Length-Mass Relationships for Macroinvertebrates in the Choghakhor International Wetland, Iran

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Abstract

This study investigates length-mass relationships for 17 families of freshwater macroinvertebrates collected in the Choghakhor Wetland (Central Iran). Body length, head width, distance between eyes and mass (dry weight and wet weight) were used to estimate biomass with linear, log-linear and exponential models. The results show better performance of the log-linear model (ANOVA, $p = 0.03$), as compared to the other two (ANOVA, $p > 0.05$). A cross-validation test demonstrated that all three models performed reasonably well, in terms of both statistics and the accuracy of prediction. However, for more than 60% of the relationships the *p*-value for the log-linear model was greater than for the other two, suggesting that the accuracy of this model is in general superior. Body length was generally demonstrated to be a good indicator to estimate biomass. However, for some taxa, the measurement of sclerotized structures (i.e., the distance between the eyes and the head width) were also found to be suitable biomass indicators. The latter is beneficial for the estimation of biomass with individuals that have been kept for a long time under laboratory conditions and/or those that show damaged parts of their bodies. The present study provides the first set of length-mass relationships for macroinvertebrates in the Middle-East region, and its findings are expected to contribute to the estimation of biomass in aquatic environments that are affected by semi-arid conditions and different degrees of anthropogenic stress.

Keywords: Wetland, Macroinvertebrates, Models, Length-Mass Relationships, Iran.

Introduction

Freshwater macroinvertebrates play an important role in aquatic and terrestrial food chains, contributing to the decomposition and recycling of organic material, the removal of environmental toxins, the control of primary productivity and the food supply to higher trophic levels (Wallace and Webster 1996). The majority of freshwater macroinvertebrate species live fixed on rocks, plants and other components of aquatic ecosystems. Overall, macroinvertebrates have limited dispersal capacity as compared to vertebrates inhabiting freshwater ecosystems (e.g. fish, mammals). Changes in macroinvertebrate community structure and demographics are usually considered reliable indicators of habitat disturbances, and are usually correlated to changes in the chemical status of aquatic ecosystems (Rico et al. 2016). Macroinvertebrate biomass is a key variable in quantifying a variety of energetic processes of food webs, including trophic dynamics, carbon cycling, and transfer of energy between aquatic and terrestrial environments (Parker and Huryn 2006). The population density of organisms (number of macroinvertebrates per area unit) is usually applied in aquatic monitoring studies as a proxy of macroinvertebrates´ biomass. However, this variable has limited ecological relevance and does not establish a direct relationship with overall ecosystem functions (Rudolf et al. 2014). For example, insect larvae belonging to the Trichoptera order usually have a large contribution to the overall biomass as compared to other aquatic larvae in similar orders (e.g. Ephemeroptera) due to their large size and long aquatic phase (Rudolf et al. 2014). Therefore, they can play a different role in terms of making a more important food contribution to secondary predators.

Macroinvertebrates biomass can be measured directly by weighing the living individuals or indirectly by using length-mass relationships. Since the direct method of estimating macroinvertebrate biomass is often hampered by difficulties in weighing small organisms, the indirect method of using length-mass relationships is regarded as a more accurate option (Brito et al. 2015; Stoffels et al. 2003; Zamani-Ahmadmahmoodi et al. 2017). In the indirect method, computational statistics, such as regression models, including linear, log-linear and exponential models have been used in neotropical and temperate regions to correlate both variables (Miserendino 2001). The semi-arid conditions in Middle-East regions contribute to particular ecosystem features (e.g. high daily thermal variability, large oxygen fluctuations, and desiccation periods); and they are also responsible for particular biological traits (e.g. drought resistance in life stages, improved dispersal capacity, and short life-cycles). All these features can lead to different biodiversity and macroinvertebrate length-mass relationships in comparison to other regions.

So far, the majority of monitoring studies and available length-mass relationships for freshwater macroinvertebrates have been performed in cold or temperate regions, while such information for arid and semi-arid regions, such as the Middle East region, is lacking. In the present study we measured the macroinvertebrate biomass in the Choghakhor wetland, Central Iran, and constructed length-mass relationships for different taxa, utilizing linear, log- linear, and exponential models. A cross-validation test was used to compare the validity of the results of these models and to determine the optimal model for measuring the biomass macroinvertebrates in freshwater ecosystems of the Middle-East.

Materials and methods

Study area

Choghakhor wetland constitutes one of the biosphere reserves of the earth (Zamani-Ahmadmahmoodi et al. 2017). It is located within the Borujen, Chaharmahal and Bakhtiari provinces in Iran, and covers an area of more than 1,500 hectares. It is protected by the

Environmental Protection Agency of Iran due to its national and international biological values (Fathi et al. 2013). This wetland has five water inflows (i.e. rivers with spring sources) and one water outlet. The surroundings of the outlet are mostly dedicated to agriculture. The most important sources of water in the wetland are the springs of Bagh Khan, Tang Siah, Sibak, Shir Poshteh and Galu Gerd. The water of these ecosystems has been diverted to Isfahan and Qom provinces for drinking purposes; however, in recent years, this issue has been under intense debate, and this water transfer has been stopped (Fathi et al. 2013). Nine monitoring stations were selected based on the land use, accessibility, possibility of sampling, and habitat. The monitored sites comprised two stations inside the wetland, six stations at the springs, and one station at the outlet (Fig. 1; Online resource 1, Table S1).

The sampling, identification and morphometric evaluations of macroinvertebrates were scheduled for late summer in the nine stations (September of 2016). The rationale behind this decision is that: (1) by late summer the majority of macroinvertebrates have completed their growth in ecosystems similar to the present one (Martin et al. 2014); (2) the volume of water, due to lack of precipitation and human consumption, is at its minimum amount, facilitating sampling; and (3) there is a maximum of density and diversity of these organisms in the aquatic ecosystem (Aazami et al. 2015).

Macroinvertebrate sampling

Macroinvertebrate samples were collected using the Surber sampling method (net mesh size 250 microns, 30 × 30 cm cross section), with three replicates at each sampling station. Immediately after sampling, the specimens were transferred into glass vials and fixated with 4% formalin. Subsequently, they were transported to the laboratory and classified using a magnifying glass, forceps, and binocular (NTB-2B model) with 25× magnification. Whenever it was possible, macroinvertebrates were identified to the genus level according to Hartmann (2007), and Oscoz et al. (2011); however, in many instances, the lowest taxonomic resolution was family. Finally, the identified samples were stored in glass containers with 30 mL of alcohol (96%) for up to 50 days in order to reduce the error in measuring the corresponding mass (Mährlein et al. 2016).

Length and mass measurements

The body length (from the head to the end of the tail), head width (the widest part of the head capsule), and the distance between the two eyes (the shortest distance between the two eyes) of each organism were measured as shown in Fig. 2, using a caliper sensor with accuracy of 0.001 mm. To measure the wet mass of the living organisms, samples were taken from the alcohol solution and kept on filter paper for 10 minutes under laboratory conditions so that the excess of alcohol, absorbed by the bodies, was eliminated. Subsequently, the samples were placed in an oven for 24 hours at 60 °C and weighed again by a digital scale with a high precision degree (0.0001 mg) to calculate the dry weight.

Statistical analyses

Three regression models (i.e. linear, log- linear, and exponential) were fitted to calculate the relationships between the mass and the length of the samples (Team 2013). The linear model reads:

$$M = a + b.L + \varepsilon \quad \varepsilon \approx \mathrm{N}(0, \delta)$$

where, a is a constant, the parameter b is the slope of the regression model, M and L are the measured mass and the length of the organisms, respectively, and ε is the model error.

The log-linear regression model comes from the power function $M = aL^b$ and reads:

$$LnM = Lna + bLnL + \varepsilon \quad \varepsilon \approx \mathrm{N}(0,\delta)$$

In the log-linear model, the geometric mean was used instead of the arithmetic mean; therefore, a correction factor was needed to correct the error of the mean change, which was obtained according to Hayes and Scott Shonkwiler (2006):

$$SF = \frac{1}{n}.\sum_{i=0}^{n} e^{\varepsilon_i}$$

where, *n* is the number of samples and ε_i is the error from the fitted log-linear model. The error of the mean change (SF=smearing factor) was considered to be negligible since the values of mass and length for macroinvertebrates are small (Mährlein et al. 2016).

The exponential regression model comes from the function $\mathrm{M} = \mathrm{ae}^{\mathrm{bL}}$, and was converted to the following linear model:

$$LnM = Lna + b.L + \varepsilon \quad \varepsilon \approx \mathrm{N}(0,\delta)$$

To evaluate the ability of each model in predicting the exact results and also comparing the results of the three models, cross-validation tests were used (Mährlein et al. 2016). In order to do so, 50% of the data were randomly selected and used for training, and the other 50% were used for the validation tests. In order to compare the predicted and actual results, the significance level of the cross-validation test was calculated by using a paired t-test. It should be noted that the cross-validation was repeated 1000 times, and the average of the repetitions was calculated as the basis of comparison. The regression coefficients, computed for the three models, were compared with an ANOVA test. All analyses were performed using the R software (see Mährlein et al. 2016).

Results

In total, 4480 macroinvertebrates were identified, including 11 orders and 26 families. Among them, individuals belonging to 9 orders and 17 families, which had whole body with all appendices, were preserved for further morphometric evaluation. The sampled communities were dominated by Gammaridae (75%, relative frequency) and Baetidae (13%), followed by Erpobdellidae (2%), Chironomidae (1.7%) and Hydropschidae (1.5%; see Online resource 1, Table S2).

The mean length of the body, the distance between the eyes and the head width for the monitored organisms are shown in the Online resource 1 (Table S3). Lowest parameter values were recorded for individuals in the Elmidae and Haliplidae families, while the largest individuals corresponded with those in the Leuctridae, Hydropsychidae, and Tipulidae families (Online resource 1, Table S3). The highest and lowest mean body mass corresponded to Gammaridae and Oligochaeta, respectively. Dry and wet body mass values are provided in the Online resource 1 (Table S4).

Table 1 gives the results of different length-mass relationships using the linear model. The length-mass relationships considered were: body length and mass; head width and mass; eye length and mass, and the same taking into account the dry mass. It is worth mentioning that the values of the relationships with R^2 of less than 0.50 were not included as were not considered robust enough. In Table 1, the lower the model's reliability error SF, the higher the accuracy of the model (Mährlein et al. 2016). Tables 2 and 3 show the results of the length-mass relationships computed with the log-linear and the exponential models, respectively.

The slope of the regression lines, obtained from the three models, were compared. The results show better performance of the log-linear model (ANOVA, $p = 0.03$), as compared to the other two (ANOVA, $p > 0.05$). This suggests that, in general, the log-linear model is the most appropriated one for calculating length-mass relationships. The SF criterion, the R^2, and the cross-validation test generally supported the same conclusion (Table 4).

Discussion

Although it has been reported that the members of Gammaridae family may have less predominance in aquatic ecosystems over 1600 meters above the sea level (Eisenring et al. 2016), the present study demonstrates that this family is the most abundant organism in Choghakhor wetland. This may be explained by specific climatic conditions and food resources related to high loads of plant materials. The largest number of them was found in the region where the vegetation was scattered, with no agricultural practice or sediment materials related to human activities (in the river source stations). After Gammaridae, the most frequent taxa were the Ephemeroptera order, mostly dominated by individuals in the Baetidae family. This family is generally widespread in freshwater ecosystems, and its distribution has been related to the availability of food sources under different climate conditions (Middlemiss 2014; Eisenring et al. 2016). The Diptera order was dominated by individuals in the Tipulidae family. The dominance of this family is related to their high tolerance to temperature fluctuations (Todd 1996), their wide geographic distribution (De Jong et al. 2008), and fast growth and large size as compared to other similar taxa in the study region (Alexander 2016). For some taxa, the measures of sclerotized structures (i.e., the distance between the eyes and the head width) were found to be suitable parameters to estimate biomass. They may also be useful for biomass estimation with individuals that have been kept for a long time in laboratory as well as the ones whose bodies have been damaged. The dry mass of some macroinvertebrate families could not be measured to the three decimal digits due to the high moisture content; therefore, the wet mass was used in some cases to investigate the morphological relationships presented in this study. The coefficient of variation (CV) is derived from dividing the standard deviation by the mean, and expressed as percentage yielding a dimensionless quantity. In this way, variables with different units can be utilized to compare the data dispersion. On the basis of these results, we found that the average wet mass CV of macroinvertebrates (35.75) is much bigger than that for body length (17.58), indicating a less spread of the length data. Consequently, the body length measurement of the living organisms can be considered as useful complement to the mass evaluation in order to determine the macroinvertebrate's biomass. For some macroinvertebrate families (e.g. Leuctridae), the distance between the two eyes can be used as useful parameter to estimate the mass per area unit. However, for damaged organisms, head capsule length, rather than body length, may be used to estimate biomass (Nakagawa and Takemon 2014). Also, our results showed that the correlation models using wet mass and body length were generally more robust than those using dry mass (larger number of models with R^2 above 0.5), confirming that wet mass is a better predictor for macroinvertebrate's biomass. These findings are useful for macroinvertebrates with very low value of dry mass, for which measurements are not straightforward.

The results of Tables 1-3 reveal that a fixed statistical model does not suit all the studied families for the evaluation of length-mass relationships. According to the present results, for some families (e.g. Elmidae), the log-linear model was found to have the best performance, while for some others (e.g. Leuctridae), the exponential model was the most suitable one, and for other families (e.g. Chironomidae), the linear model offered higher R^2 values. These findings are consistent with the findings of two earlier studies (Mantyka-Pringle et al. 2014; Martin et al. 2014), who also offer different optimal modeling choices for different taxa. It is worthy of note that length–mass relationships differ according to habitats and ecosystems (Mährlein et al. 2016), and therefore different models can be suitable for the same taxon in different regions.

The cross-validation test, through the mean of best performance, demonstrated that all three models performed reasonably well, in terms of both statistics and the accuracy of prediction.

However, for more than 60% of the relationships examined by this test, the p-value for the log-linear model was greater than for the other two (Table 4), suggesting that the accuracy of this model is in general superior, as indicated in other studies (Miserendino 2001; Miyasaka et al. 2008; Thakur 2015).

Regarding Gammaridae, none of the statistical models offered an R^2 above 0.5. Therefore, it was not possible to provide suitable length-mass relationships. The variability in the observed data might be attributed to the sampling season or the heterogeneity of sampling species, which included organisms with different age, gender, and size. Similar difficulties to evaluate such relationships, for some macroinvertebrate families, including Gammaridae, have been reported by Berezina (2007), who tried to compare length-mass relationship for several families collected from a larger biogeographical scale. In a similar study performed in the Mediterranean region, no significant statistical relationships were found for the brackish water species *Gammarus insensibilis* Stock, 1966 (Rosati et al. 2012).

For four taxa (Haliplidae, Elmidae, Leuctridae, Oligochaeta), the body length - mass relationships obtained with the log-linear model were very significant, with R^2 higher than 0.80. The differences in accuracy of the correlations calculated for the different families is related to availability of food, reproductive cycle and other environmental conditions (e.g. temperature, light). Moreover, it seems that in regions where human pressure and ecosystem impairment are high, the relationships between wet mass and total body length may be affected. This may have introduced some variability to our results, as the Chaghakhor wetland is subject to a range of anthropogenic and environmental stressors e.g. water scarcity and water diversion, increased industrial pollution, increased hunting pressure on aquatic ecosystems, agricultural intensification, climate change and, recently, the spread of some invasive species. Further research is required to quantify the contribution of these factors to changes in macroinvertebrate morphological traits, so that multiple stressors can be quantitatively linked to biomass estimations. As a novel result, this study shows that relationships between body length and wet mass offered the best estimation of biomass, as compared to those performed with dry mass. Moreover, the cross-validation test shows the optimal model choice for each family, and a good accuracy of the presented models to estimate macroinvertebrates biomass in wetland ecosystems.

Conclusions

The assessment of aquatic biomass is an important but resource consuming task, which can be complemented with the use of indirect measures (e.g. length-mass relationships). The present study provides the first set of length-mass regression models for macroinvertebrates collected in aquatic ecosystems of the Middle East region. Body length, head width and distance between eyes have been successfully used to estimate body mass by using linear, log-linear and exponential models. This study offers optimal model choices to perform biomass estimations for 17 macroinvertebrate families and indicates an overall higher efficiency of the log-linear model in comparison to the other two. The current study confirms that the wet mass is a superior predictor for the estimation of macroinvertebrates biomass than the dry mass, as has also been described for fish and other higher aquatic organisms. Finally, the results of this study are expected to contribute to the evaluation of morphological differences between macroinvertebrates from different regions, and to the monitoring of the ecological status of freshwater ecosystems in the Middle East region.

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Author Contribution Statement

All authors read and approved the manuscript.

Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Compliance with ethical standards

This article does not contain any studies with human participants performed by any of the authors.

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Tables

Table 1: Results of the linear regression model.

Taxon	a	Ln a ± SE	b ± SE	SF	R^2
BL → M regression model					
Chironomidae	1.002	0.001±0.002	0.004±0.000	1.000	0.78
Psychodidae	1.002	0.001±0.002	0.003±0.001	1.001	0.57
Tipulidae	1.007	0.006±0.007	0.003±0.000	1.001	0.64
Haliplidae	0.999	-1.000± 0.001	0.003±8.012	1.001	0.83
Elmidae	0.999	-1.000± 0.001	0.000±0.000	1.002	0.88
Limnephillidae	0.955	-4.604±0.011	0.008±0.001	1.002	0.72
Baetidae	1.074	0.071±0.030	0.026±0.003	1.000	0.72
Heptagenidae	1.288	0.253±0.016	0.007±0.001	1.001	0.51
Taeniopterygidae	0.999	-1.000± 0.001	0.004±0.004	1.000	0.75
Leuctridae	1.001	0.000±0.000	0.000±0.000	1.001	0.93
Oligochaeta	0.999	-1.000± 0.001	0.000±0.000	1.002	0.89
HW → M regression model					
Haliplidae	1.001	0.002±0.007	0.002±0.000	1.002	0.83
Elmidae	0.999	-1.000± 0.001	0.017±0.004	1.000	0.90
Heptagenidae	1.211	0.191±0.019	0.114±0.021	1.001	0.54
Taeniopterygidae	0.999	-1.000± 0.001	0.002±0.000	1.001	0.77
EL → M regression model					
Haliplidae	0.999	-1.000± 0.001	0.005±0.002	1.001	0.51
Elmidae	0.999	-1.000± 0.001	0.003±0.000	1.000	0.93
Heptagenidae	1.210	0.190±0.013	0.117±0.028	1.002	0.52
Taeniopterygidae	1.002	0.001±0.002	0.003±0.001	1.000	0.72
Leuctridae	1.214	0.193±0.015	0.001±0.000	1.001	0.93
BL → DM regression model					
Haliplidae	0.999	-1.000± 0.001	0.005±0.000	1.000	0.93
Elmidae	0.999	-1.000± 0.001	0.003±0.000	1.000	0.89
Leuctridae	1.002	0.001±0.002	9.461±3.008	1.002	0.52
EL → DM regression model					
Haliplidae	0.998	-2.002±0.005	0.008±0.002	1.001	0.76
Elmidae	0.999	-1.000± 0.001	0.003±0.000	1.000	0.84
Leuctridae	1.211	0.191±0.019	9.460±3.008	1.001	0.52
HW → DM regression model					
Haliplidae	0.999	-1.000± 0.001	3.524±8.170	1.001	0.81
Elmidae	0.999	-1.000± 0.001	0.001±0.000	1.001	0.67

Explanations: a: model constant; b: slope; SE: standard error; SF: smearing factor; R^2: coefficient of determination. BL (body length, mm), HW (head width, mm), EL (eye length, mm), M (mass, mg), DM (dry mass, mg).

Table 2: Results of the log-linear regression model.

Taxon	a	Ln a ± SE	b ± SE	SF	R^2
BL → M regression model					
Chironomidae	0.003	-5.809±0.250	1.190±0.171	1.010	0.72
Psychodidae	0.005	-5.298±0.621	0.881±0.370	1.000	0.55
Haliplidae	0.001	-6.907±0.282	1.863±0.400	1.000	0.83
Tipulidae	0.006	-5.131±0.350	0.873±0.143	1.010	0.66
Elmidae	0.001	-6.907±0.282	1.501±0.246	1.010	0.90
Limnephilidae	0.001	-6.907±0.282	2.386±0.364	1.000	0.69
Baetidae	0.056	-2.882±0.211	0.789±0.122	1.000	0.76
Heptagenidae	0.177	-1.731±0.133	0.251±0.051	1.000	0.51
Taeniopterygidae	0.001	-6.907±0.282	1.161±0.151	1.003	0.74
Leuctridae	0.001	-6.907±0.282	0.821±0.080	1.003	0.88
Oligochaeta	1.039	0.038± 0.011	3.251±0.420	1.006	0.94
HW → M regression model					
Psychodidae	0.041	-3.194±0.20	0.444±0.140	1.000	0.71
Haliplidae	0.001	-6.907±0.282	0.511±0.160	1.008	0.69
Elmidae	0.001	-6.907±0.282	1.120±0.246	1.020	0.83
Taeniopterygidae	0.001	-6.907±0.282	1.171±0.144	1.003	0.78
Leuctridae	0.002	-6.214±0.031	0.756±0.089	1.004	0.87
BL → DM regression model					
Haliplidae	7.090	1.958 ±0.650	4.921±0.911	1.022	0.88
Elmidae	0.005	-5.298±0.621	1.491±0.163	1.005	0.95
Taeniopterygidae	0.001	-6.907±0.282	0.960±0.133	1.004	0.73
Leuctridae	0.002	-6.214±0.031	0.730±0.812	1.004	0.85
M → DM regression model					
Haliplidae	27452.510	10.220±4.161	2.424±0.540	1.286	0.82
Hydropsychidae	1.429	0.356±0.450	1.540±0.122	1.034	0.88
Limnep	1.743	0.555±1.011	1.687±0.295	1.018	0.62
Chelicrophilidae	0.230	-1.469±0.232	0.981±0.061	1.004	0.92

Explanations: a: model constant; b: slope; SE: standard error; SF: smearing factor; R^2: coefficient of determination. BL (body length, mm), HW (head width, mm), EL (eye length, mm), M (mass, mg), DM (dry mass, mg).

Table 3: Results of the exponential regression model.

Taxon	a	Ln a ± SE	b ± SE	SF	R^2
BL → M regression model					
Chironomidae	0.005	-5.298±0.621	0.261±0.045	1.020	0.65
Psychodidae	0.001	-6.907±0.282	0.164±0.067	1.001	0.55
Tipulidae	0.022	-3.816±0.142	0.069±0.011	1.001	0.66
Limnep	0.003	-5.809±0.366	0.243±0.037	1.000	0.69
Baetidae	1.001	9.995±0.106	0.091±0.012	1.000	0.73
Heptagenidae	0.255	-1.366±0.053	0.023±0.005	1.000	0.50
Taeniopterygidae	0.001	-6.907±0.282	0.146±0.016	1.002	0.80
Leuctridae	0.001	-6.907±0.282	0.112±0.006	1.000	0.96
Oligochaeta	9.571	2.258±0.751	0.293±0.036	1.001	0.94
HW → M regression model					
Psychodidae	0.139	-1.973±0.142	1.834±0.566	1.000	0.71
Haliplidae	0.001	-6.907±0.282	0.518±0.204	1.010	0.58
Elmidae	0.001	-6.907±0.282	2.794±0.988	1.004	0.64
Taeniopterygidae	0.001	-6.907±0.282	0.915±0.093	1.001	0.84
Leuctridae	0.001	-6.907±0.282	0.724±0.045	1.002	0.95
BL → DM regression model					
Haliplidae	1.731	0.548±1.012	2.392±0.484	1.001	0.85
Elmidae	6.451	1.864±0.611	1.399±0.485	1.071	0.65
EL → M regression model					
Elmidae	0.001	-6.907±0.282	7.784±1.449	1.011	0.88
Taeniopterygidae	0.001	-6.907±0.282	0.838±0.101	1.001	0.78
Leuctridae	0.001	-6.907±0.282	0.728±0.050	1.002	0.95
HW → DM regression model					
Haliplidae	6.454	1.864±0.617	1.399±0.485	1.073	0.65
M → DM regression model					
Haliplidae	1.531	0.425±0.520	5609.710±6846	1.021	
Elmidae	0.001	-6.907±0.282	52.931±6.844	1.079	
Hydropsychidae	0.001	-6.907±0.282	52.918± 36.841	1.079	
Limnep	0.001	-6.907±0.282	50.944± 9.569	1.016	
Chelicrophilidae	0.002	-6.214±0.031	30.551± 2.493	1.000	

Explanations: a: model constant; b: slope; SE: standard error; SF: smearing factor; R^2: coefficient of determination. BL (body length, mm), HW (head width, mm), EL (eye length, mm), M (mass, mg), DM (dry mass, mg).

Table 4: Results of the cross validation test for the length-mass relationships.

Taxon	*Linear*			*Log-linear*			*Exponential*		
	md	*t*	*p*	*md*	*t*	*p*	*md*	*t*	*p*
Chironomidae	0.18	0.06	0.473	-2.12	-0.05	0.505	-8.37	-0.11	0.499
Tipulidae	3.91	0.09	0.484	-5.52	-0.12	0.434	0.55	0.01	0.462
Simuliidae	-0.03	-0.02	0.448	-3.95	-0.08	0.507	-2.05	-0.04	0.458
Gammaridae	0.01	-0.02	0.42	-0.11	-0.06	0.441	-3.93	-0.11	0.452
Hydropsychidae	-0.08	0.02	0.427	5.01	-0.13	0.412	-0.08	0.02	0.427
Limnephilidae	0.01	-0.01	0.483	-1.49	-0.04	0.482	-0.18	-0.02	0.474
Chelicorophium	-0.06	0.09	0.424	-7.21	-0.01	0.397	-5.25	0.01	0.422
Baetidae	0.08	0.02	0.469	-1.59	-0.03	0.455	-0.93	-0.02	0.493
Heptageniidae	-0.05	0.01	0.472	0.83	0.02	0.442	0.71	0.01	0.471
Taeniopterygidae	0.01	0.02	0.454	-4.88	-0.05	0.476	-1.49	-0.01	0.461

Explanations: *md* is the average of the difference between the measured values and the predicted ones. *t* and *p* refer to the *t*-value and the *p*-value of the paired *t*-test results, respectively. Values refer to averages obtained from predictions after 1000 model iterations.