

Article

How Do Sampling Methods Affect the Body Size–Abundance Relationships of Benthic Macroinvertebrates in Freshwater Ecosystems? A Comparative Analysis

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Abstract: Body size is a key trait of species, populations, and organisms; it relates to abundance, home range, metabolic rates, and stressors. As reported by Damuth's law, the relationship between body size and abundance is negative with a slope value of -0.75 . In aquatic ecosystems, particularly for benthic macroinvertebrates, the slope value deviates from the expected value of -0.75 . This variation may depend on abiotic and biotic factors as well as methods that are used to sample benthic macroinvertebrates. "Leaf-bags" and "Surber-net" are the most used methods and they provide different data about the body size structures and abundance of the sampled benthic macroinvertebrates. Here, we used the slope of the body size–abundance relationships to compare the benthic macroinvertebrate assemblages sampled with two sampling methods (Leaf-bags and Surber-net). We found that Leaf-bags sampled a greater number of smaller taxa than Surber-net, which collected individuals of larger sizes. In our study, differences in the benthic macroinvertebrate body size structures collected with these two sampling methods were evident even if the experiment was performed at a limited spatio-temporal scale. Therefore, Leaf-bags and Surber-net should be used as complementary rather than alternative techniques since they sample integrated information from the benthic macroinvertebrate communities.

Keywords: Apennine river ecosystems; benthic macroinvertebrates; body size–abundance relationships; sampling methods; Leaf-bags and Surber-net

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1. Introduction

Body size is a key component in the diversity and structure of animal assemblages as it encapsulates many ecological traits at species, population, and individual levels. This is because body size is strongly associated with many physiological, ecological, and behavioral traits [1–3]. The relationship between body size and abundance (or density) is one of the most important, which according to Damuth's Rule, scales with a scaling exponent of -0.75 [4,5]. Body size (or mass) is also related to metabolic rate with a scaling exponent of 0.75 (i.e., metabolic theory in ecology—MTE, [1]). Furthermore, the body sizes of species are negatively related to abundances in local and global communities. Such body size–abundance relationships have remained at the core of our understanding of ecology for more than 30 years [3,6,7].

The relationship between body size and abundance is recognized in both terrestrial and aquatic ecosystems. In aquatic habitats, this body size–abundance relationship has been explored in a wide variety of taxa from microbial to fish species, worldwide [7]. More recently, on the basis of these studies, freshwater ecologists have used body size–abundance relationships in macroinvertebrate organisms as alternative methods to investigate benthic community structures in different aquatic ecosystems [8], such as lagoons [9–11], lakes [12–15], and streams [16,17]. Most of these studies reported values of body size–abundance relationships that deviate from the putative universal slope of -0.75 . However, little attention has been paid to whether these differences are systematic or are because of methodological causes.

In stream ecosystems, macroinvertebrate organisms that are small aquatic animals, such as insect larvae, snails, worms, beetles, etc., have been widely studied because they are excellent indicators of water quality [18,19]. Moreover, macroinvertebrate species are characterized by a high functional diversity that allows them to be categorized into functional feeding groups based on their diverse morphological and behavioral functions that characterize the ways they acquire food. This functional diversity is strongly related to the habitats or ecosystems where they live, making the macroinvertebrates sensitive to environmental conditions depending on their taxonomy and traits [20–22]. There are different methodologies to sample macroinvertebrate organisms in stream ecosystems (for an exhaustive discussion, see [23–26]); however, Surber-net and Leaf-bags represent the two most widely used sampling methods for freshwater macroinvertebrates [27]. Surber-net samples the macroinvertebrates actively by moving the gravel on the bed of the river and catching everything that is disturbed in a net. Surber-net is a relatively quick sampling method, and it can be completed on the riverbanks at several locations. Conversely, for Leaf-bag sampling, the leaves are left in the aquatic ecosystems for several days, usually for 30 days [28–31], and are then collected from the sampling sites. Thus, compared to Surber-net, the Leaf-bags sampling method is a passively long-term process where macroinvertebrates are trapped for a long period in relation to the Surber-net sampler. We could say that, while Surber-net takes a “snapshot” of the condition of the watercourse at a precise moment, Leaf-bags record the state of the stream for a certain period of time. However, it is also true that Leaf-bags have strong bias toward taxa related to the natural substrates they are mimicking.

Here, we compared these two sampling methods by collecting macroinvertebrates within permanent reach of the Apennine River (Aterno River) in Italy [23,32]. Because of their different natures and temporal gradients, these samples will allow us to understand how body size–abundance relationships vary across sampling methods. Hence, the main goal of our study was to identify the relationships between the body size and abundance of macroinvertebrate communities between two distinct sampling methods and to discover temporal or spatial differences across a spectrum of body size diversity in macroinvertebrate species.

2. Materials and Methods

2.1. Study Area

The field experiment was conducted in a third-order reach ($42^{\circ}12'21.683''$ N, $13^{\circ}38'27.895''$ E; altitude: 507 m a.s.l.) of the Aterno River (Abruzzo, Central Italy) at about 60 Km from the spring source. This area is located in the “Sirente-Velino” regional natural park. The sampling site is characterized by a permanent water flow and direct and predictable anthropogenic impacts are absent. The environmental, hydraulic, and chemical–physical characteristics were well-known and already reported in the literature [23,32]. For the above reasons, we considered this sampling site useful for testing the sampling methods for the body size–abundance relationships of freshwater macroinvertebrates.

2.2. Experimental Design and Sampling Protocols

Benthic macroinvertebrate invertebrate assemblages were sampled using (in parallel) leaf-bag and Surber-net techniques. These two techniques are the most used sampling methods for collecting freshwater macroinvertebrates worldwide. Here, we applied both techniques using the most common characteristics and features used by freshwater researchers worldwide. We started the experiment in July 2010; in each sampling occasion, we used 18 leaf-bags, and when we retrieved the leaf-bags, we took 3 benthic Surber-net samples collected by using a Surber-net (sampling area: 0.12 m² and mesh-size: 200 µm, accordingly), for a total of 4 sampling dates (n. of leaf-bags = 72; n. Surber-net = 12).

Leaf-bags were made using *Phragmites australis* [(Cav.) Trin. ex Steud.] leaves collected along the banks of the Aterno River in the summer–autumn period when they were close to abscission. Leaves were transported to the laboratory, spread over a table, and left for a week in a bright and ventilated room to complete the drying process. Leaves were sprouted from the apical and basal parts, cut into fragments of about 10 cm in length, and placed in a thermostatic oven for about 72 h at 60 °C to allow the complete loss of the hydration water. Dried leaves were weighed in equal groups on the analytical balance with precision ± 0.001 g and weighing accuracy ± 0.005 g. The leaf-bags were made with 0.5 cm × 0.5 cm mesh sizes to allow the passage of invertebrates inside, and at the same time limit the loss of leaf fragments. The standardized initial dry mass for each leaf bag was 3.000 g (± 0.005 g).

After about 30 days, Leaf-bags were retrieved with accuracy in order to avoid the loss of small pieces of leaf debris and invertebrates. Each Leaf-bag was placed in a previously labeled polyethylene case and was transferred to the laboratory using a thermostatic container. In the laboratory, samples were placed in a thermostatic room at a constant temperature to keep the invertebrate component intact. Leaf-bags were subjected to the sorting procedure, which essentially consisted of the separation of the invertebrate component from leaf debris and inorganic sediment. In a rigid plastic container with dimensions equal to 40 cm in length, 25 cm in width, and 10 cm in height, the nylon nets of leaf-bags were cut and the leaf debris was carefully removed from the container, cleaned with water from the mineral sediment, and placed on absorbent paper for subsequent drying. Successively, all of the water in the container with the organisms was filtered with a 200 µm mesh-sized net, the collected invertebrates were sealed in a labeled plastic container, fixed, and stored in a 70% alcohol solution for subsequent identification. Benthic invertebrates were recognized at the lowest possible taxonomic level and counted using a stereomicroscope (Leica MZ6, 0.63×–4.0×).

The taxa identified were also assigned to functional feeding group(s) (FFG) following Tachet et al. [33]. Benthic invertebrates sampled with Surber-net underwent the same sorting, conservation, and identification treatment as described above for the leaf-bags.

The length of each specimen was measured under a stereomicroscope (Leica MZ 12.5) via an image analysis system (Leica QWin). After storing the specimens in micro-titer plates and drying them in a stove at 60 °C for 72 h, macroinvertebrates were weighed using a microbalance (Sartorius MC21S). The organisms belonging to the same species and habitat were grouped and placed in labeled ceramic crucibles. These were put in a muffle furnace for 6 h at 500 °C [34]. The ash content was estimated and the individual biomass was recorded as ash-free dry weight (AFDW).

2.3. Statistical Analysis

The data were divided according to the sampling methods used and two datasets were obtained. Subsequently, the biomass values recorded for the 4 periods considered were also isolated. To limit the effect of discretization, a K-learn algorithm [35] was applied to the 10 datasets obtained (Leaf-bags, Surber-net, Leaf-bags per 4 sampling periods, and Surber-net per 4 sampling periods) to establish the best division into size classes. On average, the best classification was the classification in 50 size classes; finally, each

dataset was discretized into 50 size classes. In this way, the sizes of the classes depended on the size range of each dataset and the applied classification allowed the correct fitting of the body size–abundance distribution functions for each dataset. In a descriptive way, a linear model was fitted to the raw data of the average body size relative to the average abundance per taxon, in order to test the body size–abundance relationship of freshwater macroinvertebrate between the two sampling methods, both variables were log10 transformed. According to [17], this approach for studying the size–abundance relationships was based on calculating the local size–abundance relationship (LSDR), which plots the average size and average abundance of each taxon. Least-squares regression (LSR) was fitted on the average of the ash-free dry weight and the average abundance (number of individuals) within each size class. The comparisons were (i) between the two sampling methods; (ii) between the two sampling methods and the pooled dataset; and (iii) among the sampling periods for each sampling method. We used least-square regression (LSR) to describe the body size–abundance relationships; LSR is more appropriate with respect to the reduced major axis regression in which the measurement error in the independent variable is less than that of the dependent variable. The effect of the sampling period was tested through an OLS bisector model in which the sampling period represents the instrumental variable. The analysis was carried out after combining the classes of each period into two datasets, one for the Surber-net method and the other for the leaf-bags method. Finally, the effect of the sampling period was tested by using one-way ANOVA with the period as a factor and by an ANCOVA with the variable “period” as a covariate.

3. Results

The two datasets differed in the number of individuals collected; 5179 individuals were counted for the leaf-bags sampling methods and 1082 for the Surber-net samples. The sub-datasets of the four sampling periods showed a more regular distribution for the leaf-bags method, in relation to the number of individuals recorded for each sampling period and the body size range recorded (Figure 1).

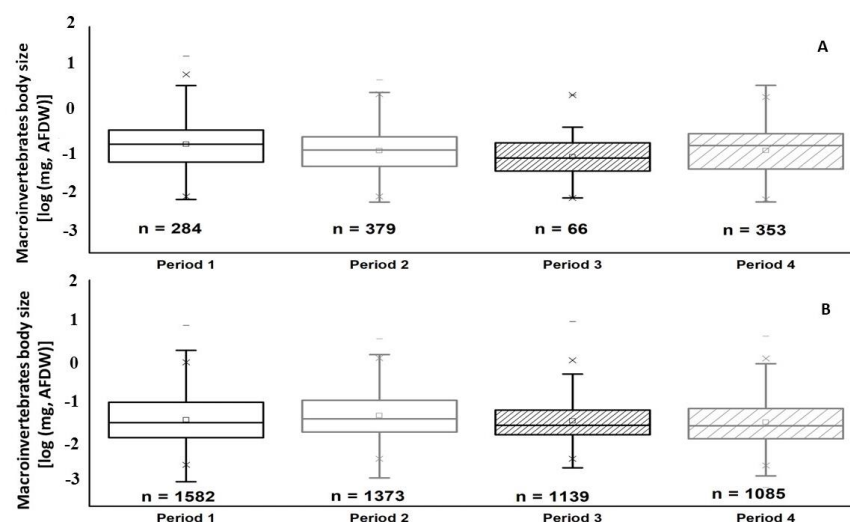


Figure 1. Boxplots of macroinvertebrate body sizes (after the log transformation of individual Ash-Free Dry Weight, mg) of the specimens collected through the two sampling methods and 4 sampling periods considered; (A) describes the results obtained by using the Surber-net sampling method and (B) describes the results by using the Leaf-bags sampling method.

Both the LSDR of Leaf-bags and Surber-nets showed significant body size–abundance relationships. The scaling slopes were higher than the energetic equivalent hypothesis value of -0.75 with -1.8 and -1.4 , respectively (Table 1; Figure 2). The body size–abundance relationships that were constructed according to sampling periods (Fig-

ures 3 and 4) show that with the “Leaf-bag” method the slope is preserved (Figure 3), while with the “Surber-net” method, there is a loss of parallelism (slope change) and higher variability in the intercept values (Figure 4) is highlighted.

Table 1. Results of the LSR analyses of the log10 abundance in relation to the log10 body weight in two considered sampling methods (Surber-net and Leaf-bags).

Sampling technique	Intercept Value	Intercept SE	Slope Value	Slope SE	C.I. Min	C.I. Max	Adjusted R-Square	<i>p</i>
Surber-net	1.253	0.109	−1.416	0.163	−1.497	−1.334	0.851	***
Leaf-bags	1.226	0.065	−1.817	0.116	−1.875	−1.759	0.92	***

Note: *p* value signification code: $0 < *** < 0.001$.

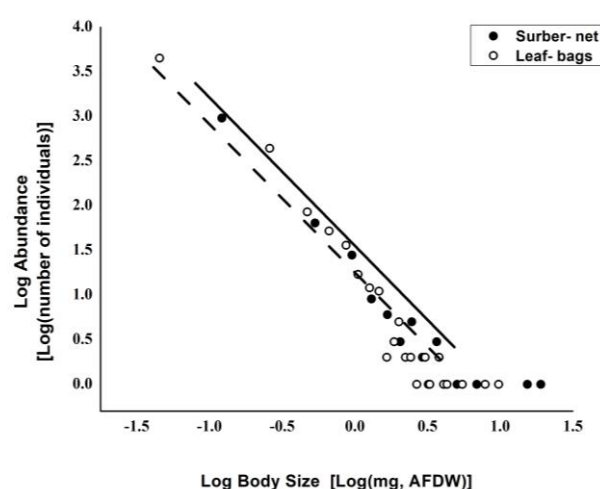


Figure 2. Log10–log10 relationship between the average body size class (Ash-Free Dry Weight, mg) and abundance (number of individuals in each class) of macroinvertebrates sampled with the two sampling methods (Surber-net and Leaf-bags); all sampling periods are included.

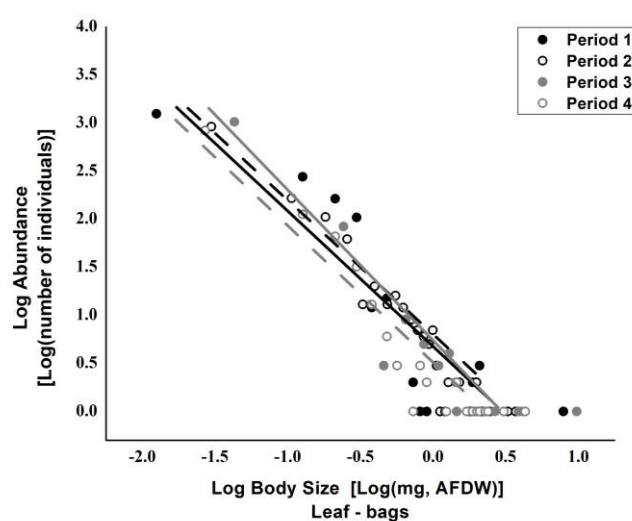


Figure 3. Log10–log10 relationship between the average body size classes (Ash-Free Dry Weight, mg) and abundance (number of individuals in each class) of macroinvertebrates sampled in each sampling period (4 periods) by using the Leaf-bags method.

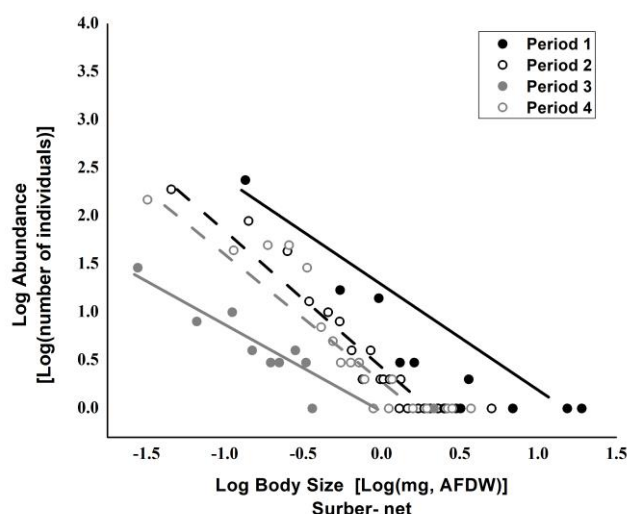


Figure 4. Log10–log10 relationship between the average body size classes (Ash-Free Dry Weight, mg) and abundance (number of individuals in each class) of macroinvertebrates sampled in each sampling period (4 periods) by using the Surber-net method.

Considering the sampling period as an instrumental variable in an OLS bisector, we can see how the slope values increase; in particular, the slope value of the data relating to the “Surber-net” dataset is 0, presenting a value of 1.09×10^{-6} while for the “Leaf-bags” dataset, the slope value is -0.448 (Table 2).

Table 2. Results of the bisector OLS analyses of log10 abundance in relation to log10 body weight for the 2 considered sampling methods (Surber-net and Leaf-bags) for each sampling period.

Surber Net					
Source	GDL	SS	MSE	F	Pr > F
Model	1	0.008	0.008	0.022	0.882
Error	37	13.300	0.359		
Correct total	38	13.308			
Leaf bags					
Source	GDL	SS	MSE	F	Pr > F
Model	1	0.011	0.011	0.010	0.923
Error	45	51.305	1.140		
Correct total	46	51.316			

The ANCOVA models confirmed the dependence of the “Surber-net” method on the sampling period ($R^2 = 0.777$) while the “Leaf-bags” method ($R^2 = 0.898$) was fundamentally insensitive to the period variable. The “Period X Biomass” interaction was significant for all four periods considered for the “Surber-net” method while it was not significant for the “Leaf-bags” method (Table 3).

Table 3. ANCOVA table with parameter values and significance of the method X sampling period interactions.

Source	Value	SE	t	Pr > t	Lower Limit (95%)	Upper Limit (95%)
Intercept	0.422	0.107	3.935	0	0.202	0.641
Body-size_Surber-net_Period 1	−0.472	0.174	−2.704	0.012	−0.829	−0.114
Body-size_Surber-net_Period 2	−1.497	0.132	−11.303	<0.0001	−1.769	−1.226

Body-size_Surber-net_Period 3	−0.19	0.188	−1.007	0.323	−0.576	0.196
Body-size_Surber-net_Period 4	−1.390	0.215	−6.448	<0.0001	−1.831	−0.948
Intercept	0.476	0.083	5.769	<0.0001	0.310	0.642
Body-size_Leaf-Bags_Period 1	0.166	0.175	0.953	0.346	−0.186	0.518
Body-size_Leaf-Bags_Period 2	0.197	0.101	1.947	0.058	−0.007	0.401
Body-size_Leaf-Bags_Period 3	0.292	0.111	2.639	0.011	0.069	0.515
Body-size_Leaf-Bags_Period 4	0	0	−	−	−	−

The one-way ANOVA confirmed the results shown by ANCOVA; the datasets resulting from the sampling period are in fact significantly different from each other for the “Surber-net” method and not for the “Leaf-bags” method, for which it was not possible to reject the null hypothesis of equality of the means (Table 4).

Table 4. ANOVA table relating to the methods considered (Surber-net and leaf-bags), divided according to the sampling periods.

	DF	Sum of Squares	Mean Square	F Value	Prob > F
Model Leaf-Bags	4	11.576	2.894	3.901	0.376
Error Leaf-Bags	1076	798.216	0.742		
Total Leaf-Bags	1080	809.793			
Model Surber-net	4	1.135	0.378	4.201	0.005 ***
Error Surber-net	5175	466.194	0.090		
Total Surber-net	5178	467.329			

Note: p value signification code: $0 < *** < 0.001$.

For each performed analysis (ANOVA and ANCOVA), the assumptions of heteroscedasticity and normality of the data were positively tested.

The results of all of the applied analyses confirm the differences observed between the two sampling methods. The “Leaf-bag” method allows one to take more abundant samples while the “Surber-net” method is more sensitive to the “period” variable (and likely any other accessory variable).

4. Discussions

A general principle of the benthic community structure can only be found if the similarity between assemblages can be unambiguously established from the sampling method. The patterns of the body size–abundance relationships across the two sampling methods showed that slopes were significantly steeper (i.e., −1.46 and −1.83 for leaf-bags and Surber-nets, respectively) than predicted by [4,5,36]).

Our patterns were consistent with several studies that suggested that the relationship between body size and abundance is much steeper than the value of −0.75 for aquatic macroinvertebrate communities in stream ecosystems (e.g., [16,37–39]). However, the steeper slopes were not consistent with other studies that have shown a shallower scaling exponent for aquatic benthic invertebrates communities [10,11,40,41]. Moreover, the steep slopes found were not consistent with the energetic equivalent rule (EER), providing evidence against the universality of EER. Thus, it shows that the amount of energy used is not the same for all species, with small species showing an increased proportion compared with the larger species [3].

In this respect, our study clearly shows that thoroughly collected stream communities with wide ranges of taxa and body sizes have inverse body size–abundance proportionalities. This may not arise from a decreasing number of larger species but from size-dependent physiological processes, such as metabolism. Because the EER predicts that body size–abundance relationships arise from metabolic scaling, environmental

factors may alter the size-dependent metabolic demands and the size–abundance relationships as a consequence [42]. Therefore, the shallower slope of LSDR in both freshwater channels implies a shallower slope of the metabolic scaling with the body size [1].

In our study, we demonstrated that the overall body size–abundance relationship in the two sampling methods (Leaf-bags *vs.* Surber-net) from April, May, June, and July showed different patterns with different slopes. The body size–abundance relationships of the assemblages sampled with leaf-bags showed similar slopes across all of the sampling periods (April: -1.43 , May: -1.60 , June: -1.37 , and July: -1.51). These slopes were always significantly steeper than predicted by Damuth [4,5,36].

Conversely, the slopes of the body size–abundance relationships of the Surber-net sampling method were across the following sampling periods: April: -1.08 , May: -1.40 , June: -0.80 , and July: -1.31 . The slope from April was isometric and close to -1 in value, deviating from that predicted by Damuth [4,5,36]. Furthermore, the slopes from May and July were even steeper than the -1 value, whereas the slope from June was consistent with the -0.75 value predicted by Damuth [4,5,36].

Taxa with wide ranges of body sizes do not show spatial–temporal persistence and are prone to larger-scale changes in habitat complexities. If body size differences are the causal bases for differences in resource uses, body size could be considered the major factor defining the niche of a species [43]. Once body size differences emerge in an ecosystem, they may reinforce differential resource uses between species and contribute to the long-term coexistence of species. The leaf-bags method actually seems to create the relative percentage of invertebrates colonizing leaf species that are similar, irrespective of the geographical origin of the leaves. However, Surber-net is able to sample invertebrates that colonize different leaf species from different leaf types. For this reason, it is possible that, when the species have similar nutritional qualities, such as being sampled with the leaf-bags method, the differences between taxa colonizing both of these leaves are minimal. Moreover, leaf-bags can have different nutritional qualities, depending on the leaves used; for this reason, a higher heterogeneity of the colonizing species should be observed. It is also true that many benthic taxa are food generalists that modify their diets according to food availability (e.g., [43–45]).

The steep slopes of the body size–abundance relationships we found represent the efficiencies of energy transfer from small and abundant individuals to fewer large predators [46], with clear implications for ecosystem functioning [47,48]. Shallow exponents (less negative) indicate the efficient transfer of energy (or higher availability of energy at the base of the food web) by supporting a relatively higher proportion of larger individuals, while steeper exponents (more negative) indicate inefficient energy transfer (or reduced energy availability at the base of the food web) with relatively fewer large individuals [46,49]. The sampling methods of Leaf-bag showed stable steep slopes across all months, while Surber-net showed shallower slopes across all months relative to the leaf-bags sampling method. This implies that Surber-net is able to capture a larger proportion of large-sized individuals in comparison with the Leaf-bags sampling method. Indeed, there was a reduction in the abundance of large-sized individuals across both sampling methods but even more for the Leaf-bags method. Therefore, as demonstrated in previous research, Leaf-bags and Surber-net should be used as complementary rather than alternative techniques since they collect integrated information on the benthic invertebrate communities and ecosystem functions [23,28,50,51]. Although in many cases the use of Surber-net leads to a greater collection of organisms, the Leaf-bags method has the advantage of being less invasive, especially in spatially limited freshwater ecosystems. However, for the analysis concerning body size–abundance relationships, it would be desirable to continue the experimentation using an implementation of the “classic” Leaf-bags method (*sensu* [52]). The new approach developed with the use of leaf-nets (LN) [24, 25 and 26], allows for quantitative data (i.e., density, not only the abundances of the sampled individuals) that are directly comparable with the densities of the organisms collected with Surber-net.

Furthermore, experimental studies on aquatic macroinvertebrates demonstrated that small organisms are the first to colonize new habitats, followed by medium- to large-sized species [53], which does not concur with the idea of species invasion around an ‘optimum body size’ [54]. Small organisms are able to rapidly track changes in a fluctuating environment, becoming abundant in short-lived niches [55] in almost benthic habitats. Taxa with wide ranges of body sizes do not show spatial–temporal persistence and are prone to larger-scale changes in habitat complexities. If body size differences are the causal bases for different uses of resources, in the same way, body size could be considered the major factor defining the niche of a species [43]. On the other hand, the substrate plays an important role in macroinvertebrate species [40], and in vegetation, it can retain higher organic matter [55], such as high densities of seagrass and macrophytes [56,57]. However, comparing microhabitats based on the substrate is not possible when sampling with the leaf-bags method. This is because the different macroinvertebrate species that inhabit these microhabitats are underrepresented in the leaf-pack technique, whereas they can be sampled by Surber-net.

In general, our results corroborate the fundamental importance of choosing the sampling method when undertaking a sampling action for a biological monitoring aim and scientific research purposes, confirming the plasticity of the allometric approach for ecological issues [58].

The negative linear relationship between body size and abundance of benthic macroinvertebrates is confirmed in our experiment and this relationship is much more marked than those found in other freshwater ecosystems [17].

In particular, the slope of the line representing the data collected with the Leaf-bags method is steeper than that of Surber-net, demonstrating that the Leaf-bags sampled a greater number of smaller taxa than Surber-net, which, in turn, collected more individuals of larger sizes. Differences in the community body size structures sampled with the two methods are evident, but because of the limited spatial–temporal scale of our experiment, the results are currently not generalizable.

Therefore, as demonstrated in previous research studies, leaf-bags and Surber-net should be used as complementary rather than alternative techniques since they collect integrated information on the benthic macroinvertebrate communities and ecosystem functions [23,28,50,51].

Although in many cases the use of a sampling method such as Surber-net leads to a quantitatively greater collection of organisms, Leaf-bags have the advantage of being less invasive, especially in spatially limited freshwater ecosystems. Another advantage of using leaf-bags involves simultaneously taking information from both the community structure and ecosystem processes (for example, the leaf litter breakdown).

This study shows a potential method to monitor the functional traits of the macroinvertebrate species underlying ecological processes across time, space, habitat type, and functional diversity. The use of body size–abundance relationships for describing the community structure and linking functional diversity with ecosystem functioning is well known. Therefore, developing a sampling technique that can approach better size–abundance relationships can improve our ability to make predictions on size-based community architectures. We may be able to predict how time, space, habitat type, and functional diversity changes may change aquatic ecosystems in the future. This of course will benefit research, as well as management policies of aquatic ecosystems for their conservation of biodiversity.

5. Conclusions

Our study corroborates the fundamental importance of choosing the sampling method when undertaking sampling actions for biological monitoring and scientific research purposes, confirming the plasticity of the allometric approach for ecological issues [58]. The negative linear relationship between body size and abundance of benthic macroinvertebrates is confirmed in our experiment; this relationship is much more marked

than those found in other freshwater ecosystems. In particular, the slope of the line representing the data collected with the Leaf-bags method is steeper than that of Surber-net, demonstrating that Leaf-bags sampled a greater number of smaller taxa than Surber-net, which, in turn, collected more individuals of larger sizes. Differences in the community body size structures sampled with the two methods are evident, but because of the limited spatial-temporal scale of our experiment, the results are currently not generalizable.

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