Biology, Ecology and Diversity

Biomass estimation of *Triplectides egleri* Sattler (Trichoptera, Leptoceridae) in a stream at Ducke Reserve, Central Amazonia

Janaina Gomes de Brito*, Renato Tavares Martins, Kleicy Maciel Soares, Neusa Hamada

Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil

ABSTRACT

Biomass is a fundamental measure for understanding the structure and functioning (e.g. fluxes of energy and nutrients in the food chain) of aquatic ecosystems. We aim to provide predictive models to estimate the biomass of *Triplectides egleri* Sattler, 1963, in a stream in Central Amazonia, based on body and case dimensions. We used body length, head-capsule width, interocular distance and case length and width to derive biomass estimations. Linear, exponential and power regression models were used to assess the relationship between biomass and body or case dimensions. All regression models used in the biomass estimation of *T. egleri* were significant. The best fit between biomass and body or case dimensions was obtained using the power model, followed by the exponential and linear models. Body length provided the best estimate of biomass. However, the dimensions of sclerotized structures (interocular distance and head-capsule width) also provided good biomass predictions, and may be useful in estimating biomass of preserved and/or damaged material. Case width was the dimension of the case that provided the best estimate of biomass. Despite the low relation, case width may be useful in studies that require low stress on individuals.

Introduction

Abundance is the most common measurement used to understand the functioning (e.g. fluxes of energy and nutrients in the food chain) of aquatic ecosystems (Callisto et al., 2001; Townsend et al., 2003). However, individuals and taxa have different sizes and, consequently, different ecological importance, e.g. one large shredder (*Triplectides*) may be more important on leaf breakdown than two or more small shredders (Woodward et al., 2005). Thus, biomass measurements are fundamental to understand processes (e.g. energy transformation) in aquatic ecosystems. Moreover, biomass is essential to evaluating secondary production, life history, trophic relationships between the functional groups, and the role of invertebrates in leaf decomposition (e.g., Benke and Huryn, 2010; Brand and Miserendino, 2012; Luek et al., 2015).

Biomass can be estimated directly (weighing individuals) or indirectly using body and/or case dimensions (Smock, 1980; Burgherr and Meyer, 1997). Direct methods for determining biomass generally do not allow use of individuals for further experiments (Cressa, 1999a). In addition, in determining biomass of aquatic invertebrates that have small size and are numerous require

a considerable amount of time and resources. Thus, indirect methods may be the best alternative for estimating the biomass of these organisms (Smock, 1980).

Among the indirect methods for determining biomass, regression models examining dry mass and body dimensions are the most often used (Smock, 1980; Meyer, 1989). Regression models are accurate, fast and inexpensive; however, the accuracy of regression methods is typically taxon-specific and varies among populations (Benke et al., 1999; Johnston and Cunjak, 1999). In general, low efficiency has been recorded in models created for different regions and even in models for closely related taxa (e.g., family). Moreover, general models are not effective for estimating biomass at the order level, such as in Trichoptera, mainly due to the high variability in the bodies of individuals (Sample et al., 1993; Cressa, 1999a).

Models for estimating the biomass of aquatic insects have been developed primarily for populations in temperate regions (e.g., Smock, 1980; Meyer, 1989; Benke et al., 1999; González et al., 2002). In the Neotropics, length-mass relationship models have been created for insect populations in streams in Argentina (Miserendino, 2001), Brazil (Becker et al., 2009; Martins et al., 2014), and Venezuela (Cressa, 1999a). In Brazil, biomass estimation models have been obtained mainly for shredder organisms, in particular *Phylloicus* (Trichoptera: Calamoceratidae; Becker et al., 2009; Martins et al., 2014).

* Corresponding author.
 E-mail: janaina.gomesdebrito@gmail.com (J.G. de Brito).

Fig. 1. Case built from a small twig by a *Triplectides egleri* (Trichoptera: Leptoceridae) larva in a Central Amazonian stream, Brazil. Red line indicates where the width of the case (= extent of the excavated area, at the ventral region) was measured.

Triplectides (Trichoptera: Leptoceridae) are also important shredders in the Amazon region (Landeiro et al., 2010; Martins et al., 2015). Due to this feeding habit, this genus has the potential for use in laboratory studies to evaluate the role of invertebrates in leaf breakdown in Amazonian streams. Our objective is to provide predictive models based on body and case dimensions for estimating the biomass of *Triplectides egleri* Sattler, 1963, which is the only species of this genus reported in the study area (Barro Branco stream).

Material and methods

Study area

This study was conducted with a population of *T. egleri* in the Barro Branco stream, located in the Ducke Reserve (2°55′ and 03°01′ S, 59 53′ and 59°59′ W), Manaus municipality (Amazonas state). The Ducke Reserve comprises 10,000 hectares (100 km^2), drained by a dense network of low-order streams and covered by "*terra firme*" (unflooded upland) tropical rain forest (Ribeiro et al., 1999). The streams in this area are shaded by dense riparian vegetation and have acidic (pH = 4.63 ± 0.08) well-oxygenated (6.62 ± 0.06 mg L^{-1}) water, with a low capacity to conduct electrical current (10.71 ± 0.41 μS cm^{-1}), and an average temperature of 24.52 ± 0.52 °C (Martins et al., 2014).

Sample collection

Triplectides egleri larvae were collected in July 2014. Sampling of individuals was performed manually in pool areas of the stream with accumulation of litter. We sampled 57 larvae of different sizes and transported them to the laboratory in a Styrofoam box with stream water and litter; in the laboratory they were removed from their cases and analyzed individually (Becker et al., 2009). Because two larvae got out of their cases, after collection, the sample size for case dimensions was 55.

Body dimensions used to predict the biomass of individuals were: body length, head-capsule width and interocular distance (Becker, 2005; Becker et al., 2009). Body length was measured as the distance between the anterior region of the head and the distal region of the abdomen. Head-capsule width was measured as the widest portion of the head. Interocular distance was measured at the smallest distance between the eyes.

Case length and width can be used to estimate biomass because these insects build a portable tubular case from small twigs (Sattler, 1963). *Triplectides egleri* excavate the ventral region of the case at its opening, probably to facilitate locomotion. Case width was therefore measured as the extension of the excavated portion (Fig. 1). Case length was measured as the distance between the anterior (case opening) and the posterior portion of the case.

The body and case dimensions were determined from digital images (Leica stereomicroscope M165, accuracy = 0.001 mm) obtained with Leica Application Suite (LAS) software version 3.8. After measurement, larvae were placed in an oven (OdontoBras

Mod-EL-1.6) for 72 h at 50 °C and cooled in a desiccator for 24 h. Biomass (dry weight) of each individual was measured with a high-precision digital scale (Metter Toledo, model AB265-S/FACT; accuracy = 0.01 mg).

Data analysis

To evaluate the relationship between biomass and the body or case dimensions of *T. egleri*, we used regression analysis based on the least squares method (Zar, 2010). Three regression models were fitted to the data: (a) linear ($y = a * x + b$), (b) exponential ($y = a * e^{bx}$; in linear form: $\ln y = \ln a + b * x$), and (c) power ($y = a * x^b$; in linear form: $\ln y = \ln a + b * \ln x$). In all models, y is the biomass (mg), x is the size of the body or case (mm), and a and b are regression constants describing the allometric relationship between variables y and x. The fit of the regression models was assessed by the coefficient of determination (R^2) expressed in proportion or percentage (%) (Zar, 2010). The coefficient of variation was obtained according to equation: CV = (SD/mean)*100, where SD is the standard deviation. Results of CV were presented in percentage (%).

Cross-validation analyses were performed to assess the predictive power of the models obtained from our data. We follow the method used by Martins et al. (2014). Thus, our data set was divided in half, one of the subsets was used to create models (training set) and the other was used to evaluate the performance of predictive models (test set). Moreover, to assess whether biomass estimation models proposed for other leptocerids (*Nectopsyche* genus) are satisfactory to estimate *T. egleri* biomass, we used the equations proposed by Cressa (1999a,b), which were obtained to Trichoptera larvae from the Venezuelan Amazon. The reliability of cross-validation was assessed by comparison of predicted results with the biomass obtained through weighing of the larvae. The significance of our cross-validation was tested using a paired *t*-test. The cross-validation was repeated 100 times and the result was expressed as an average value. All analyses were performed with the R program (R Core Team, 2014).

Results

Dry weight of *T. egleri* had a high coefficient of variation (CV = 108%), with values from 0.18 to 11.30 mg (Table 1). Among body dimensions, body length had the highest range (3.97–18.25 mm) and coefficient of variation (36%). Case dimensions also had high coefficients of variation as compared to body dimensions (Table 1).

All regression models used to estimate *T. egleri* biomass were significant (Table 2). The best fit between biomass and body or case dimensions was obtained using the power model, followed by the exponential and linear models. For the linear, exponential and power models, body length was the dimension that best predicted biomass. Among the case dimensions, case width provided the best estimate in all models (Table 2).

To linear models, the predicted biomass using models for body length was underestimated (~ −11%; Table 3). On the other hand, the predicted biomass using others body and case dimensions were overestimated (~18–64%). To exponential and power, the predicted biomass was overestimated when we used body (Exponential: ~12–27%; Power: ~8–24%) and case (Exponential: ~35–52%; Power: ~32–49%) dimensions. To all models, no significant differences between predicted biomass using cross-validation models and observed biomass was recorded (Table 3). On the other hand, all models using the models proposed to other Neotropical leptocerids (*Nectopsyche*) and Trichoptera (Cressa, 1999a,b) were significantly different in relation to *T. egleri* biomass and, values were underestimated up to 98% (Table 4).

Table 1
Range, mean, standard deviation (SD), coefficient of variation (CV = (SD/mean)*100, in %) and number of observations (N) for body mass and body and case dimensions of *Triplectides egleri* (Trichoptera: Leptoceridae) larvae from a Central Amazonian stream, Brazil.

	Range	Mean	SD	CV	N
Body dimensions					
Body length (mm)	3.97–18.25	10.34	3.68	35.60	57
Head-capsule width (mm)	0.39–0.80	0.80	0.23	28.60	57
Interocular distance (mm)	0.22–0.72	0.48	0.15	30.06	57
Case dimensions					
Case length (mm)	13.00–146.40	32.65	18.53	56.75	55
Case width (mm)	0.56–3.88	2.03	0.69	34.10	55
Body mass					
Dry mass (mg)	0.18–11.30	2.69	2.91	108.09	57

Table 2
Linear, exponential and power models for the relationship between body mass (mg) and body and case dimensions (mm) of *Triplectides egleri* (Trichoptera: Leptoceridae) larvae from a Central Amazonian stream, Brazil.

Function	Models	a	b	R^2	N	p
Linear	DM~BL	−3.990	0.646	0.663	57	<0.001
	DM~HW	−4.157	8.564	0.444	57	<0.001
	DM~ID	−4.093	13.996	0.482	57	<0.001
	DM~CL	1.191	0.044	0.065	55	0.034
	DM~CW	−2.857	2.697	0.424	55	<0.001
Exponential	ln(DM)~BL	−2.352	0.270	0.816	57	<0.001
	ln(DM)~HW	−2.808	4.060	0.711	57	<0.001
	ln(DM)~ID	−2.739	6.556	0.754	57	<0.001
	ln(DM)~CL	−0.409	0.026	0.176	55	0.001
	ln(DM)~CW	−1.907	1.148	0.531	55	<0.001
Power	ln(DM)~ln(BL)	−5.821	2.755	0.835	57	<0.001
	ln(DM)~ln(HW)	1.257	3.058	0.728	57	<0.001
	ln(DM)~ln(ID)	2.648	2.852	0.746	57	<0.001
	ln(DM)~ln(CL)	−4.976	1.596	0.395	55	<0.001
	ln(DM)~ln(CW)	−1.026	2.238	0.546	55	<0.001

DM, dry mass; BL, body length; HW, head-capsule width; ID, interocular distance; CL, case length; CW, case width.

Discussion

In general, body length was the best dimension for estimating the biomass of *Triplectides egleri*, explaining 66–75% of biomass variation. Other studies have also reported that body length is an efficient dimension for estimating the biomass of other genera of Trichoptera (e.g., Towers et al., 1994; Benke et al., 1999; Miserendino, 2001; Becker et al., 2009; Martins et al., 2014). This dimension typically provides good estimates because most of the body of the insect larvae (holometabolous) is not sclerotized and tends to increase gradually in each instar, accompanying the changes in the mass of individuals (Johnston and Cunjak, 1999).

Interocular distance and head-capsule width also provided good estimates of biomass (>44%). For example, the best predictor of biomass in the exponential model was the interocular distance. In general, sclerotized structures (e.g., head) show low variation within each instar and tend to have a poor fit with biomass as compared to body length (Towers et al., 1994; Burgherr and Meyer,

Table 3
Predictive performance of cross-validation models to estimate biomass of *Triplectides egleri* from a Central Amazonian stream. Difference indicates percentage of difference between predicted and observed data. Positive differences indicate that the predicted values were higher than the observed ones. *T* and *p* refers to paired *T*-tests between observed and predicted values. Values refer to averages obtained from predictions and tests repeated 100 times.

Model	Body and case dimensions	Difference (%)	R^2	T-Test		
				t	Gl	p
Linear	BL	−10.68	0.59	1.84	28	0.428
	ID	18.12	0.43	1.62	28	0.435
	HW	28.22	0.32	1.90	28	0.392
	CW	33.83	0.38	4.16	27	0.089
	CL	64.30	0.08	5.23	27	0.107
Exponential	BL	11.52	0.56	1.68	28	0.424
	ID	18.42	0.38	2.66	28	0.277
	HW	27.07	0.24	2.34	28	0.339
	CW	35.04	0.25	2.21	27	0.394
	CL	52.30	0.12	2.92	27	0.218
Power	BL	8.43	0.67	2.31	28	0.345
	ID	17.42	0.41	2.60	28	0.285
	HW	23.56	0.25	2.28	28	0.345
	CW	32.33	0.33	3.44	27	0.228
	CL	48.89	0.28	2.92	27	0.351

BL, body length; HW, head-capsule width; ID, interocular distance; CL, case length; CW, case width.

Table 4

Predictive performance of biomass estimation models from *Nectopsyche* (Leptoceridae) and Trichoptera proposed by Cressa (1999a, 1999b) to estimate biomass of *Triplectides egleri* from a Central Amazonian stream. Difference (%) indicates percentage of difference between predicted and observed data. Negative differences indicate that the predicted values were lower than the observed ones. *T* and *p* refers to paired *t*-tests between observed and predicted values. Values refer to averages obtained from predictions and tests repeated 100 times.

Reference	Taxon	Preservation method	Difference (%)	R^2	*t*-test		
					t	Gl	*p*
Cressa (1999a)	*Nectopsyche*	Not preserved	−85.03	0.26	19.17	28	<0.001
Cressa (1999b)	*Nectopsyche*	Freezing	−95.00	0.28	20.66	28	<0.001
Cressa (1999b)	*Nectopsyche*	Formaldehyde 4%	−98.49	0.32	22.54	28	<0.001
Cressa (1999b)	*Nectopsyche*	Kahle	−98.23	0.30	21.61	28	<0.001
Cressa (1999b)	Trichoptera	Freezing	−97.77	0.27	21.39	28	<0.001
Cressa (1999b)	Trichoptera	Formaldehyde 4%	−96.87	0.28	21.69	28	<0.001
Cressa (1999b)	Trichoptera	Kahle	−98.05	0.26	21.31	28	<0.001

BL, body length; HW, head-capsule width; ID, interocular distance; CL, case length; CW, case width.

1997). However, sclerotized structures are resistant to damage in terms of breaks or deformities induced by preservation methods, and may be very useful for biomass estimation of preserved or damaged individuals (Johnston and Cunjak, 1999).

In our study, case dimensions provided poor fit with biomass as compared to body dimensions, not corroborating the studies of Cressa (1999a) and Martins et al. (2014), who observed similar accuracy for case and body dimensions for predicting biomass of *Nectopsyche* and *Phylloicus* (Trichoptera). The strong relationship between case dimensions and biomass of *Nectopsyche* and *Phylloicus* may be related to use of sand grains and leaves to build their cases (Glover and Floyd, 2004; Prather, 2003). Usage of these materials, which are small or can be cut to size determined by larvae, allows construction of a case according to the size of the larvae. On the other hand, *Triplectides* individuals build their cases by drilling small twigs (Sattler, 1963), behavior which limits the fit between case and biomass because it depends on the larvae finding small twigs suitable to their size, which does not always occur (Camargos and Pes, 2011).

The power model provided the best fit between the biomass and body and case dimensions of *T. egleri*. However, the difference in relation to the exponential model was less than 2% (except for case length). Other studies have reported that the difference between power and exponential models in Trichoptera biomass explanation is low – usually less than 6% (Becker et al., 2009; Martins et al., 2014) – and that differences observed using different regression models tend to decrease with increasing number of observations (Wenzel et al., 1990).

In aquatic invertebrates, the "*b*" value of the mass-length curve adjusted by the power regression model is close to 3, ranging between 2 and 4 (Towers et al., 1994; Benke et al., 1999; González et al., 2002). Specifically, for insects, the average value of "*b*" tends to be lower than 3 (Benke et al., 1999; Smock, 1980). In this study, "*b*" values for the relationship between body dimensions and biomass followed this pattern, indicating that the body narrows with an increase in body length (Benke et al., 1999). For other Leptoceridae genera (*Oecetis* and *Nectopsyche*) of temperate regions, the "*b*" value has been found to be greater than 3, indicating that bodies of these organisms become proportionally larger as the length increases (Benke et al., 1999). In the tropics, Cressa (1999a) recorded a "*b*" value of 1.35 for *Nectopsyche* individuals. The "*b*" values of equations for biomass estimation of *T. egleri* and *Nectopsyche* indicates that larvae of both genres tend to be longer than wide as the individuals grow. However, the low "*b*" value obtained for *Nectopsyche* indicates that the body shape of this genus changes more intensely during the larval development. Moreover, may have a large ontogenetic variation between different species comprising the genus. These differences may account for the high underestimation of biomass *T. egleri* when we used the equations proposed by Cressa (1999a,b). Thus, our results reinforce the importance of obtaining models for organisms (genus or species) studied from a particular geographic region (Martins et al., 2014).

Compared to the results obtained in other studies, we conclude that our regression models (power and exponential) describe with high precision the relation between body dimensions and biomass of *T. egleri*. Thus, they are useful tools for determination of biomass and can be used in studies that aim understanding the role of shredder invertebrates on ecological processes in Amazonian streams.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

References

Becker, G., 2005. Life cycle of *Agapetus fuscipes* (Trichoptera, Glossosomatidae) in a first-order upland stream in central Germany. Limnologica 35, 52–60.

Becker, B., Moretti, M.S., Callisto, M., 2009. Length-dry mass relationships for a typical shredder in Brazilian streams (Trichoptera: Calamoceratidae). Aquat. Insects 31, 227–234.

Benke, A.C., Huryn, A.D., Smock, L.A., Wallace, B.J., 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the Southeastern United States. J. N. Am. Benthol. Soc. 18, 308–343.

Benke, A.C., Huryn, A.D., 2010. Benthic invertebrate production-facilitating answers to ecological riddles in freshwater ecosystems. J. N. Am. Benthol. Soc. 29, 264–285.

Brand, C., Miserendino, M.L., 2012. Life cycle phenology, secondary production, and trophic guilds of caddisfly species in a lake-outlet stream of Patagonia. Limnologica 42, 108–117.

Burgherr, P., Meyer, E.I., 1997. Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. Arch. für Hydrobiol. 139, 101–112.

Callisto, M., Moretti, M., Goulart, M., 2001. Macroinvertebrados bentônicos como ferramenta para avaliar a saúde de riachos. Rev. Bras. Recur. Hídr. 6, 71–82.

Camargos, L.M., Pes, A.M.O., 2011. The grass is always greener on the other side: *Triplectides* Kolenati, 1859 (Leptoceridae) and *Marilia* Müller, 1880 (Odontoceridae) occupying cases of other Trichoptera species. Acta Limnol. Bras. 23, 353–356.

Cressa, C., 1999a. Dry mass estimates of some tropical aquatic insects. Rev. Biol. Trop. 47, 133–141.

Cressa, C., 1999b. Dry mass estimation of tropical aquatic insects using different short-term preservation methods. Rev. Biol. Trop. 47, 143–149.

Glover, J.B., Floyd, M.A., 2004. Larvae of the genus *Nectopsyche* (Trichoptera: Leptoceridae) in eastern North America, including a new species from North Carolina. J. N. Am. Benthol. Soc. 23, 526–541.

González, J.M., Basaguren, A., Pozo, J., 2002. Size-mass relationships of stream invertebrates in a northern Spain stream. Hydrobiologia 489, 131–137.

Johnston, T., Cunjak, R., 1999. Dry mass-length relationships for benthic insects: a review with new data from Catamaran Brook, New Brunswick, Canada. Freshw. Biol. 41, 653–674.

Landeiro, V.L., Hamada, N., Godoy, B.S., Melo, A.S., 2010. Effects of litter patch area on macroinvertebrate assemblage structure and leaf breakdown in Central Amazonian streams. Hydrobiologia 649, 355–363.

Luek, A., Morgan, G.E., Ramcharan, C.W., 2015. Biomass of benthic invertebrates unaffected by industrial damage to lakes despite effects on species composition. Hydrobiologia 744, 101–114.

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 31, 337–342.

Martins, R.T., Melo, A.S., Gonçalves Jr., J.F., Hamada, N., 2015. Leaf breakdown in urban streams of Central Amazonia: direct and indirect effects of physical, chemical and biological factors. Freshw. Sci. 34, 716–726.

Meyer, E., 1989. The relationship between body length parameters and dry mass in running water invertebrates. Arch. für Hydrobiol. 117, 191–203.

Miserendino, M.L., 2001. Length-mass relationships for macroinvertebrates in freshwater environments of Patagonia (Argentina). Ecol. Austral 11, 3–8.

Prather, A.L., 2003. Revision of the *Neotropical caddisfly* genus Phylloicus (Trichoptera: Calamoceratidae). Zootaxa 275, 1–214.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/.

Ribeiro, J.E.L.S., Hopkins, M.J.G., Vicentini, A., Sothers, C.A., Costa, M.A.S., Brito, J.M., 1999. Flora da Reserva Ducke: Guia de identificação das plantas vasculares de uma floresta de terra firme na Amazônia Central. Editora INPA, Manaus.

Sample, B.E., Cooper, R.J., Greer, R.D., Whitmore, R.C., 1993. Estimation of insect biomass by length and width. Am. Midl. Nat. 129, 234–240.

Sattler, W., 1963. Eine neue *Triplectides-Art (Leptoceridae, Trichoptera)* aus dem brasilianischen Amazonasgebiet, ihre Metamorphosestadienund Bemerkungen zu ihrer Biologie. Beitr. zur Neotropischen Fauna 3, 20–33.

Smock, L.A., 1980. Relationships between body size and biomass of aquatic insects. Freshw. Biol. 10, 375–383.

Towers, D.J., Henderson, I.M., Veltman, C.J., 1994. Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. N. Z. J. Mar. Freshw. Res. 28, 159–166.

Townsend, C.R., Dolédec, S., Norris, R., Peacock, K., Arbuckle, C., 2003. The influence of scale and geography on relationships between stream community composition and landscape variables: description and prediction. Freshw. Biol. 48, 768–785.

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A., Warren, P.H., 2005. Body size in ecological networks. Trends Ecol. Evol. 20, 402–409.

Zar, J.H., 2010. Biostatistical Analysis. Pearson Education, New Jersey.