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PRIMARY RESEARCH PAPER

Length–weight relationships for some plecoptera and ephemeroptera from a carbonate stream in central Apennine (Italy)

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Abstract The relationship between dry weight and body length for larvae of Plecoptera (*Leuctra* spp., *Isoperla grammatica*, *Nemoura cinerea*) and Ephemeroptera (*Baetis* spp., *Habrophlebia fusca*, *Paraleptophlebia submarginata*, *Ecdyonurus helveticus*, *Rhithrogena semicolorata*), collected from a carbonate stream in the Apennine (central Italy), is reported. The power equation $f(x) = Ax^B$ has been applied to fit the curves of dry weight vs. body size (length) in the ranges 0.03–13.00 mg and 2–14 mm, respectively; a total of 674 larvae were examined. The power model was in very good agreement with experimental data. Moreover, the error between measured and estimated weight was in the 4–20% range. The data on *Isoperla grammatica*, *Leuctra* spp., *Rhithrogena semicolorata* and *Baetis* spp. were compared to those in a previous study in a different geographical setting (south-western Germany's Black Forest) obtaining similar results but with lower errors. We used and compared two methods: the weighted least-square method (WLS) and an analysis of covariance (ANCOVA). The values of the *A* and *B* coefficients obtained with the two methods were very similar (<6% discrepancy for either *A* or *B*). We found the best fits for all the examined Plecoptera (species, genus, and order level), while the results for Ephemeroptera were varied, with loose fits at the order level and also for Leptophlebiidae collectively considered.

Keywords Biomass · Body length · Dry weight · Weighted least-square · ANCOVA · Aquatic insects

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Introduction

Biomass is an important parameter to study community structure, distribution of resources, species, matter, and energy fluxes (Harvey & Godfray, 1987; Blackburn et al., 1993). Moreover, several studies have indicated that secondary production has a fundamental role in the dynamic-quantitative characterization of energetic transformations that occur in aquatic ecosystems (e.g., Downing & Rigler, 1984; Benke et al., 1999; Huryn & Wallace, 2000; Stead et al., 2005).

Insects and other invertebrates may form a major portion of secondary production in stream habitats (Wright et al., 1985; Habdija et al., 1995) and many investigations targeting stream ecosystem structure and dynamics have relied heavily on insects (Marques & Barbosa, 2001; Bowman et al., 2005). However, the quantification of invertebrate production is difficult to achieve. Community-based, comprehensive estimations using a large number of specimens is a cumbersome task, and may still not resolve the uncertainty. Several mathematical models have been developed to bypass this problem, estimating invertebrate- or insect-based secondary production from biomass, using actual measurements on a relatively limited set of specimens. However, accurate or even precise community-wide estimations of invertebrate biomass remain difficult to obtain empirically. In fact, the literature reports of high variability from different studies, even for closely related taxa (Johnston & Cunjak, 1999; and references therein). Hence, the development of a reliable predictive model is necessary to obtain accurate mass estimates.

Invertebrate biomass may be determined using a number of approaches. The three major methods are direct biomass estimation on living (wet weight) or preserved specimens (dry weight), biovolume, and length–mass relationships (Burgherr & Meyer, 1997). The first two methods are either inaccurate (if sample size is small) or cumbersome (if a reliably large sample size, or even a census, is sought). Also, all methods directly quantifying the biomass of individual specimens (or groups of individual specimens) have shortcomings. For example, wet weight does not account for variability in the contribution of water to biomass (Burgherr & Meyer, 1997), preservation in formalin destroys the lipid component (Leuven et al., 1985; Johnston & Mathias, 1993), and biovolume tends to underestimate the biomass of larger organisms (Burgherr & Meyer, 1997).

Despite some problems still unsolved, such as the uncertain source of intra- and inter-taxon variability (Johnston & Cunjak, 1999), the length–mass relationship has the advantage of being both fast and precise (Benke et al., 1999). Not surprisingly, several quantitative relationships to estimate biomass (as dry or fresh weight) from body length have been developed for aquatic invertebrates, both at the larval (Rogers et al., 1977; Smock, 1980; Mason et al., 1985; Eggert & Burton, 1994; Johnston & Cunjak, 1999) and adult stage (e.g., Sabo et al., 2002).

Regression analysis has been the technique most commonly used to quantify the length–mass relationship (Sabo et al., 2002; and references therein). The power function in particular seems to provide the best fit to the data, yielding errors <20% between measured and estimated biomass (Wenzel et al., 1990), and is currently the most commonly used approach in quantitative length–mass determinations (Benke et al., 1999; Sabo et al., 2002).

The aim of this paper is to obtain the regression curves of body-length versus dry-weight for larvae of stream Plecoptera and Ephemeroptera collected in an unimpacted, carbonate-rich stream (Fig. 1) in the central Apennine (Italy), using the power equation with either weighted least-square (WLS) or an analysis of covariance (ANCOVA) procedure resulting from the use of discrete classes of body length. Measured biomass and biomass estimated with power length–mass equations were compared. The results were also compared with those in Meyer (1989), a similar investigation carried out for invertebrates in the Steina, a Black Forest mountain stream in southwestern Germany, with which this study shares four taxa.

Methods

A total of 674 larvae of Ephemeroptera ($n = 366$) and Plecoptera ($n = 308$) (Fig. 2) were collected between February 1998 and December 1999, in the

Fig. 1 Location of the study area

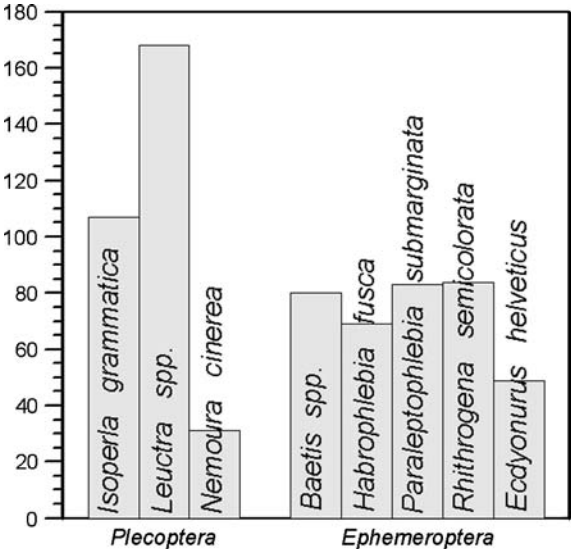


Fig. 2 Histogram representation of the number of specimens (by taxon) used in the investigation

stream Raio (42°17′56.36″ N, 13°18′06.34″ E—808 m asl) a first-order tributary of the river Aterno) in the Region of Abruzzo (Fig. 1). The stream is rich in invertebrates, thus providing a wide range of values of body size and weight. All taxa of Plecoptera (Fochetti et al. in press) and Ephemeroptera (http://www.faunaeur.org,) are common in the study area and have a pan-European distribution, potentially leading to a wide applicability of the empirically estimated length–mass relationships. All organisms were sampled using a macrobenthos net (0.47-mm mesh size); samples were stored in the laboratory and cultured at 8°C in small trays for a few hours before body size determination. Each individual was examined under a Wild *M7* stereoscope; body size of living animals was measured with a micrometer slide (Wild). The length of each larva was determined from the front end of the head capsule to the end of the last abdominal segment, following methods adopted elsewhere (Eggert & Burton, 1994). Body length was used instead of head capsule width, as the former varies more gradually with general body size and measurement errors are smaller with respect to organism size (Bird & Prairie, 1985; Johnston & Cunjak, 1999). Each living larva was then individually inserted in a numbered test tube and introduced in an oven with air vents. The procedure was chosen to avoid weight alteration due to the loss of lipids and dehydration associated with techniques such as ethanol preservation (Collier & Winterbourn, 1990; Waringer, 1992). Larvae were dried for 48 h at 60°C (Burgherr & Meyer, 1997). The low temperature and the long drying time were chosen to avoid loss of weight by fat evaporation (Hynes, 1982). Finally, dry biomass for each individual larva was determined with a Gibertini E42 balance (balance error = ±0.1 mg). A pre-weighted aluminum foil was used to reduce the accumulation of electrostatic charges that could alter larval weight (Burgherr & Meyer, 1997).

Weight versus length curves were fitted with the following power law:

$$f(x) = A\,x^{B} \qquad (1)$$

(Rogers et al., 1977; Smock, 1980; Sabo et al., 2002); variables were ln-transformed to linearize the power relationship as:

$$\ln f(x) = \ln A + B \ln x \qquad (2)$$

where A and B are constants, x is the body length in mm and $f(x)$ is the dry weight in mg (Benke et al., 1999).

We used two methods to perform the fits: the weighted least-square method (Young, 1981) and an analysis of covariance (ANCOVA). We define the parameter weight w_i (not to be confused with the weight of organisms expressed in mg) as

$$w_i = 1/\sigma_i \qquad (3)$$

where σ_i is the standard deviation of the average weight of a given class i of body size (see *Appendix*). Since there are also errors in body size measurements it is possible to use a least-square method when both variables have uncertainties (Orear, 1982; Lybanon, 1984; Zar, 1996) but in our cases the error in body length is consistently 0.5 mm for all size classes; such an error is constant because it is due to instrument error (a graduated microscope slide). Though the balance error was constant, the calculated weight average and its standard deviation (σ) for each class size produced a variable error on weighted biomass (last column of *Appendix*). Therefore, the parameter σ was used in Eq. 3 to give greater weight w to low error σ and smaller w to high error σ.

Hence, we performed a weighted method (WLS) only for one variable (dry weight). We used also a covariance analysis (ANCOVA) for comparison purposes, where we associated the σ_i of *Appendix* to the value couple ($f(x_i)$, x_i); we used neither the balance error nor the size error in the statistical analyses because they are constants. Correlation coefficients R^2 were compared with a modified *t*-test with Fisher-transformed R^2 values (Zar, 1996).

Special procedures were followed for unusual conditions, specifically: for younger larvae (with weight close to the error of the balance) groups of 2–5 individuals were weighted at the same time, (19 events); for same-size classes (13 events), where only one individual was collected, the higher standard deviations obtained for the same taxon across all size classes was assigned to avoid an overestimate in the weighted least-square method (Young, 1981); the balance error (±0.1 mg) was associated with the size classes where a number of same-weight individuals (5 events) were collected.

Results

The number of collected aquatic insects with their body sizes (mm), average weights (mg), and associated errors (standard deviation σ expressed in mg) are reported in the *Appendix*. The two statistical analyses used in this study, the WLS and the ANCOVA, produced very similar results (Table 1).

The correlation coefficients obtained with the WLS method were very high, ranging from 0.90 (all Ephemeroptera) to 0.99 (*Leuctra* spp.), whereas, with ANCOVA, the lowest R^2 was 0.93 (for Leptophlebiidae and all Ephemeroptera) and the highest was 0.99 for eight taxa; the *P* values associated with the R^2 values were all <0.0001.

The coefficients *A* and *B* obtained with the two methods were highly similar (Table 1). In particular the differences of values of *A* ranged from 0% for *Nemoura cinerea* (Retzius) to 5.8% for Plecoptera, and the differences of values of *B* ranged from 0% for Heptageniidae and *Nemoura cinerea* to 6.4% for Plecoptera. The errors of the *A* constant [$\Delta(lnA)$] in the power Eq. 1 ranged from 0.37% for *Rhithrogena semicolorata* (Curtis) to 1.85% for Leptophlebiidae with the WLS method, and from 0.01% for *Rhithrogena semicolorata* to 0.56% for *Nemoura cinerea* using the ANCOVA.

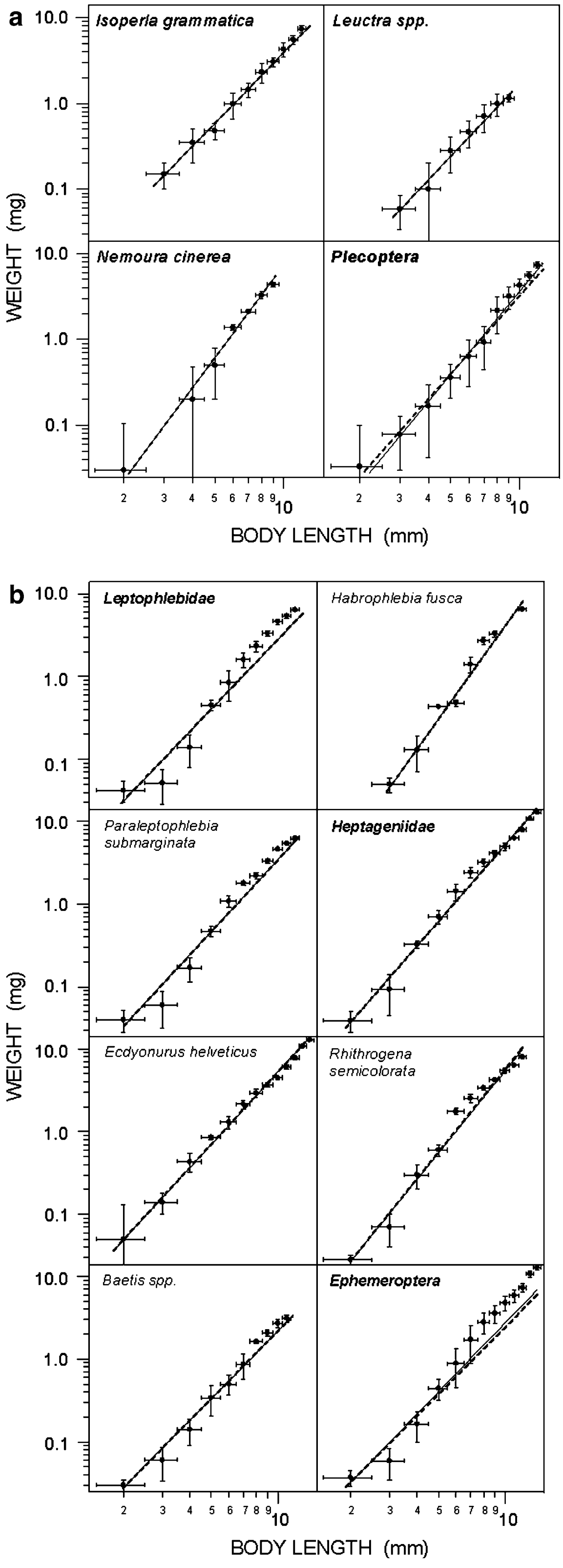
The errors of the *B* constant (ΔB) in the power Eq. 1 ranged from 0.67% for *Rhithrogena semicolorata* to 2.94% for Leptophlebiidae with the WLS method, and from 0.15% for *Rhithrogena semicolorata* to 0.72% for Heptageniidae using the ANCOVA.

Figure 3a and b shows all data of *Appendix* with the comparisons of regression curves obtained with the ANCOVA and WLS methods. Taxon-specific WLS-estimated biomass closely followed measured

Table 1 *A* and *B* values of Eq. 1 with the respective errors and correlation coefficients are reported

Taxa	ln(*A*)	ln(*A*)*	Δ(ln*A*)	Δ(ln*A*)*	*B*	*B**	Δ*B*	Δ*B**	R^2	R^2*
Plecoptera	−6.134	*−5.776*	0.056	*0.022*	3.221	*3.015*	0.036	*0.015*	0.931	*0.986*
Isoperla grammatica	−4.947	*−4.935*	0.052	*0.017*	2.743	*2.735*	0.032	*0.011*	0.940	*0.992*
Nemoura cinerea	−6.265	*−6.265*	0.110	*0.035*	3.588	*3.588*	0.060	*0.019*	0.947	*0.989*
Leuctra spp.	−5.942	*−5.939*	0.049	*0.016*	2.818	*2.814*	0.033	*0.010*	0.986	*0.989*
Ephemeroptera	−5.348	*−5.290*	0.061	*0.017*	2.756	*2.682*	0.057	*0.016*	0.895	*0.931*
Leptophlebiidae	−5.393	*−5.384*	0.100	*0.023*	2.789	*2.786*	0.082	*0.018*	0.934	*0.926*
Habrophlebia fusca	−7.176	*−7.082*	0.041	*0.017*	3.733	*3.762*	0.030	*0.011*	0.923	*0.977*
Paraleptophlebia submarginata	−5.386	*−5.393*	0.061	*0.019*	2.872	*2.875*	0.047	*0.015*	0.959	*0.952*
Heptageniidae	−5.393	*−5.400*	0.027	*0.027*	3.057	*3.057*	0.021	*0.022*	0.981	*0.993*
Ecdyonurus helveticus	−5.015	*−5.002*	0.038	*0.013*	2.900	*2.894*	0.023	*0.008*	0.913	*0.993*
Rhithrogena semicolorata	−5.871	*−5.895*	0.022	*0.006*	3.284	*3.314*	0.022	*0.005*	0.968	*0.992*
Baetis spp.	−5.429	*−5.442*	0.022	*0.006*	2.689	*2.705*	0.020	*0.006*	0.908	*0.987*

The columns with * and/or italic characters show the results of analysis of covariance (ANCOVA), in the others columns are the results of weighted least-squared method (WLS). The *A* and *B* values with their standard deviations have the dimensions of mg and mg mm^{-1}, respectively. The *P* level associated with R^2 was <0.0001 in all cases



◀**Fig. 3** (**a**) Regression curves in $\log_{10}$ scale for Plecoptera (*Isoperla grammatica*, *Leuctra* spp., *Nemoura cinerea* and the cumulative result for all Plecoptera). Dashed bold lines represent the fits obtained with an analysis of covariance (ANCOVA) and thin lines represent the fits obtained with the weighted least-square method (WLS). (**b**) Regression curves in $\log_{10}$ scale for Ephemeroptera (Leptophlebiidae, *Habrophlebia fusca, Paraleptophlebia submarginata*, the cumulative result for Heptageniidae, *Ecdyonurus helveticus*, *Rhithrogena semicolorata*, *Baetis* spp. and the cumulative result for all Ephemeroptera). Dashed bold lines represent the fits obtained with an analysis of covariance (ANCOVA) and the thin lines represent the fits obtained with the weighted least-square method (WLS)

biomass for Plecoptera, regardless of taxonomic level (relative difference between estimated and measured biomass was about 10% in Table 2). Results for Ephemeroptera were varied, typically <12% for species and genera [with the exception of *Paraleptophlebia submarginata* (Stephens)], but 19.8% for Leptophlebiidae and 39.2% for Ephemeroptera collectively considered (Table 2).

The error in weight (standard deviation of weight) for all tested organisms increased with body size in the lower range of body size, and approached asymptotically 1.00 mg for body size >7 mm (Fig. 4). We did not find any relationship between number of organisms and standard deviations of weight.

Discussion

The statistical robustness of the results obtained with either method (WLS or ANCOVA) is very high, supporting the reliability and general applicability of the power equation and the associated WLS or ANCOVA analyses to estimate biomass from linear body size measurements (Johnston & Cunjak, 1999).

Ecologically speaking, the high agreement (high R^2 values in Table 1; small differences between estimated and measured biomass in Table 2) at the genus level (*Leuctra* spp. and *Baetis* spp.) suggests that the species that comprise the genera share similar biomass-size growth patterns, including at the early stages (younger size classes—Fig. 3a, b).

The agreement between measured and estimated biomass (Table 2; dots with deviation ranges and power model curves, respectively, in Fig. 3a) was

Table 2 Estimated (using data from Table 1) and measured weights (using data in *Appendix*), difference and relative (%) biomass for all larvae collected using results from weighted least-squared method

Taxa	Estimated	Measured	Absolute	Relative (%)
All Plecoptera	418.647	447.800	−29.153	−6.5
Isoperla grammatica	259.030	275.300	−16.270	−5.9
Nemoura cinerea	87.870	83.830	4.040	4.8
Leuctra spp.	79.744	88.675	8.931	10.1
All Ephemeroptera	342.821	563.470	−220.649	−39.2
Leptophebiidae	87.831	109.510	−21.679	−19.8
Habrophlebia fusca	32.108	30.398	−1.710	−5.6
Paraleptophlebia submarginata	65.366	79.110	13.744	17.4
Heptageniidae	383.297	400.880	−17.583	−4.4
Ecdyonurus helveticus	134.456	126.310	−8.146	−6.4
Rhithrogena semicolorata	259.266	274.565	15.299	5.6
Baetis spp.	46.837	53.090	6.253	11.8

All values in the first three columns are expressed in mg

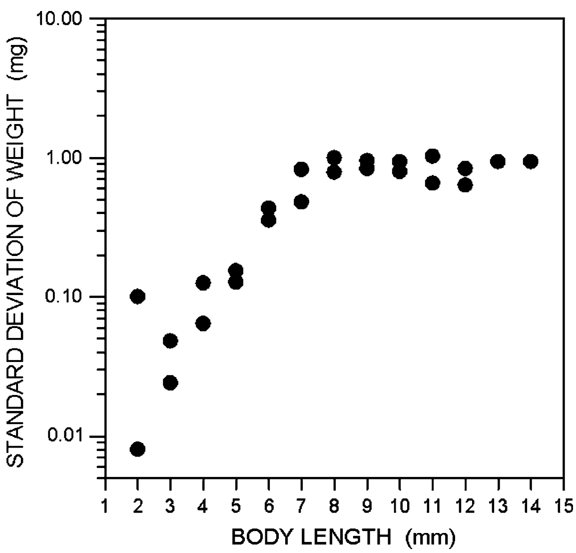


Fig. 4 Relationship between standard deviation of weight (from *Appendix*) and organism size for all tested specimens, regardless of taxon

very high for genus- and species-level Plecoptera. WLS- and ANCOVA-estimated biomass also were virtually the same for *Isoperla grammatica* (Poda), *N. cinerea*, and *Leuctra* spp, suggesting high predictive power of the power equation, with *A* and *B* estimated with either method accurately describing the length–biomass growth pattern of these three taxa. The high agreement among patterns also suggests that length–biomass growth for these three taxa proceeds linearly throughout the size classes considered.

The ANCOVA-based model underestimated the biomass of Plecoptera collectively considered for the largest body size classes (average ± standard deviation of each measured value not overlapping with the dashed line, describing the ANCOVA-based model in Fig. 3a). The discrepancy between measured biomass and the WLS-based estimated biomass remained qualitative (average ± standard deviation of each measured value overlapping with the continuous thin line, describing the WLS-based model in Fig. 3a), suggesting that, despite an apparent higher precision of the ANCOVA-based power equation (ANCOVA-based R^2 typically higher that WLS-based R^2 in Table 1), the WLS method may provide a better estimate of biomass from body length for the largest-bodied (i.e., later instars) Plecoptera.

The measured vs. estimated discrepancy was significant for both methods for the larger Ephemeroptera collectively considered (Fig. 3b); however, the discrepancy was more marked for the ANCOVA-based model, supporting the general trend found for Plecoptera.

The typically higher ANCOVA-based R^2 (Table 1) may not reflect a real difference with WLS-based R^2 because of the inherent different mathematical procedures, precluding a quantitative comparison of R^2 values. However, R^2 values typically >0.9 suggests that the fit is very high for either method.

As for Plecoptera, the WLS-based model agrees almost perfectly with the ANCOVA-based model for all taxonomic levels below order for Ephemeroptera (Fig. 3b and Table 1). However, contrary to Plecoptera, both models tend to over- or underestimate biomass in all cases except for *Ecdyonurus helveticus*

(Eaton) (Fig. 3b). The observed difference between Plecoptera and Ephemeroptera may be due to intrinsic biological differences between the two orders rather than to method-sensitive procedural differences. For example, the examined taxa of Plecoptera tend to have relatively regular growth patterns, with elongation broadly following biomass accrual (Fig. 3a). The mildly S-shaped log–log fit curves in Fig. 3b for some taxa of Ephemeroptera (e.g., *P. submarginata*, *Baetis* spp.), instead suggest that growth is allometric, with younger larvae elongating faster than accruing biomass, and later instars tending to accrue biomass while elongation slows down. Our findings for Ephemeroptera support earlier observations that body mass increases relatively more rapidly than body length in late than early instars (e.g., Merritt et al., 1982; Nolte, 1990). However, it is noteworthy that *Habrophlebia fusca* (Curtis) and *R. semicolorata* exhibit an S-shaped curve followed by a "sudden" model overestimation of biomass for the largest body size classes examined (Fig. 3b). The biological meaning of such biomass accrual slowdown for these taxa (if any) remains unknown, and a discussion in this regard is beyond the scope of this work.

The higher variability in length–mass patterns at order level for Ephemeroptera than Plecoptera may reflect the higher variability (in turn possibly reflecting a combination of species-specific allometric growth and differential contribution of several species at genus level) at species and genus level for Ephemeroptera (Fig. 3a, b). Significant underestimations for small sizes and overestimations for the larger sizes of Ephemeroptera at order level strongly suggest that species-specific variability leads to a gross discrepancy between measured and estimated biomass for multi-species taxa, possibly rendering the method (whether WLS- or ANCOVA-based) unreliable and not applicable at order level.

The results for *I. grammatica*, *Leuctra* spp., *R. semicolorata*, and *Baetis* spp. obtained with a WLS-based power equation were compared to those obtained by Meyer (1989) for the same taxa (Table 3). Though the *A* and *B* values were roughly comparable for all taxa, variability with our WLS method was much lower, suggesting that the WLS method can safely counterbalance the accuracy lost when body sizes are organized into relatively coarse classes. Slightly different specimen handling methods [e.g., biomass determined after 48 h of desiccation at 60°C for our study but after 36 h of desiccation at 104°C for Meyer (1989)] do not appear to have influenced power equation parameters and significance levels, as most differences were either very small or, when quantifiable, were not significant (Table 3). The slight discrepancy between our and Meyer's (1989) *A* value for *Baetis* spp. may be due to different species comprising the two taxa in Italy's central Apennine and Germany's Black Forest, respectively. Different species may also account for the significant difference in the obtained R^2 values for *Leuctra* spp. However, less numerous specimens

Table 3 Comparison of the WLS-based power equation parameters with those obtained by Meyer (1989) (in bold) for the four taxa shared in the two investigations

Taxa	ln(*A*)		Δ(lnA)		*B*		Δ*B*	
Isoperla grammatica	−4.947	**−5.072**	0.052	**0.186**	2.743	**2.697**	0.032	**0.107**
Leuctra spp.	−5.942	**−5.901**	0.049	**0.295**	2.818	**2.713**	0.033	**0.199**
Baetis spp.	−5.429	**−6.252**	0.022	**0.152**	2.689	**3.238**	0.020	**0.088**
Rhithrogena semicolorata	−5.871	**−5.675**	0.022	**0.251**	3.284	**3.345**	0.022	**0.131**
Taxa	***n***		**Range**		R^2		***P***	
Isoperla grammatica	107	**65**	3–12	**1.7–12.8**	0.940	**0.909**	0.177	
Leuctra spp.	168	**66**	3–9	**1.8–10.7**	0.986	**0.743**	<0.001	
Baetis spp.	80	**177**	2–11	**1.5–11.2**	0.908	**0.885**	0.389	
Rhithrogena semicolorata	84	**106**	2–12	**3.0–9.3**	0.968	**0.980**	0.109	

The *A* and *B* values with their standard deviations have the dimensions of mg and mg mm^{-1} respectively. The *P* level associated with each R^2 was <0.0001 in all cases. Sample size (*n*) for this study as in Appendix; reported ranges are for body sizes in mm. The reported *P* values refer to pair-wise comparisons between our and Meyer's (1989) R^2 values spread over a wider range of body length (Table 3) may also account for Meyer's (1989) significantly lower R^2 value for *Leuctra* spp. Conversely, Meyer's (1989) higher sample size for *R. semicolorata* counterbalanced a more restricted body size range, leading to statistically comparable R^2 values. The direct between-study comparisons in Table 3 suggest that sample size and body size range can influence the outcome of the length–mass relationship considerably, as also argued elsewhere (e.g., Johnston & Cunjak, 1999). Consequently, though the results in Table 3 suggest a broad geographical applicability of the power equation, caution must be exerted in view of the slightly different sample sizes and length ranges. Thus, similar sample sizes and length ranges, in addition to high taxonomic resolution, are greatly desirable to reliably compare mathematical models of length–mass relationships.

Conclusion

The weighted least-square (WLS) and the analysis of covariance (ANCOVA) are powerful methods to estimate biomass using the weight–size power relationship; mainly when the weight measurement is more accurate than body size. In these cases in each body size class it is possible to have a high number (a few dozens: see Appendix) of weight values. These methods allow to obtain reliable *A* and *B* values while maintaining a low degree of variability (i.e., error). Also, regressed biomass estimates remain consistent with experimental data (i.e., measured biomass). Consequently these results could be particularly helpful in biomass evaluations.

Though a large number of specimens are typically desirable or even necessary for biomass–length estimates, the weighted least-square method is very effective in detecting taxon-specific "deviations" from order-level patterns using a limited number of specimens per size class.

Both methods are particularly suited for lower taxonomic levels (i.e., genus and species), while the higher error at higher taxonomic levels (e.g., family or order) suggests that the methods should be used with caution at such high taxonomic levels, as inherent interspecific variability may affect total biomass estimates in non-negligible ways.

Our results support the need to standardize all aspects of methods (from specimen handling to mathematical procedures) if reliable comparisons across taxa and across geographical locations are sought.

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