what what we eat eats: the effects of prey diet on growth, development, behavior and survival of a secondary consumer. Hydrobiologia, v. 852, 55-67. https://doi.org/10.1007/s10750-024-05637-9.

Zilli, F.; Barco, J.D.; Vanzetti, A., 2017. Biometry of neotropical invertebrates inhabiting floodplain rivers: unraveling bionomy. Iheringia. Série Zoologia, v. 107, e2017014. https://doi.org/10.1590/1678-4766e2017014.