



# A conceptual model for unbiased calculations of invertebrate abundances from freeze core samples

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**Abstract** Freeze coring is a commonly used method for the investigation of the bed sediment fauna of rivers. It is considered to produce quantitative numbers of invertebrate abundance in different depth layers. Calculations of abundance use total volume of the freeze core sample as spatial reference. This definition of sample volume is incorrect. In the present, study freeze core samples are shown to consist of two parts: (1) a cylindrical inner core in which the pore water has turned into ice and (2) all parts of sediment, which protrude from this inner core. Invertebrates are fixed only within the inner core since the protruding parts contain no pores and therefore no invertebrate habitat. In samples from gravel rivers, the

protruding parts form a considerable bias, which depends on the size of the core and the coarseness of the sediment. In the present study, total volume of individual core segments varied between 97 and 248% of actual sample volume. The inner core can be measured directly to avoid the bias. The procedure is proposed for future studies to produce unbiased, comparable values of invertebrate abundance and consequently reliable data on vertical distribution.

**Keywords** Vertical distribution of invertebrates · Quantitative sampling · Benthic zone · Hyporheic zone · Gravel bed rivers

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## Introduction

Freeze coring was introduced by Stocker & Williams (1972) to investigate the vertical distribution of invertebrates in stream beds. Their experiments with the new method, however, could not produce sufficient data about invertebrates, but they pointed out its potential. Stocker & Williams (1972) concluded although the method is not completely successful in its primary objective, it does lend itself to an accurate description of the vertical distribution of coarse sediments. The method was developed further and was mainly applied to gain volumetric samples of bed sediments (Walkotten, 1973; Carling & Reader,

1981). It became a routinely used method for this purpose (Bunte & Abt, 2001) despite some criticism that freeze coring does not sample different grain sizes proportionally and that large particles are not represented correctly because of comparatively small sample sizes (Kondolf & Lisle, 2016).

Moreover, the method was adopted as a means of collecting samples of stream invertebrates by Pugsley & Hynes (1983). The authors considered freeze coring as the only method to obtain truly quantitative samples of invertebrates from different depth layers of sediment. To optimise sampling efficiency, Bretschko & Klemens (1986) introduced the so-called electro-positioning—applying an electric field prior to sampling and thus immobilising invertebrates—to prevent flight reactions and gave a strict definition of sampling space.

Over the last decades, researchers applied freeze coring in a number of studies on the vertical distribution of benthic and hyporheic fauna. Vertical distributions were described for different river types, habitats and seasons (Marchant, 1988; Strommer & Smock, 1989; Maridet et al., 1992; Adkins & Winterbourn, 1999; Weigelhofer & Waringer, 2003; Varricchio et al., 2005). Reactions of the invertebrate fauna to disturbances were investigated (Olsen & Townsend, 2005; Sternecker et al., 2013). When Fraser & Williams (1997) compared results from different methods of hyporheic sampling, they used their results from freeze coring as a standard to evaluate the quantitative performance of other methods. One of the authors concluded in a later article (Williams, 2000) that in terms of removing an exact, representative portion of habitat (to obtain absolute measures) only the freeze corer is qualified. Moreover, if a larger sample volume, together with a description of invertebrates and the undisturbed sediments in which they live, is required, then the freeze corer (preceded by electro-positioning) would be the choice (Williams, 2000).

In principle, a sound definition of sampling space is the basis for the comparison between samples from different methods and different localities. Therefore, a quantitative sample has to be well defined in all three directions of space (Bretschko & Klemens, 1986). Pugsley & Hynes (1983) chose the diameters of their cores to calculate a reference area. Klemens (1983) measured total volume of each core section and divided it by its respective height to express

abundances as individuals per area. Bretschko & Klemens (1986) recommended total sample volume as spatial reference. Total volume was widely accepted by researchers as spatial reference and became a standard in freeze core analysis. Abundances were expressed as number of individuals per litre substrate (Maridet et al., 1992; Fraser & Williams, 1997), per 9 l substrate (Adkins & Winterbourn, 1999), per  $\text{dm}^3$  substrate (Scarsbrook & Halliday, 2002; Weigelhofer & Waringer, 2003; Olsen & Townsend, 2005). Moreover, some authors divided substrate volume by height of the core section following Klemens (1983) to express abundances as number of individuals per  $\text{m}^2$  (Winkelmann et al., 2003; Varricchio et al., 2005).

### Research question and research aims

The present study targets the criticism of Kondolf & Lisle (2016) that freeze core samples have a ragged edge and consequently their volume is not well defined in space (formulated for the calculation of grain size distributions) for the definition of sampling space for invertebrates. The use of total core volume for the current definition of reference volume is based on the assumption that the animals colonising the stream substrate occur throughout the entire sample and that invertebrate abundances from the entire sample can be considered representative for the river. The aim of this paper is to challenge this assumption and to show that consequently total sample volume is not an adequate spatial reference for quantitative samples of invertebrates.

Based on a systematic analysis of the freeze coring process, a conceptual model is introduced. The model is used to identify the relative error associated with total volume. Its validity is tested in the field. Field data do not include actual invertebrate densities from the sampling site. A comparison between total core volume and the actual volume, from which invertebrates are sampled, is provided to emphasise the relevance of the error. As freeze coring studies have been applied over decades without these considerations and have consequently produced imprecise or erroneous data on abundance and vertical distribution of invertebrates, the importance of this research is underlined.

## Methods

### Sampling procedure

To obtain a freeze core sample, a hollow metal pole is driven into the river bed sediments. After a chosen settling period sampling begins. One or more styro-foam insulators are attached to the submerged part of the pole with rubber bands. Insulators must contact the substrate to reduce the heating effect of streamflow or else the top 10 cm of the core may not be frozen successfully (Pugsley & Hynes, 1983). Liquid Nitrogen ( $-196^{\circ}\text{C}$ ) is then poured into the pole. Consequently, the water in the vicinity freezes and forms a mass of sediment, ice and invertebrates, which is extracted from the streambed after sufficient time for freezing. Freeze core samples are, if taken properly, columnar in shape. They are usually divided vertically into layers to investigate vertical distributions. Pugsley & Hynes (1983) use the diameters of the layers to calculate abundances in their study without elaborating on the measurements. In subsequent studies, diameter is replaced by total core volume.

### Conceptual model

The sampling process can be analysed in detail. It starts when liquid Nitrogen is poured into the hollow pole. As soon as the pole is cooled down by the Nitrogen, the pore water in the immediate vicinity freezes and forms an ice layer. This layer is very thin at the beginning. Its shape is that of a hollow cylinder around the pole. It grows regularly outwards as long as Nitrogen is added keeping its cylindrical shape to form an inner core of frozen water including sediment particles and invertebrates (Fig. 1).

When the sample is extracted from the streambed, this inner core containing ice and sediment is preserved and (additionally) all sediment parts protruding irregularly from the mantle of this inner core (Fig. 2). Animals are only sampled within the inner core (where they are fixed in ice) and lost from all protruding parts (from which they are washed off during extraction of the sample). Since the dimensions of the ice layer around the pole depend not only on freezing time but also on temperature and flow velocity of the surrounding water, its shape may deviate from cylindrical form, if for example depth layers have a significantly different hydraulic

conductivity since the warmer river water counteracts the freezing process. However, for horizontal cross sections at all given depths its outline is a smooth circle around the pole (Fig. 2, right).

Based on these observations, freeze core samples can be modelled in the following way: Each layer of a freeze core sample consists of two parts: (1) an inner core of ice and sediment with a cylindrical or near cylindrical shape and a smooth mantle. It constitutes the actual invertebrate sample. Its dimensions can be measured with relative ease and its volume can be calculated based on these measurements. And (2) the sediment parts protruding from this inner core. These are free of ice and therefore with no animals attached to them. For this reason, they form no part of the reference volume for the calculation of invertebrate abundance.

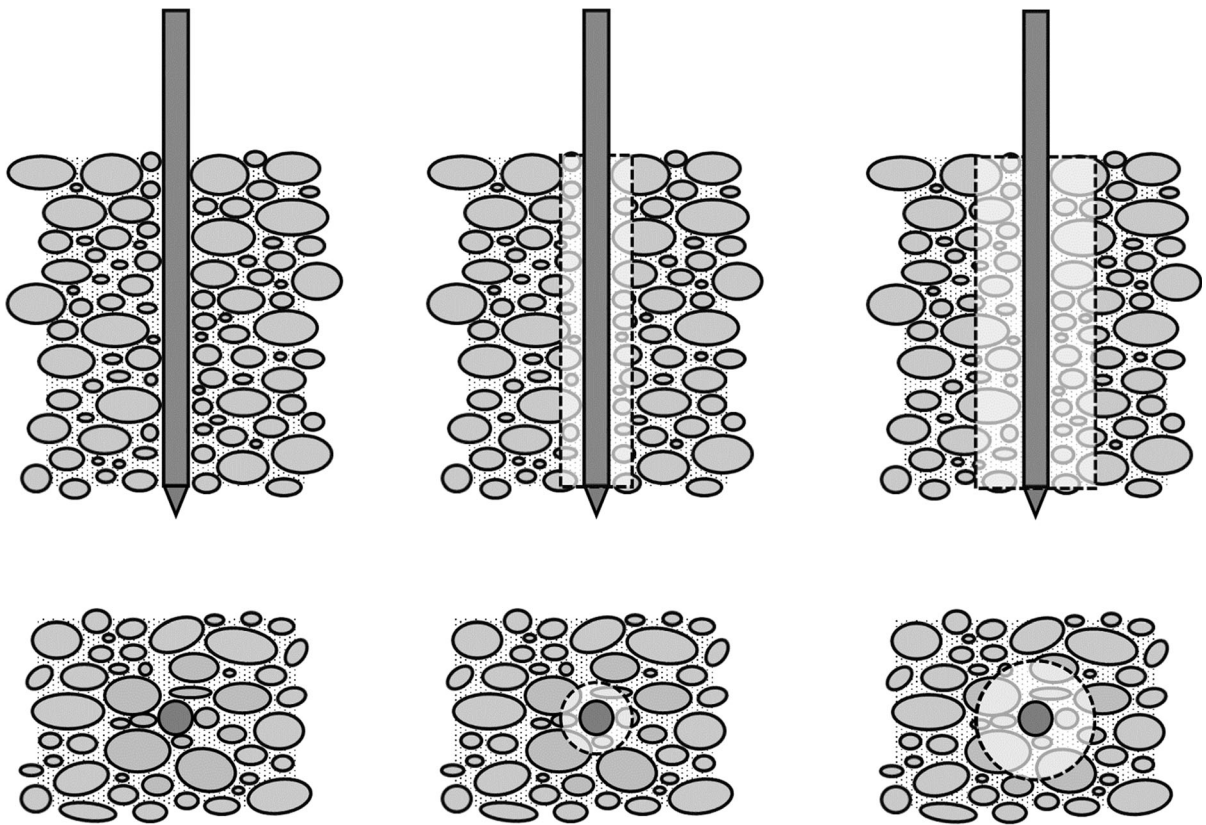
The model suggests a systematic error in estimations of invertebrate abundances (animals per volume) calculated from total volume and that improved estimations can be based on the volume of the inner core.

The basic assumption for the development of the model is that in the sampling process the inner core of ice (and sediment) grows continuously and regularly outwards as illustrated in Fig. 1. Provided that the assumption is correct, (1) parts protruding from a freeze core are free of ice and (2) a well-defined inner core with a circular outline is visible at all cross sections.

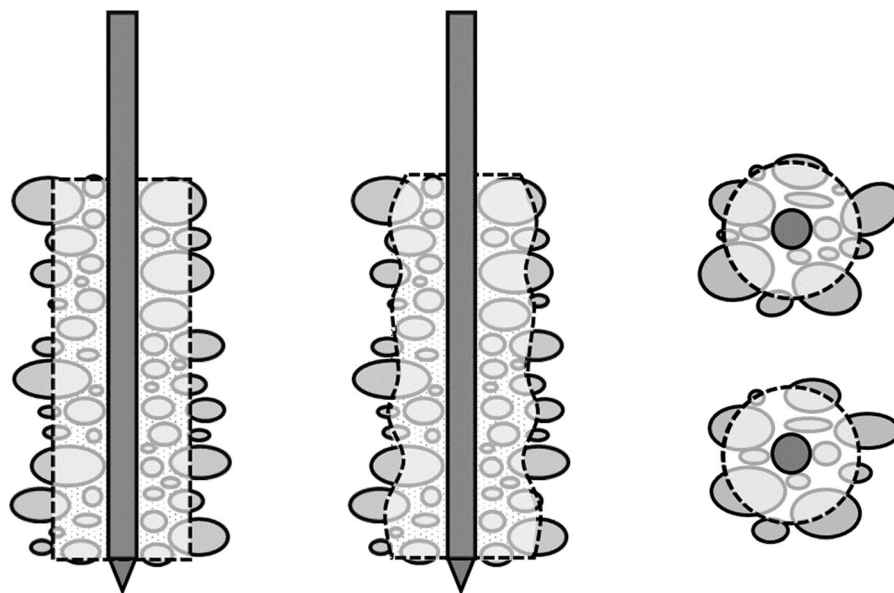
The model was tested with a field experiment in the Danube/Austria. The first aim was to verify the two above-mentioned points. The second aim was to examine its relevance by comparing values for sample volume predicted by the model to total volume.

### Field experiment

The field experiment was carried out on 23 November 2017 during a sampling session for the project PP BDA on the river Danube near Hainburg, approximately 25 km downstream of Vienna, Austria. Water temperature was about  $6.6^{\circ}\text{C}$ . Six freeze core samples were taken according to the method developed by Humpesch & Niederreiter (1993) for deep rivers (in its essence the method described by Pugsley & Hynes (1983) with the addition of necessary equipment for sampling in greater depth). The pole used for sampling had an outer diameter of 7 cm. It could be extended to



**Fig. 1** Schematic plot of freeze core sampling—left: the corer in the sediment; middle: the ice layer forms; right: the ice layer grows



**Fig. 2** Schematic cross sections of extracted samples—left: vertical cross section of a cylindrical core; middle: vertical cross section of a non-cylindrical core; right: two horizontal cross sections

the necessary length for deeper parts of the river (water depth was between 4 and 5 m) and was operated from a dredging vessel. The extracted freeze cores had a length of about 1 m. Total volume for individual cores lay between 33.17 dm<sup>3</sup> and 68.29 dm<sup>3</sup> (arithmetic mean was 50.57 dm<sup>3</sup>). They were put onto a table with a smooth surface and photographed. The surface of the freeze cores was examined for traces of ice and for animals clinging to it. Cores were divided vertically from top to bottom into nine layers of 10 cm and a bottom layer, which included all sediment deeper than 90 cm from the surface. Starting at the bottom, one layer after another was carefully removed from the pole. Before removing the layer, a picture of the vertical cross section was taken and the diameter of its inner core was measured with the help of a ruler. The measurement of diameter was repeated several times to note potential deviations from circular form of the inner core. Since the deepest layers were not well defined regarding length and the uppermost layers of the retrieved cores were not well preserved due to technical problems (loss of large particles because of insufficient insulation near the sediment surface), those were excluded from the analysis.

Still in the field, total mass of each layer was measured using digital scales. Samples were packed and taken to the lab. Dry mass was measured after samples had dried completely. Afterwards samples were sieved for grain size distributions.

Data on actual invertebrate densities could not be recorded, since the primary aim of the sampling session was to gain data on sediment and core shape.

#### Data analysis

Total volume was calculated as the volume of sediment (dry mass divided by the density of the sediment) plus the volume of water (total wet mass minus dry mass divided by the density of water) (Eq. 1):

$$V(\text{total}) = V(S) + V(W) = \frac{M(S)}{\rho(S)} + \frac{M(\text{total}) - M(S)}{\rho(W)}, \quad (1)$$

where  $V(S)$  is the volume of sediment,  $V(W)$  is the volume of water,  $M(S)$  is the mass of dry sediment,  $M(\text{total})$  is the total wet mass,  $\rho(S)$  is the density of the

sediment, and  $\rho(W)$  is the density of water. The values used for  $\rho(S)$  and  $\rho(W)$  were 2.65 and 1, respectively.

To calculate the volume of the inner core of ice and sediment, its near cylindrical shape was transformed into a perfect cylinder of a mean radius. This mean radius was calculated as the arithmetic mean of the radius measured at the top of the layer and that at the bottom of the layer. The volume of the pole was subtracted from the volume of this cylinder (Eq. 2):

$$V(\text{core}) = r(\text{mean})^2 * \pi * h - r(p)^2 * \pi * h, \quad (2)$$

and alternatively the inner core was transformed into a truncated cone and the volume was calculated as the volume of the truncated cone minus the volume of the pole (Eq. 3):

$$V(\text{core}) = \frac{1}{3} * (r(1)^2 + r(1) * r(2) + r(2)^2) * \pi * h - r(p)^2 * \pi * h, \quad (3)$$

where  $r(1)$  is the radius at the bottom;  $r(2)$  is the radius at the top (2nd layer);  $r(\text{mean}) = (r(1) + r(2))/2$  is the mean radius;  $r(p)$  is the radius of the pole;  $h$  is the height

Volume of the inner core of ice and sediment was compared with respective total volume for each layer. The differences were tested for (statistical) significance using a paired  $t$  test and a Wilcoxon signed-rank test.

A relative error was calculated as the ratio of total volume to volume of the inner core. It was the factor by which the inner core—the correct spatial reference according to the conceptual model—was overestimated by the traditional method of using total volume.

## Results

### Verification of the model

The examination of the six freeze cores from the river Danube showed that the protruding parts of sediment were indeed free of ice. The close up (Fig. 3) shows the protruding parts to be free of ice. No animals were clinging to the protruding parts. They were entirely free of invertebrates.

The outline of the inner core was a relatively smooth circle at all horizontal cross sections (Fig. 4), as predicted by the model. Diameters could be





**Fig. 3** Freeze core sample—close up

measured with 5-mm precision. Differences between parallel measurements of diameter were in all cases within the limits of the chosen resolution of 5 mm and could therefore be ignored.

#### Size of the freeze cores

The freeze core samples contained coarse sediment to a large extent. The largest particle of the individual cores had a diameter of the  $b$ -axis ( $D_{\max}$ ) of between 90 and 108 mm. Representative diameters varied considerably between layers in some cases (Fig. 5).  $D_{90}$  of individual layers varied between 31 and 104 mm and  $D_{50}$  between 11.8 mm and 64 mm. Due to the low water temperatures during sampling, large freeze cores could be obtained. Diameters of the inner core were between 16 and 28 cm (Table 1).

The differences in diameter of the inner core between the bottom and the top of each layer were generally small. They were mostly less than 5% of the total diameter and exceeded 10% only on one occasion (Table 1). Volume of the inner core was calculated for each layer using the formula for a cylinder as well as that for a truncated cone. Differences between the values obtained from the two formulas were negligible. They were within the range of millilitres and did not affect the values in the given resolution.

#### Differences between volume of the inner core and total volume

Volume of the inner core varied little between neighbouring layers, since the edges of the inner core



**Fig. 4** Freeze core sample—cross section. The interrupted lines show the extent of the sampling space

were smooth without abrupt changes in diameter in all obtained freeze cores. Total volume on the other hand showed abrupt changes in some cases (Fig. 6). Relative errors in individual freeze core segments, expressed as the factor by which sampling volume was overestimated and consequently invertebrate abundances underestimated, ranged from 0.97—practically no error, even slightly underestimated volume—to 2.48—overestimated by nearly two and a half. Mean values for individual freeze cores ranged from 1.76 to 1.27 (Fig. 7).

The differences between volumes of the inner core and total volume were significant in all individual cores ( $P$  values  $< 0.05$  for the paired  $t$  test and also for the more conservative Wilcoxon signed-rank test).

## Discussion

Total core volume is not an adequate estimate for sampling volume. It is not well defined in all three directions of space as claimed by Bretschko & Klemens (1986). The irregular shape of a freeze core with ragged edges does not represent a well-defined section of the streambed. More importantly, it contains volume (all protruding parts of sediment), from which invertebrates are not sampled. Based on the present conceptual model sampling, space can be defined much more accurately as the volume of the inner core of ice and sediment. The validity of the model was tested successfully in the field. Field data show that the use of total volume adds a significant bias to reference volume, introduced by the volume of protruding parts of sediment. This bias can be expressed as a relative

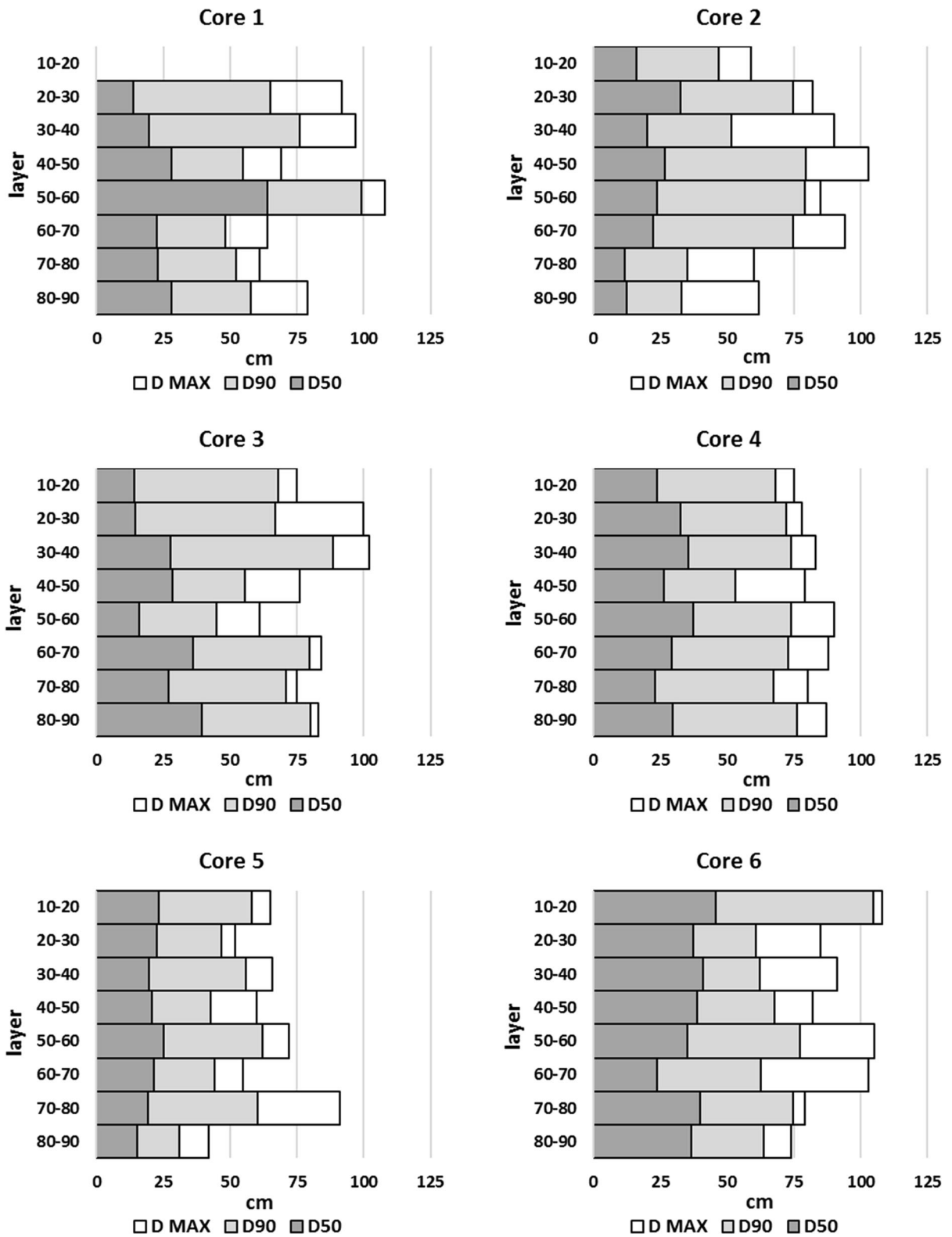


Fig. 5 Sediment composition of the vertical layers from the different cores ( $n = 6$ )

**Table 1** Diameters of the inner core (values in cm)

Depth	Core 1	Core 2	Core 3	Core 4	Core 5	Core 6
10		22	27.5	25	28	20
20	22	23	27.5	25	28	20
30	20.5	24.5	28	24.5	28	20
40	19	25	27	24	28	20
50	17	25.5	26	22.5	27.5	20
60	16	24	24	22	27.5	20
70	16	23	24.5	22	28	19
80	16	20	25	22	28	17.5
90	16	19.5	23	20.5	26	17

error—the factor, by which the actual volume of the invertebrate sample is overestimated.

#### Sources of the bias

The magnitude of the relative errors observed in the study depends on four factors.

The grain size of the sampled sediment (1), because in coarser sediment protruding parts are potentially larger and the magnitude of the inner core (2), since in larger cores the mass of protruding particles can be expected to be smaller relative to the inner core.

The sampled sediment from the river Danube was relatively coarse with maximum grain sizes of more than 10 cm. As suggested by the conceptual model, the bias introduced by the added sediment volume was considerable.

Because of the low water temperatures during sampling, relatively large freeze cores were obtained. Diameters of the inner core exclusive of protruding particles ranged from 16 to 28 cm. Total volume of individual 10 cm sections ( $n = 47$ ) ranged from 2.07 to 9.77 dm<sup>3</sup>. Sizes of freeze cores reported from different authors were generally much smaller: 10 to 15 cm in diameter (Pugsley & Hynes, 1983), 0.15 dm<sup>3</sup> to 0.6 dm<sup>3</sup> (Adkins & Winterbourn, 1999) or 0.01 dm<sup>3</sup> to 0.59 dm<sup>3</sup> for 15-cm segments (Scarsbrook & Halliday, 2002). Since the magnitude of the bias is inversely correlated with sample size, much larger relative errors than those documented in this study can be expected from smaller samples.

The two factors can be combined by expressing them as the ratio of the coarsest sediment relative to the size of the core.  $D_{90}$  was chosen as an indicator of

maximum coarseness divided by the diameter of the core (Fig. 8). The coefficient of determination between this factor and relative error in the sampled segments was 0.35 and 0.18 when two extreme points were treated as outliers. This indicates that about 18 to 35% of the variability in observed relative errors could be explained by coarseness of sediment relative to the size of the core. The remaining variability can be attributed to random factors.

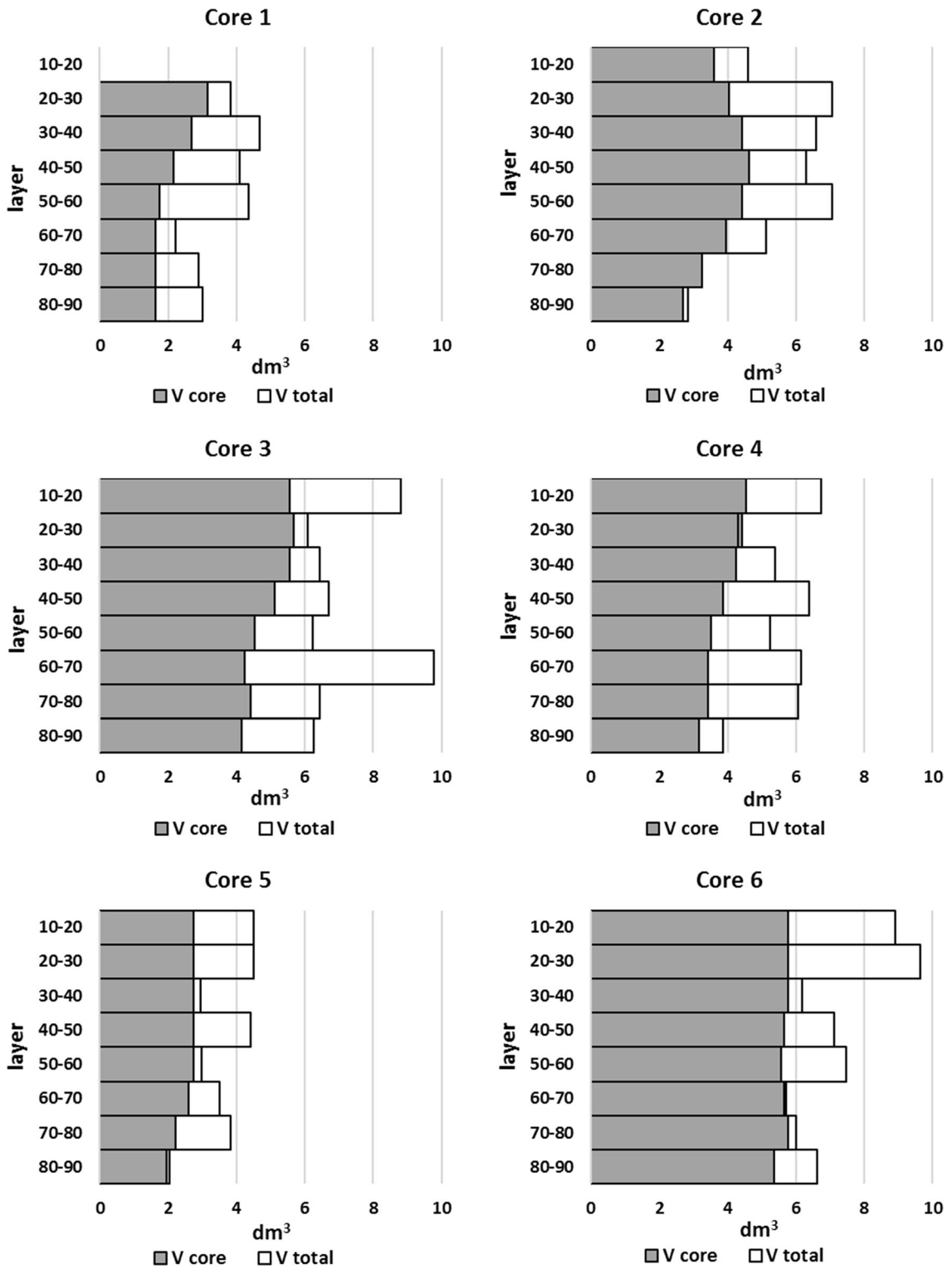
A random factor (3) depends on how much of the coarse sediment is protruding from the inner core. If the largest part of coarse particles is contained within the inner core, the bias will remain relatively low. If large parts of those particles protrude, the bias will increase.

Added randomness (4) is introduced by the fact that vertical borders between segments are not smooth. If the border runs through a large stone, this stone will go either to the layer above or below the border adding to the total volume of the one and reducing that of the other layer without having much impact on the actual sample volume because the ice of the pores remains in its own layer. This does not influence the bias directly but produces a non-directional error. A larger sediment particle, which lies on the border between two layers, will go to the layer, in which its larger part lies, leaving a hole in the other layer. The losses of volume for a layer caused by those holes are compensated by the gains caused by the protruding parts of those stones, which remain in that layer. Since gains and losses do not necessarily add up to zero, an additional error is introduced. Unlike the bias of overestimating sample volume introduced by the other factors, this error is non-directional. It can either add to this bias or reduce it. In one layer of the study (20–30 mm of core 4) the bias was even overcompensated resulting in an estimation for total volume, which was slightly lower than actual sample volume.

#### Generalisation

The conceptual model, validated in the study, allows some general inferences. The use of total volume biases the calculation of abundances. The bias is correlated with the grain size of the sampled sediment and the magnitude of the ice/sediment core. It may be negligible in large cores extracted from fine sediment deposits, but is considerable in cores from coarse





**Fig. 6** Volume of the inner core and total volume of the vertical layers from the different cores ( $n = 6$ )

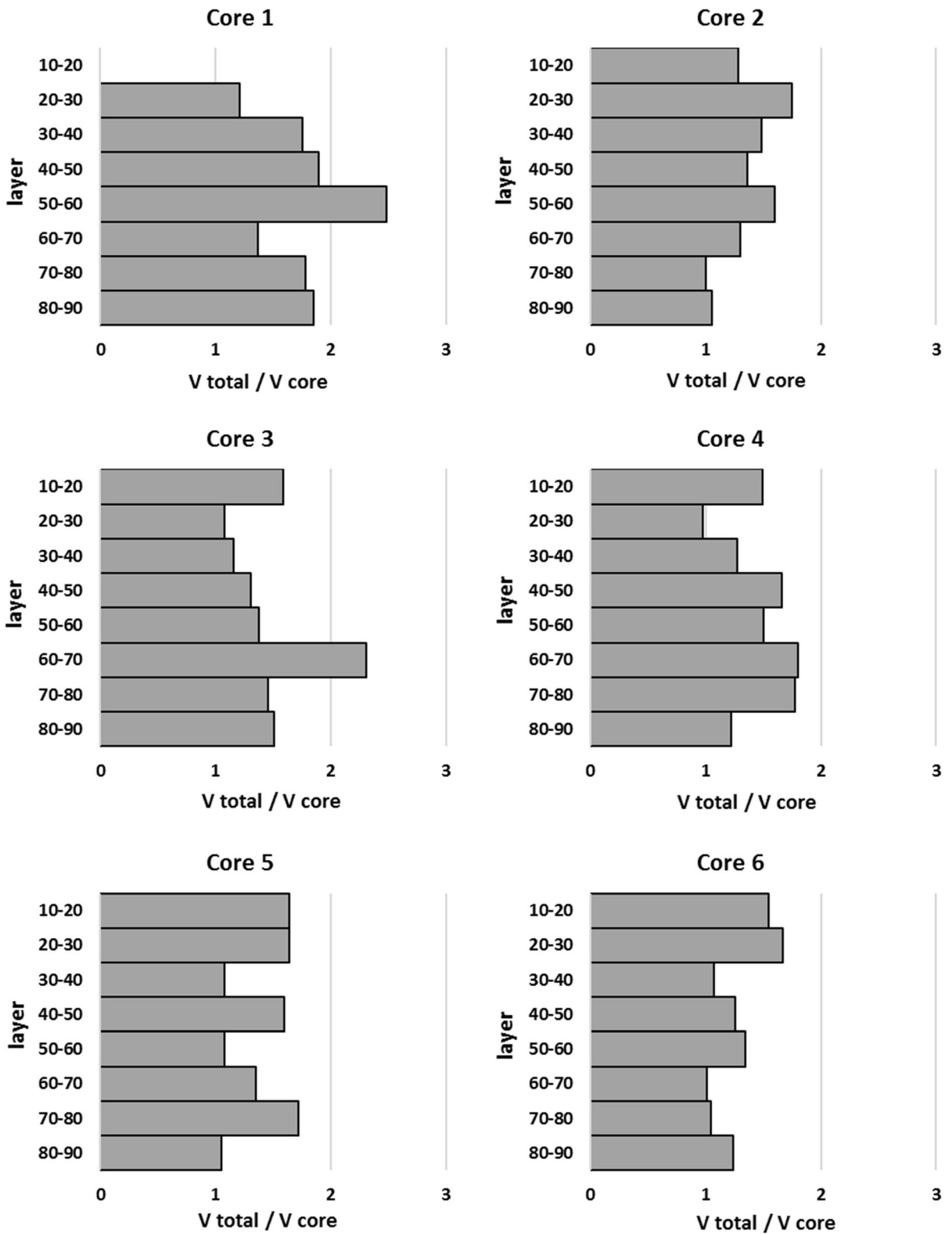
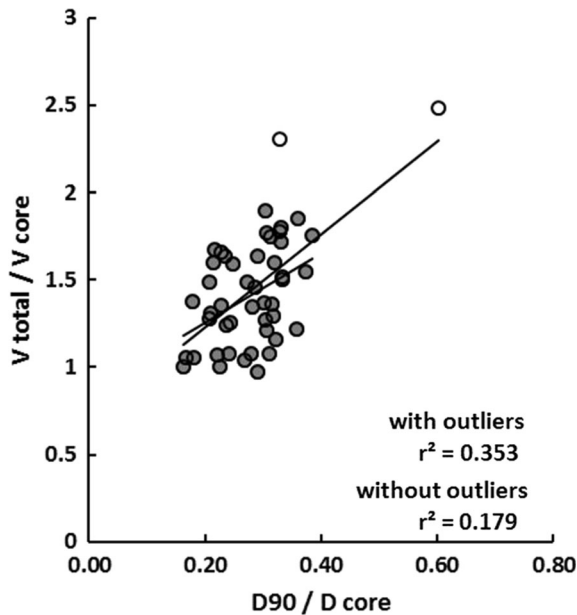


Fig. 7 Relative errors from the vertical layers of the different cores ( $n = 6$ )



**Fig. 8** Coarseness of sediment in relation to magnitude of the core and relative error produced by using total volume (outlier points in white)

gravel and cobbles where large particles are protruding at the sides (Fig. 9).

In such streams and rivers, invertebrate abundances calculated from total core volume are systematically underestimated. Moreover, those calculated abundances must not be compared quantitatively between different layers of one core, because their respective biases depend not only on sample size and particle size but also on the described random factors, which cannot be controlled for and therefore show a considerable variation (Fig. 10).

The proposed conceptual model can explain results from previous studies. Stocker & Williams (1972) reported invertebrate abundances from freeze coring (calculated using total volume as reference) to be significantly lower than those from two other methods and attributed the fact to flight reactions. The differences were so marked that they did not present their invertebrate data. Underestimated abundances by freeze coring were documented by Marchant (1988). Mean abundances from freeze cores at three different sites were only 27% to 39% of the respective abundances from parallel surber samples. The difference was least pronounced (39%) at the site, where the largest cores had been obtained. This observation is in accordance with the fact that larger cores tend to have

smaller biases. Similar ratios between freeze cores and surber samples were reported by Adkins & Winterbourn (1999) for different taxonomic groups.

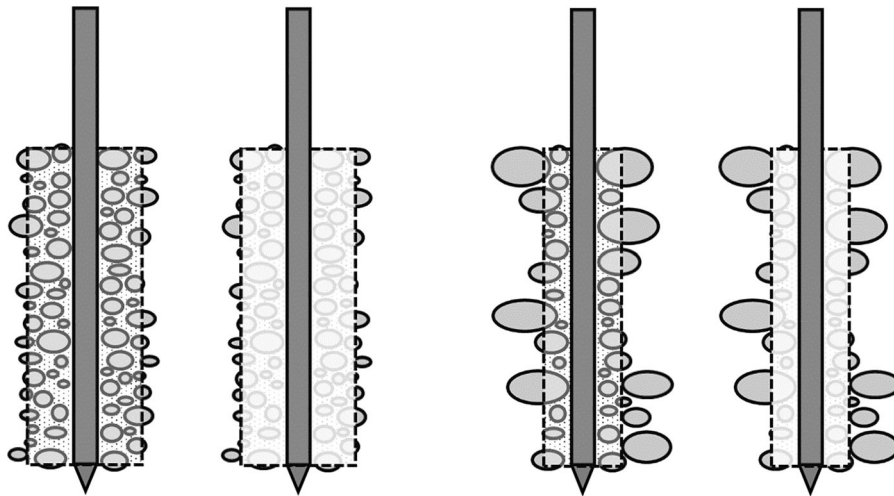
A method to correct for the observed underestimation of abundances especially in coarse sediments was used by Omesová & Helešic (2004). They subtracted particles larger than 70 mm from total volume, because “the occurrence of a boulder in the sample does not always reflect the situation in the streambed, being much more a result of randomness”. This approach may lead to more plausible values in some cases but it is not a ‘clean solution’. The unknown bias of the volume of all protruding parts of particles cannot be corrected by the combined volume of all large particles, a value, which is—as demonstrated in the present study—only weakly correlated with it. The approach only shifts the problem. The exclusion of large particles is likely to overcompensate for the volume of protruding parts in coarse sediments, leading to underestimated sample volume.

Using total volume leads to biased calculations of abundance and it is generally impossible to assess the magnitude of this bias in freeze core samples and in their individual depth layers. Observed vertical distributions described in the published literature as percentages of the total numbers in the core may be reasonably accurate if the coarseness of the investigated sediment is similar between depth layers. If the sediment is significantly different between layers, percentages from coarser layers, especially from the top layer of consolidated sediments, are likely to be underestimated.

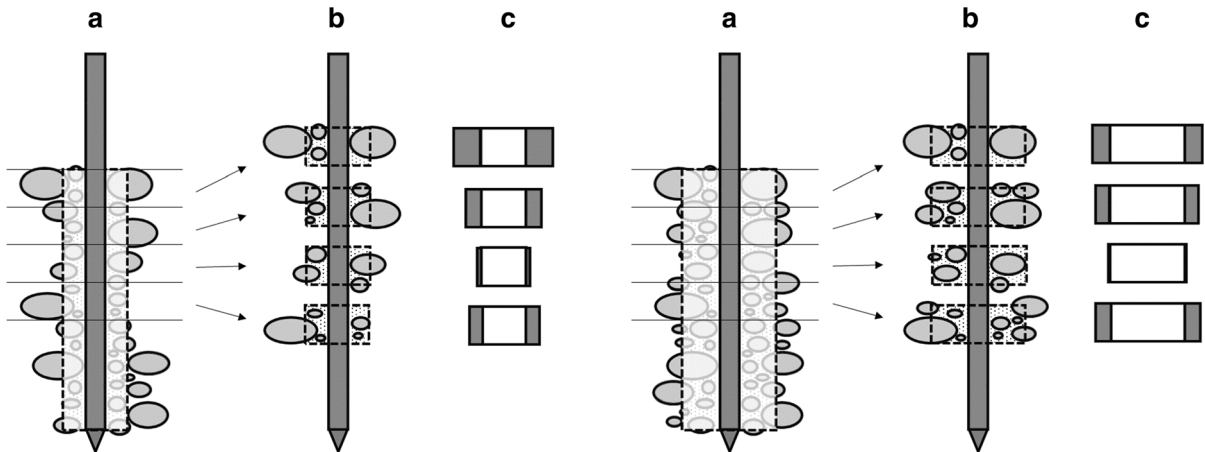
The cores of the present study are examples of large cores from coarse substrate, the situation, which is sketched on the right side of Fig. 10. The observed errors as well as their variation are consequently relatively small. When smaller cores of similar sediments are obtained, larger errors and a higher variation between layers can be expected, leading to potentially distorted pictures of vertical distribution. In future studies, the magnitude of relative errors as well as the distortion of vertical distribution can be assessed by comparing uncorrected values with corrected ones.

#### Avoiding the bias/practical application

The obvious procedure for estimating the real sample volume is to measure it directly. This has been done in



**Fig. 9** Cores and their biases (schematic)—left: a large core from fine sediment; right: a small core from coarse sediment



**Fig. 10** Different biases of a smaller core (left) and a larger core (right) from the same substrate—**a** the sample, **b** four sections separated, **c** sample space (white) and bias (grey) of different sections

the present study successfully by examining the freeze core sample visually and measuring the diameter of its ice/sediment core with the help of ruler at the bottom and at the top of each layer. The measurements with a ruler are straightforward and take little time. In order to minimise measurement errors, it is recommended to take several measurements to come up with a meaningful representative diameter. The volume of each layer is approximated as that of a truncated cone.

It is possible that in some cases animals (for example river limpets) are not washed off when the sample is retrieved and may cling to the protruding particles although they are not fixed in ice. They must

be collected and excluded from the quantitative sample, since they are not sampled from the reference volume.

## Conclusion

The common practice of using total core volume as spatial reference in freeze coring adds a systematic error to estimations of invertebrate abundance. This systematic error depends on sample size as well as on grain size. It is considerable in coarse sediments and especially large when cobbles are attached to thin ice

cores. Since the systematic error varies considerably between depth layers of an individual sample, data on vertical distributions of invertebrates are also prone to bias.

The present model can serve as a sound basis for future investigations. Measuring the diameter of the ice/sediment core of the sample directly avoids the systematic error. It leaves only the random error associated with the measurement. This random error is small and can be minimised by taking several measurements of diameter to describe the shape of the core. Unlike the bias associated with total volume, the random error does not affect mean values of parallel samples. Abundances calculated in this way can then be used to gain unbiased information on vertical distributions and total abundances.

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