# Effects of incomplete sampling on macroinvertebrate secondary production estimates in a forested headwater stream 

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#### Abstract

Estimates of secondary production depend on the efficiency of sampling methods in capturing abundances and body lengths of the entire macroinvertebrate community. The efficiency of common sampling methods in fulfilling these criteria is poorly understood. We compared the effects of a Surber sampler ( $250 \mu \mathrm{~m}$ mesh size) and a Freeze corer in capturing abundance, biomass, and secondary production of macroinvertebrates in a forested headwater stream. We then examined how the use of nets with different mesh sizes could affect estimates of secondary production. Macroinvertebrate abundance was three times lower, and biomass was three times higher with the Surber than with the Freeze


[^0]corer. Neither method captured the entire length distribution, and incomplete sampling of body lengths and abundance resulted in underestimating total secondary production by $48 \%$ (Surber) and $49 \%$ (Freeze corer). We estimated that reducing the mesh size from 250 to $100 \mu \mathrm{~m}$ would reduce the underestimation of production from $\sim 48$ to $\sim 12 \%$ due to the inclusion of smaller individuals. Our results improve the efficiency of common sampling methods, allowing a reliable quantification of the role of macroinvertebrates in stream ecosystem functioning.

Keywords Macroinvertebrate • Secondary production • Benthos • Surber sampler • Freeze corer

## Introduction

The contribution of the metazoan community to stream energy fluxes can be quantified by secondary production, i.e., the generation of new heterotrophic biomass over time (Benke \& Huryn, 2010). The relative simplicity of the calculation combined with its large informative power contributed to its widespread use, from answering research questions on food web dynamics (Benke \& Huryn, 2010; Dolbeth et al., 2012; Cross et al., 2013) to assessing ecosystem response to environmental change and human stressors (Buffagni \& Comin, 2000; Chadwick \& Huryn, 2007; Brabender et al., 2016; Brauns et al., 2022). Over time, the method for calculating secondary
production has been further refined (e.g., CPI correction, optimization of sampling effort, estimation of uncertainty) to improve its accuracy and precision (Krueger \& Martin, 1980; Morin et al., 1987; Mothiversen \& Dall, 1989).

The accuracy of secondary production estimates depends on the reliability of biomass and abundance quantification, which, in turn, depends on how efficiently a sampling method captures the abundance and body length distribution of the entire macroinvertebrate community (Méthot et al., 2012).

Net-based methods (e.g., Hess and Surber samplers) collect samples from large areas but fail to collect small individuals when used with meshes that are too coarse (Hauer \& Resh, 2007). This leads to an underestimation of abundance and biomass (Gaufin et al., 1956; Waters, 1966), affecting secondary production estimates (Schmid-Araya et al., 2020).

The fraction of invertebrates not retained by the $500 \mu \mathrm{~m}$ mesh net but by the $40 \mu \mathrm{~m}$ mesh (Fenchel, 1978; Higgins \& Thiel, 1988) is termed "meiofauna." This includes "permanent meiofauna," i.e., individuals bearing the typical meiofaunal traits (such as nematodes, rotifers, tardigrades, harpacticoid copepods, and gastrotrichs), but also "temporary meiofauna," i.e., individuals that can potentially reach macrofaunal size (e.g., chironomids). Benke et al. (1984) found that the $600-\mu \mathrm{m}$ mesh size net missed the smallest organisms, which accounted for the largest proportion of total production. Waters (1979) suggested that incomplete sampling of the lower end of the body length spectrum would underestimate secondary production by $5-10 \%$. More recent studies confirmed this (Stead et al., 2005; Tod \& Schmid-Araya, 2009; Majdi et al., 2017) and showed that secondary production is underestimated by $5-50 \%$ when using a $500 \mu \mathrm{~m}$ mesh size because permanent and temporary meiofauna are not properly collected.

Coring techniques (e.g., PVC corers, Freeze corer (Stocker \& Williams, 1972)) do not require nets during the collection phase, thus, potentially capturing the entire community inhabiting the surface and subsurface sediment. Although they are more advantageous in collecting macroinvertebrates, the processing poses certain drawbacks. First, fixation and extraction may damage soft-body organisms (Balsamo et al., 2020); second, the number of replicates, and thus the area sampled, is limited by the time required to process the sample. The limited sample
replicates and the relatively small sampled area may lead to underestimating larger and rarer individuals.

Taken together, the choice of the sampling technique affects the composition, abundance, and secondary production of benthic samples. In this study, we aimed to assess the extent to which different sampling techniques affect the estimation of secondary production of benthic invertebrates in streams and to suggest improvements, when applicable. For this purpose, we sampled benthic macroinvertebrates and individuals in the temporal meiofauna size range in a forested headwater stream for one year using a Surber sampler and a Freeze corer. Then, we compared abundance, biomass, and body size distribution and quantified the effects of sampling techniques on benthic secondary production.

We hypothesized that the Surber sampler would underestimate seasonally fluctuating abundances due to its inefficiency in collecting the smallest individuals. In contrast, the Freeze corer would miss the larger and rarer individuals because of the smaller sampled area. As a result, the Freeze corer will underestimate diversity and biomass, but it will provide a more accurate estimation of secondary production by effectively capturing early instars of macroinvertebrates that fall into the size range of meiofauna. Finally, we expect to determine the optimal mesh size of the net required to collect the most productive body sizes and, consequently, determine the mesh size that should provide the most reliable secondary production estimates.

## Materials and methods

## Sampling

Macroinvertebrates were sampled bimonthly from August 2019 to June 2020 in a 300 m reach of a 2 nd order forested headwater stream in Germany (Drängetalbach, $51^{\circ} 48^{\prime} 21.02^{\prime \prime} \mathrm{N}, 10^{\circ} 43^{\prime} 51.82^{\prime \prime} \mathrm{E}$ ). The Drängetalbach is a forested, nutrient-poor stream with a bed dominated by coarse gravel and cobbles (Table 1, Online Resource 1).

Macroinvertebrate samples were collected using the Surber sampler and the Freeze corer (Fig. 1a, Online Resource 1) at five randomly located spots at each sampling event. The Surber was equipped with a $250 \mu \mathrm{~m}$ mesh net, and samples were collected by stirring the top 5 cm of the sediment from an area of 625
$\mathrm{cm}^{2}$. The collected material was repeatedly rinsed to separate organic and inorganic fractions. Subsequently, the organic fraction was preserved in $70 \%$ ethanol.

After collecting the Surber samples, we installed the tubes for the Freeze corer (tubes area: $12.6 \mathrm{~cm}^{2}$, UWITEC Freeze corer for sediment type 1, Fig. 1a, Online Resource 1) at a distance between 0.5 and 1.5 m from where the Surber samples were collected. We verified that flow conditions and substrate type were similar to where we sampled with the Surber sampler to retrieve similar communities using both methods. Tubes were installed at a sediment depth of 45 cm and left for at least 72 h to allow macroinvertebrate communities to recover from disturbance (Pugsley \& Hynes, 1983).

To minimize the contact between flowing water and sediment during the freezing process, we positioned a PVC "freezing plate" with a foam rubber rim (Fig. 1a and b Online Resource 1) on top of the sediment. After this, we injected at least 30 L of liquid nitrogen into the five tubes for approximately 45 min . In summer, when temperatures were higher, we increased the freezing time to 60 min . Cores (example in Fig. 1b, Online Resource 1) were extracted using a tripod and sliced into three segments ( 0 to $5 \mathrm{~cm}, 5$ to $15 \mathrm{~cm}, 15$ to 30 cm depth). Slices were stored at $-20^{\circ} \mathrm{C}$. Larger cobbles and pebbles were separated, weighed, and measured in situ for granulometric analysis. We collected 30 samples ( 6 sampling campaigns $\times 5$ spots) with the Freeze corer and 30 samples with the Surber. We focused on the first 5 cm of the sediment core to be comparable with the Surber. Temperature, pH , and conductivity were measured during each sampling (Multi 3630 IDS SET F, Xylem Analytics GmbH, Weilheim, Germany). Water samples were collected and filtered through $0.22 \mu \mathrm{~m}$ filters (Sartorius, Minisart Syringe Polycarbonate Filters) to determine nitrate $\left(\mathrm{NO}_{3}\right)$, nitrite $\left(\mathrm{NO}_{2}\right)$, ammonium $\left(\mathrm{NH}_{4}\right)$, reactive phosphorous (SRP), and dissolved organic carbon (DOC). Additional water samples were collected to determine chlorophyll-a (Chl-a) and dissolved oxygen ( $\mathrm{O}_{2}$ ) concentrations (Table 1, Online Resource 1).

## Sample processing

We extracted invertebrates collected with the Freeze corer from a subsample in the laboratory.

Subsampling large saturated sediment samples is challenging because of the heterogeneous distribution of pore space, which must be considered when upscaling abundance results (Huber \& Hauer, 2020). In addition, environmental factors such as water temperature, flow velocity, and substrate type influence the shape and volume of the retrieved cores (Huber \& Hauer, 2020) even if sampling effort (i.e., time, amount of liquid nitrogen) is kept constant. This impedes establishing a priori an amount of sample to process. Given these limitations, we adopted a hybrid approach based on the observation of Adkins (1997) and Omesová \& Helešic (2004) to process the subsamples and upscale the results.

Briefly, a pre-weighted sample was thawed, mixed, and washed with tap water through a nested column of sieves ( $2 \mathrm{~mm}, 1.12 \mathrm{~mm}$, and $20 \mu \mathrm{~m}$ ) to remove larger stones. The sample retained on the smallest sieve ( $20 \mu \mathrm{~m}$ ) was collected, and invertebrates were extracted with a flotation method (details are reported in Online Resource 1). Through this technique, we were able to extract also individuals from the permanent meiofauna fraction, which includes rotifers, nematodes, copepods, ostracods, cladocerans, tardigrades, mites, and gastrotrichs. However, as our objective was to compare the efficacy of the two sampling methods in capturing the "macroinvertebrate" fraction, we did not include them in our analysis.

After the extraction, the Freeze corer samples were oven dried at $60^{\circ} \mathrm{C}$ for up to 48 h , and the unprocessed part was sieved for granulometric analysis.

Individuals collected with the Freeze corer and Surber were sorted, counted, and identified to the lowest possible taxonomic level under a stereomicroscope (Leica S8AP0, Wetzlar, Germany) with up to 40 -times magnification. The first 30 individuals from each taxon and sample were measured for body length or head width (Trichoptera) to the nearest 0.1 mm . Dry mass (DM) was calculated using length-mass relationships from the literature (Table 1, Online Resource 2). We evaluated that body length fell within the length range used to establish the length-mass relationship initially. For Surber samples, $5 \%$ of all body lengths were above, and $13 \%$ below the initial length range. For Freeze corer samples, $1 \%$ of all body lengths were above, and $61 \%$ were below the initial length range. We also applied length-mass relationships to individuals smaller than those used to initially establish the relationship
because we assumed that this source of uncertainty would not affect our comparison, given that the bias is low for individuals falling below the initial length range (Xiao et al., 2011).

Finally, standardization was necessary to compare Freeze corer samples with varying initial volumes. We first calculated macroinvertebrate abundances for the entire sediment core by multiplying individual abundances in the subsample with a factor that accounted for the ratio between the subsample's porosity and that of the whole core. The porosity was calculated as the weight difference between the wet and dry sediment (Stocker \& Williams, 1972). The resulting abundances were then upscaled to the volume of the Surber samples ( $V_{\text {Surber }}=3125 \mathrm{~cm}^{3}$, height $=5 \mathrm{~cm}$, Area $=625 \mathrm{~cm}^{2}$ ), maintaining the original proportion between sediment and pore volume. Although Freeze corer and Surber samples were retrieved from a 3-dimensional space (sediment depth $=5 \mathrm{~cm}$ ), the results were expressed by surface area, i.e., $1 \mathrm{~m}^{-2}$.

## Calculation of secondary production

## Freeze corer and Surber

We calculated annual secondary production (g DM $\mathrm{m}^{-2}$ year ${ }^{-1}$ ) separately for the samples collected with the Freeze corer and the Surber. Our sampling was sufficiently replicated spatially but had a lower sampling frequency than other studies (e.g., Walther \& Whiles, 2011; Wallace et al., 2015). To test the potential bias of a bimonthly compared to a monthly sampling, we conducted an additional analysis on a published dataset obtained in a stream similar to the studied here (Wild, 2022) (Online Resource 1). We found that secondary production achieved with a bimonthly sampling was, on average, $17 \%$ higher than the production achieved with a monthly sampling (Fig. 3, Online Resource 1). However, overlapping confidence intervals showed that differences in sampling frequency were not significant. Hence, we are confident that the rather coarse sampling frequency did not affect our results.

We encountered taxa whose cohorts were either overlapping or indiscernible and applied the modified size-frequency method (Hynes and Coleman, 1968; Hamilton, 1969) and corrected for cohort production intervals (CPIs) (Benke, 1979). CPIs values
were extracted from the literature (Table 2, Online Resource 2). We quantified the biomass of taxa with an average annual abundance of at least 15 ind. $\mathrm{m}^{-2}$ and we estimated secondary production for taxa with an annual abundance of at least $60 \mathrm{ind} . \mathrm{m}^{-2}$. This was necessary to ensure sufficient individuals in each size class and obtain more reliable production estimates (Brabender et al., 2016). The selection reduced the species richness from 71 to 20 in the Surber samples and from 30 to 17 in the Freeze corer samples. However, taxa included in the analyses represented 94 and $81 \%$ of the total mean biomass for the Freeze corer and Surber sampler, respectively. We calculated the annual production for each taxon using a four-step procedure. In the first step, we calculated the body size distribution for each taxon by dividing the entire length distribution into 10 equal-binned size classes and assigning each individual to its corresponding size class. Subsequently, we calculated the individual densities per size class for each sample. In the second step, we sampled the five spots collected each day with replacement ( 1000 times) and then averaged size class-specific abundances (package boot, Canty \& Ripley, 2021). This resulted in six size-abundance vectors corresponding to the six sampling campaigns, which were then averaged to create a single annual size-abundance vector. In the third step, we calculated the difference in abundance from one size class to the next and multiplied it with the mean body mass between size classes. Before summing the products for each size class, each value was multiplied by 10 , i.e., the total number of size classes. Negative increments were included except when they appeared in the first size class (Benke \& Huryn, 2007). In the last step, we corrected production values for CPIs. For some taxa, there were multiple CPI estimates due to variation in their life cycle related to, e.g., temperature or ecoregion. Thus, to account for CPI and its variability, we generated a vector of 1000 values drawn from a uniform distribution between the minimum and maximum CPI available for each taxon. Then, we multiplied this vector with the corresponding uncorrected production to obtain mean CPIcorrected production and $95 \%$ percentile confidence intervals (95CI) (Cross et al., 2013).

We found high abundances of veliger larvae, which we assumed were early instars of Ancylus fluviatilis O.F. Müller, 1774. We found no published lengthmass relationships for its veliger larvae. Therefore,
we quantified individual body mass ( mg DW) by weighing all the undamaged individuals to the nearest 0.001 mg with a microbalance (ME5, Sartorius, Surrey, UK) after drying in the oven (for 24 h at $60^{\circ} \mathrm{C}$ ). We then calculated secondary production using a $\mathrm{P} / \mathrm{B}$ ratio of 2.64 (Streit, 1976).

## Combined sample

Given that the Surber may underestimate abundances of small-sized individuals and the Freeze corer may miss the larger individuals, we combined datasets obtained with both methods to serve as a reference for comparison of the sampling efficiency of the Surber and Freeze corer. In addition, we used the combined dataset to assess how varying mesh sizes impact secondary production. Specifically, we aimed to determine the degree to which changes in mesh size affect estimates of secondary production. Combining both datasets may lead to overestimating the abundances of the taxa ( 17 out of 25 taxa) collected with both methods. To minimize this potential bias, we inspected the body size distributions of each taxon obtained with both the Freeze corer and Surber sampler and identified overlaps between the two distributions.

Depending on the extent of overlap, various approaches were employed to reduce this potential bias (see Fig. 2 Decision tree to build the combined dataset, Online Resource 1). The methods were considered complementary if the 5th and 95th percentiles of the body length distributions of both methods did not overlap. Hence, all individuals captured with both techniques were incorporated into the combined dataset. Nevertheless, the body size distribution displayed partial overlap in most cases. In these cases, we assumed that the Freeze corer was better at collecting smaller individuals, while the Surber sampler was more efficient at capturing larger individuals. Therefore, we calculated the midpoint $(m)$ of the overlapping length segment (Fig. 2c, Online Resource 1) and included all the individuals whose body length was less than $m$ from the Freeze corer dataset and all the individuals whose body length was larger than $m$ from the Surber sampler dataset. For those taxa where body size distributions completely overlapped, we assumed that the method with the wider body size range was a more accurate representation of true body size distribution. Consequently, only measurements obtained with this method were included. Finally, we
calculated taxon-specific and total secondary production as described above.

## Mesh thresholds

We followed a three-step procedure to assess the effects of different mesh sizes on secondary production (Online Resource 3). First, we calculated the contribution of each size class of invertebrates to production. We then estimated how efficiently each size class is retained by nets with different mesh sizes. Finally, we integrated the results of the two previous steps to calculate the production that could have been obtained by using different mesh sizes.

Statistical analysis
We evaluated differences in macroinvertebrate abundance and biomass between Freeze corer and Surber and sampling campaigns using a 2 -way repeatedmeasures ANOVA ( 2 RM ANOVA). The data were checked for outliers with Grubbs' test, normality with Shapiro-Wilk's test, and sphericity using Mauchly's test, and $\log _{10}$ transformed if needed. We reported the $P$-value after the Greenhouse-Geisser correction when the sphericity assumption was unmet. We performed pairwise comparisons with Bonferroni's method to test for differences due to seasonality in each sampling method. All those tests were performed in Origin (OriginPro, Version 2022. OriginLab Corporation, Northampton, MA, USA).

To test for the main effect of the sampling method on compositional differences, we conducted a permutational ANOVA (PERMANOVA) with repeated measurements by using sampling campaign as a blocking factor (function adonis2). We ran a homogeneity of dispersion test (function betadisper) (Anderson, 2006) separately for each sampling campaign. We conducted ANOVAs to compare the mean distance-to-centroid of the samples collected with the two methods. The analysis was conducted on a Bray-Curtis similarity matrix generated from $\log 10(x+1)$ transformed abundance data. Taxa most associated with a given sampling method were identified using SIMPER analysis. Differences in community composition were visualized with Non-metric multidimensional scaling (NMDS) ordination. We compared macroinvertebrate length distributions obtained with the two sampling methods with


Fig. 1 NMDS ordination of macroinvertebrate communities collected with the Freeze corer (violet triangles) and the Surber sampler (orange dots)


Fig. 2 a Mean $( \pm$ SE) monthly abundance and $\mathbf{b}$ mean ( $\pm$ SE) monthly biomass obtained with the Freeze corer and the Surber sampler
the bootstrap Kolmogorov-Smirnov test (function: ks.boot, library: Matching (Sekhon, 2011)). Finally, to assess differences among collection methods
in estimating secondary production, we compared means and $95 \%$ confidence intervals. Means with non-overlapping confidence intervals were interpreted as significantly different (Cross et al., 2013; Brabender et al., 2016; Wild et al., 2022). All com-munity-level tests were performed in R ( R Core Team 2022, version 4.2.1) with the vegan and permute library (Oksanen et al., 2020).

## Results

Community structure
We collected 81 taxa belonging to 52 families and 10 major taxonomic groups (Table 2, Online Resource 4). Communities collected with the Freeze corer and Surber differed significantly (NMDS Fig. 1; PERMANOVA, $F_{1,59}=16.8$, $P<0.001$ ). The dispersion of the communities with the two sampling methods differed significantly in April and December ('betadisper', ANOVAapril, $F_{1,8}=13.62, P<0.001$, ANOVAdecember, $F_{1,8}=$ $6.46, P=0.034)$. Specifically, Surber communities in April were more variable than Freeze corer communities, and Freeze corer communities in December were more variable than those collected with the Surber. SIMPER analysis revealed that 5 dipterans, Esolus spp. (Coleoptera), and Ancylus fluviatilis adult (Gastropoda) contributed to 30\% of cumulative dissimilarity between the sampling methods. Mean monthly abundance differed significantly between sampling methods and was $3.5-$ fold higher in Freeze corer than Surber (Fig. 2a). There was no significant effect of season on the abundance in the samples collected with the Surber (Bonferroni Test, $P>0.05$ ). In contrast, significant seasonal differences in abundance occurred within the samples collected with the Freeze corer (Bonferroni Test, $P<0.05$ ). Mean biomass was threefold higher for Surber than Freeze corer samples (Fig. 2b). No significant differences were observed for biomass in the Surber and Freeze corer samples collected during the different sampling campaigns (Bonferroni Test, $P>0.05$ ). Individual body lengths ranged between $0.20-35.00 \mathrm{~mm}$ in the Surber and $0.08-12.02 \mathrm{~mm}$ in the Freeze corer (Fig. 3), and length distributions were significantly different (Kolmogorov-Smirnov test, $P<0.001$ ).


Fig. 3 Density plot of macroinvertebrate body lengths collected with the Freeze corer (orange) and the Surber Sampler (violet). Dash lines represent the means of the respective method

Secondary production
Mean annual secondary production calculated from Surber samples ( $6.88 \mathrm{~g} \mathrm{DM} \mathrm{m}^{-2}$ year $^{-1}$, 95CI [5.698.08], Fig. 4) did not significantly differ from that of Freeze corer samples ( 6.72 g DM m ${ }^{-2}$ year $^{-1}$, 95 CI [1.98-11.46], Fig. 4). Production calculated from Surber samples and Freeze corer samples was $52 \%$ and $51 \%$ of the entire production obtained with the combined approach (Surber + Freeze corer) (13.15 g DM m ${ }^{-2}$ year $^{-1}$, 95CI [7.83-18.48], Fig. 4). Thus, the Surber and Freeze corer underestimated the entire production by $48 \%$ and $49 \%$, respectively.

Differences between sampling methods occurred in the relative contribution (\%) of major taxonomic groups to total production (Table 3, Online Resource 4), i.e., Trichoptera, Ephemeroptera, and Coleoptera had a higher relative contribution in the Surber than in the Freeze corer and in the combined samples. The largest difference occurred for Trichoptera, whose relative contribution was 2 and sevenfold higher in the Surber than in the combined and the Freeze corer samples. Ephemeroptera's mean relative contribution to production was 2 and threefold higher in the Surber than in the combined and Freeze corer samples. In contrast, Coleoptera's relative contribution was twofold higher in the Surber than in the Freeze corer and combined samples. Gastropoda contributed $\sim 11 \%$ to the entire production in the Surber, while its larval stage (Veliger) contributed to $1 \%$ of the entire production estimated with the Freeze corer. Oligochaeta


Fig. 4 Mean contribution of the major taxonomic groups to annual secondary production obtained with the Freeze corer, the Surber sampler and by combining the two methods ("Combined")
contributed $\sim 4 \%$ to the entire Surber production and $\sim 2 \%$ in the combined approach, while it was absent in the Freeze corer.

Secondary production distribution among macroinvertebrate size classes and mesh efficiency in estimating production

The contribution of the different size classes to production obtained from the combined dataset showed a right-skewed distribution with the main peak occurring between 1 mm and 2 mm (Fig. 5). Individuals with body length $\leq 2.25 \mathrm{~mm}$ accounted for $50 \%$ of total production while individuals with body length $\leq 5 \mathrm{~mm}$ accounted for $79 \%$ of the total production (Fig. 5). The probability (unitless) of being retained by the $250 \mu \mathrm{~m}$ net was $\leq 0.3$ for individuals with a body length $\leq 2.25 \mathrm{~mm}$ (Fig. 2, Online Resource 3). It increased to 0.9 for individuals with a body length $\geq 4.25 \mathrm{~mm}$ and was higher than 0.95 for individuals with a body length $\geq 5 \mathrm{~mm}$. Reducing mesh size from 250 to $100 \mu \mathrm{~m}$ increased mesh efficiency by $36 \%$ (Table 1), underestimating $12 \%$ of the entire production. Increasing mesh size from 250 to $300 \mu \mathrm{~m}$ and $500 \mu \mathrm{~m}$ decreased mesh efficiency by 6 and $27 \%$, respectively, underestimating

Fig. 5 Contribution (\%) of macroinvertebrate body length classes to total macroinvertebrate secondary production


Table 1 Efficiency of different mesh sizes in estimating macroinvertebrate secondary production

| Mesh size ( $\mu \mathrm{m}$ ) | Delta (\%) | Mesh <br> efficiency <br> $(\%)$ | Underestimation <br> of production (\%) |
| :--- | :---: | :--- | :--- |
| 50 | +48 | 100 | 0 |
| 100 | +36 | 88 | 12 |
| 250 | 0 | 52 | 48 |
| 300 | -6 | 46 | 54 |
| 500 | -27 | 25 | 75 |

Delta (\%) is the relative increase ( + ) or decrease ( - ) in the efficiency compared to a $250 \mu \mathrm{~m}$ net. Mesh efficiency is the efficiency of different mesh sizes in capturing total macroinvertebrate production (\%)
total macroinvertebrate production by 54 and $75 \%$ (Table 1).

## Discussion

Estimates of macroinvertebrate secondary production require accurate quantification of biomass and abundance, which depends on the efficiency of the sampling methods in capturing the entire body length distribution. However, the efficiency of common
sampling methods in collecting the entire size distribution has rarely been quantified, and the effects of the sampling method on secondary production have been unclear.

## Surber vs. Freeze corer

Our study in a forested headwater stream shows that the Surber and Freeze corer collected different portions of the macroinvertebrate body length distribution, resulting in significantly different total abundances and biomasses. Quantitatively, secondary production values obtained by both methods were comparable, disproving the hypothesis that the Freeze corer is more efficient at estimating production due to its higher capture capacity for smaller invertebrates. Qualitatively, however, there was a significant difference in the performance of the two methods when considering the contribution of major groups to total macroinvertebrate production. The relative contributions of Trichoptera, Ephemeroptera, and Coleoptera were significantly higher in the Surber samples than in the Freeze corer ones, which were dominated by Diptera. One possible explanation for this difference is that Surber's larger sampling area covers a greater diversity of epibenthic habitats, capturing a higher abundance of

Trichoptera, Ephemeroptera, and Coleoptera. On the other hand, the smaller mesh size of the Freeze corer efficiently collected the small-bodied Chironomidae, a numerically dominant component of stream insect communities.

The peaks in abundance observed in summer, and early autumn with the Freeze corer confirm the hypothesis that this method is more effective in capturing early instar larvae of macroinvertebrates than the Surber. Large individuals ( $>12 \mathrm{~mm}$ ) were absent from Freeze corer samples, probably because of the limited sampling area, which prevented the collection of larger but rare individuals. This resulted in a body size distribution that was more right-skewed in the Freeze corer samples than in the Surber samples. The different body size ranges captured with the two methods explains why the biomass estimates from the Surber samples were larger than those from Freeze corer samples.

Community composition differed significantly between the two sampling techniques for two reasons. First, the level of taxonomic identification was lower in the Freeze corer community because Chironomidae are more difficult to identify than Ephemeroptera, Trichoptera, and Plecoptera, which were much better captured by the Surber. Second, damage to fragile or soft-body organisms like oligochaetes resulting from sampling processing may explain the lack of those taxa in samples collected with the Freeze corer (Traunspurger \& Majdi, 2017; Balsamo et al., 2020). Furthermore, the use of the Ludox solution to extract the invertebrates inhabiting the sediment may explain the presence of only the veliger forms of Gastropoda in the Freeze corer samples and the lack of adult specimens with shells that are too heavy to float in the $1.14 \mathrm{~g} \mathrm{ml}^{-1}$ solution.

Surber and Freeze corer underestimate secondary production

The Surber sampler equipped with the $250 \mu \mathrm{~m}$ net underestimated total production by $48 \%$ due to its inability to collect small but abundant individuals. Our mesh size efficiency analysis showed that by reducing the mesh size from 250 to $100 \mu \mathrm{~m}$, production estimates could be improved by $36 \%$. Yet, smaller mesh sizes would require more labor for sorting and identifying individuals. Thus, to ensure a manageable workload and capture most small individuals
contributing to production, we recommend taking an additional sample with a $100 \mu \mathrm{~m}$ net during the warmer months when early instar larvae are most abundant.

The Freeze corer did not collect the larger, rarer organisms because of the smaller sampled area compared to the Surber sampler, resulting in a $49 \%$ underestimate of total production. Unfortunately, the diameter of the core area is limited by practical issues (e.g., freezing time, amount of liquid nitrogen) and the collection of a higher number of replicates is also limited by the time-consuming and relatively high costs of the freeze coring procedure. Therefore, for an accurate representation of larger individuals, we recommend combining the Freeze corer with net-based sampling methods that cover a larger sampling area.

Production distribution among size classes
The body size-production distribution exhibited a right-skewed pattern in the combined sample, indicating that in streams with gravel beds, methods that fail to capture the lower end of the body size distribution may lead to an underestimation of production. However, how this affects production estimates in other types of streams ultimately depends on the body size distribution in those streams.

A careful examination of the height and the position of the abundance peak in the body size distribution can provide information about where production peaks are likely to occur. The higher the peak, the greater the likelihood that individuals in the corresponding size range will contribute substantially to production. Additionally, if the peak in abundance is among smaller organisms, it is more likely to correspond to a production peak due to the higher biomass turnover rates of small organisms in comparison to larger ones.

Body size distributions have been reported to be unimodal (Morin \& Nadon, 1991; Bourassa \& Morin, 1995; Navarrete \& Menge, 1997; Principe, 2008), bimodal (Poff et al., 1993) or trimodal (Stead et al., 2005) and differences in the distribution appear to be more influence by local conditions rather than by major evolutionary/latitudinal constraints (Navarrete \& Menge, 1997). Based on these findings, the body size distribution of macroinvertebrates exhibits peaks irrespective of stream type or latitude. Nevertheless, the position and the amount of the peaks in the size
spectrum are unknown and may vary based on local conditions.

Thus, when planning sampling strategies for secondary production, we recommend performing a preliminary analysis to characterize the potential body size distribution. Based on the location of the peak within the size spectrum, sieve retention probabilities models could be used (Morin et al., 2004; Gruenert et al., 2007) to determine the mesh size of the net best suited to capture the peak. We showed that $50 \mu \mathrm{~m}$ meshes are preferable in forested gravel-bed streams when body size peaks start at 0.5 mm , while a $100 \mu \mathrm{~m}$ mesh may be appropriate for a population with peaks around 1 mm . This way, the sampling strategy for estimating secondary production could be optimized for the particular system to maximize efficiency.

Integrating the temporary meiofauna into macroinvertebrate secondary production studies

Based on body size and taxonomic criteria, stream invertebrate production studies often distinguish three compartments: (1) macroinvertebrates, (2) temporary meiofauna, and (3) permanent meiofauna. Keeping a taxonomic criterion to distinguish between the permanent meiofauna and the macroinvertebrates may be relevant, but the boundary between temporary meiofauna and macroinvertebrates has poor support. The meiofauna-macrofauna distinction comes from marine sciences and is traditionally based on a body size criterion, i.e., individuals that pass through a $500-\mu \mathrm{m}$ mesh size net but retained on $40 \mu \mathrm{~m}$ meshes are considered meiofauna (Fenchel, 1978; Higgins \& Thiel, 1988). But this distinction also has a more solid ontogenetic background in marine systems. Meiofaunal invertebrates spend their whole life cycles in the benthic zone, while macroinvertebrates have mostly planktonic larvae. Hence, their younger larval instars are not found in the benthos and are not confounded with the marine meiofauna. Keeping a distinction between macroinvertebrates and temporary meiofauna in freshwater ecosystems may give the impression that "macroinvertebrates" and "temporary meiofauna" are distinct communities, but this is mostly not the case. Historically, this issue might have led to sampling strategies that did not sample both simultaneously. Calculating production on trunked cohorts affects production accuracy, especially if production is calculated using methods such
as the size frequency that relies on a correct representation of all size classes. Removing this distinction in future studies could reduce the error derived by calculating production on trunked cohorts and improve our understanding of the functional role of macroinvertebrates in energy fluxes.

## Conclusion

Integrating functional attributes of biological communities for understanding and effectively restoring freshwater ecosystems is becoming increasingly recognized (Palmer \& Ruhi, 2019). We showed that the choice of the sampling method profoundly affects the abundance and biomass estimates of macroinvertebrates, leading to biased estimates of secondary production and energy transfer in streams. We recommend mesh sizes of $100 \mu \mathrm{~m}$ during peaks of abundances or using sieve retention probability models to select the best mesh size as an optimized sampling strategy in streams. This will improve estimates of secondary production and lead to a better understanding of the functional role of macroinvertebrates in stream ecosystems.

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Data availability All data generated during this study are included in this published article (and in its supplementary information files).

Code availability R and Power Point were used for data presentation.

## Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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