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A common error in calculating density of invertebrates in sediment core sections of unequal lengths

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Abstract – In benthic studies, an absolute measure of invertebrate density with depth in the sediment is desirable. It can be expressed as invertebrates per unit of sediment volume or invertebrates per unit of sediment surface area. Calculations are often distorted, however, by whether the core is divided into unequal or equal segments. This is demonstrated in a study conducted in the Vistula Lagoon (South Baltic Sea). We collected 30 cores, and performed an analysis of distribution of invertebrates along the sediment profiles divided into sections of different lengths, or of equal lengths. In both cases, animal density/biomass was calculated both per unit area (m^2) and per unit volume (L). When cores are divided into equal sections, various ways of data computing do not affect the final results. If, for any reason, the cores are divided into sections varying in length, the only way to obtain an accurate measure of invertebrate distribution in the sediment profile is to express the data per unit volume. In many studies, calculations have been made per unit area using unequal segments, and these calculations are erroneous. The same error is made if density is expressed as the percentage contribution of the numbers/biomass or number of specimens recorded in particular unequal sections.

Key words: Benthic invertebrates / sediments / vertical distribution / methods / bioturbation

Introduction

Burrowing and mixing of sediment (bioturbation) by benthic invertebrates alters its physical and chemical properties. It changes the flux of solutes and dissolved gases, and modifies the concentration and distribution of sediment particles (Krantzberg, 1985; Svensson and Leonardson, 1996; Mermillod-Blondin, 2011). It also stimulates microbial activity, and increases decomposition of organic matter (Hansen *et al.*, 1998; Gingras *et al.*, 2007). Hölker *et al.* (2015) reviewed the literature and showed that the activities of tube-dwelling invertebrates may have considerable effects on the functioning of entire lake ecosystems and particularly shallow ones.

It is widely believed that deep occurrence of benthic fauna in sediments is a result of the trade-off between protection from predators and quality of the environment and food. Burrowing requires energy and the deeper sediments are anoxic and possibly less nutritious, but less accessible to predators (Baker and Ball, 1995; Hölker and Stief, 2005; Persson and Svensson, 2006). Burrowing depth can also depend on the traits of particular benthic species and on ontogeny (Urban-Malinga *et al.*, 2013, 2014; Majdi *et al.*, 2014).

Insights into the distribution of invertebrates in sediments and their density in particular sediment sections are very important in the determination of the role of fauna in the functioning of the water/sediment interface. They also provide essential information concerning for example the abundance of live food for fish, or production of trace fossils for palaeolimnological investigations. Therefore, ways of calculating density distributions are particularly important.

There are several ways of expressing the density of benthos in sediments:

- number of individuals, which in fact is not density but abundance, in sampled sections of the same length (Efford, 1960; Ford, 1962; Dauer *et al.*, 1987; Olafsson, 1992; Soumille and Thiery, 1997);
- relative (percentage) density in sampled sections (Takacs and Tokeshi, 1994; Soumille and Thiery, 1997; Rae, 2013), with some not always of equal length (Shiozawa and Barnes, 1977; Dauer *et al.*, 1987; Van de Bund and Groenendijk, 1994);
- per unit surface area of bottom in sampling sections of the same length (Schiemer *et al.*, 1969;

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Milbrink, 1973; Beckett *et al.*, 1992; Soumille and Thiery, 1997);

- per unit surface area of bottom in samples of unequal length (Kajak and Dusoge, 1971; Nalepa and Robertson, 1981; Newrkla and Wijegoonawardana, 1987; Kornijów, 1997; Martin *et al.*, 1999, 2005; Narita, 2006; Persson and Svensson, 2006; Cardoso *et al.*, 2010; Urban-Malinga *et al.*, 2014);
- per unit volume of sediment in sampling sections of unequal or equal lengths (Heuschele, 1982; Strayer, 1985; Strommer and Smock, 1989; Mcelravy and Resh, 1991; Maridet *et al.*, 1992; Winkelmann *et al.*, 2003; Kornijów and Pawlikowski, 2015).

Expressing benthos density in such different ways makes it difficult, or even impossible to compare results obtained by different authors. More importantly, however, when selecting ways of calculating density, authors do not always take into account whether the cores are divided into sections of equal or unequal lengths, and if the volumes of the sections are comparable. In this paper, we demonstrate how failure to do this can affect the results obtained. We analysed the distribution of invertebrates in sediment profiles divided into sections of both different and equal lengths. In both cases, animal density was calculated both per unit area (m^2) and per unit volume (L).

Methods

The Vistula Lagoon is a brackish (salinity: 1–5 PSU) water body separated from the Baltic Sea by the Vistula Spit (54°16′N, 20°24′E). Its surface area is 838 km^2, mean depth 2.6 m and maximum depth 5.1 m. Considerable exchange of water occurs through the Strait of Baltiysk (Chubarenko and Margoński, 2008). The lagoon is strongly eutrophic. Total nitrogen concentrations in the water column vary between 1.65 and 2.31 $mg\,L^{-1}$; total phosphorus ranges from 0.089 to 0.114 $mg\,L^{-1}$, and Secchi disc transparency amounts to 39–50 cm (Kornijów and Pawlikowski, 2015). Blooms of cyanobacteria are common (Nawrocka and Kobos, 2011).

Samples were collected in April, July and October 2010 at two sampling sites located in the western and middle parts of the deep-water area at a depth of approximately 2 m. The sediments were soft, and consisted of pelitic silts. From three to five cores (28 cm^2 area each) approximately 30 cm in length were collected on each occasion by means of a UWITEC Co. (Austria) gravity corer. Each sample was immediately extruded and divided into seven sections by means of a slicer equipped with a sub-sample holder (Kornijów, 2013). The upper section was 2 cm in length, the second 3 cm and the last five were each 5 cm long. Each section was sieved separately through a 0.3 mm mesh bag. The bag with the residual sediment was gently squeezed to remove excess porewater. Still moist, it was transferred to plastic containers (with no addition of water, but with air volume above the sample at least twice as high as the sample volume) and kept in a cooler. The humidity inside the containers prevented animals from drying out. Lack of water and low temperature made it difficult for predators to move freely and potentially consume their prey.

In the laboratory, invertebrates were sorted alive without magnification in a white tray filled with water. The material was preserved with 4% formalin solution. All of the collected invertebrates were counted and then weighed (blotted wet) with a precision of ±0.1 mg. An analysis of distribution of invertebrates along the sediment profile was performed for sections of different lengths (as listed above), and those of equal lengths of 5 cm each (after combining the first two surface sections of 0–2 and 2–5 cm). The faunal density and biomass in subsequent sections were calculated in two ways: (i) per unit sediment volume (number of individuals L^{-1}, $mg\,L^{-1}$); and (ii) per unit sediment surface area (number of individuals m^{-2}, $mg\,m^{-2}$).

A Shapiro–Wilk test for normal distribution and a Levene test for equality of variances were conducted. Because the tests showed unequal variances, and because sampled sediment sections were not independent, a Friedman non-parametric analysis-of-variance (ANOVA) test (Chi-square, χ^2) was conducted to check for the impact of sediment depth on the density/biomass of the invertebrates. A *post hoc* test for Friedman ANOVA from the STATISTICA package was used to verify the significance of differences between densities/biomasses found in particular sediment sections. Statistical tests were done with StatSoft STATISTICA 10 software (StatSoft, 2011).

Results

Zoobenthos occurred down to a depth of only 25 cm. As a result, samples from the deeper sediment section of 25–30 cm were excluded from further analyses. The fauna consisted mostly of Tubificinae and larvae of Chironomidae. Tubificids were dominated by the *Potamotrix* and *Limnodrilus* genera, and chironomids by *Chironomus balatonicus* Devai, Wülker et Scholl, 1983. The total mean faunal density ranged from 8877 to 15 633 individuals m^{-2}, and the total wet weight biomass from 37 988 to 96 130 $mg\,m^{-2}$.

Vertical distribution in sediment cores divided into sections of equal lengths

Both the density and biomass of the fauna were significantly affected by sediment depth, irrespective of whether the calculations were made per unit area or per unit volume (Table 1). In both cases, the maximum density values were recorded in the two upper sections: 0–5 and 5–10 cm (Fig. 1), and the maximum biomass in the 5–10 cm section (Fig. 2). Statistically significant differences were recorded between the same sections (Figs 1 and 2).

Table 1. Friedman ANOVA (χ^2) results for the differences between densities and biomasses of zoobenthos (calculated per unit volume and per unit area) in the equal and unequal sections of sediment cores.

Parameter	Way of calculation	*N*	d.f.	*P*	χ^2 ANOVA
Equal sections					
Density	Per L	25	4	$P < 0.05$	86.9
	Per m^2	25	4	$P < 0.05$	86.9
Biomass	Per L	25	4	$P < 0.05$	78.1
	Per m^2	25	4	$P < 0.05$	78.1
Unequal sections					
Density	Per L	25	5	$P < 0.05$	97.8
	Per m^2	25	5	$P < 0.05$	99.4
Biomass	Per L	25	5	$P < 0.05$	92.3
	Per m^2	25	5	$P < 0.05$	88.3

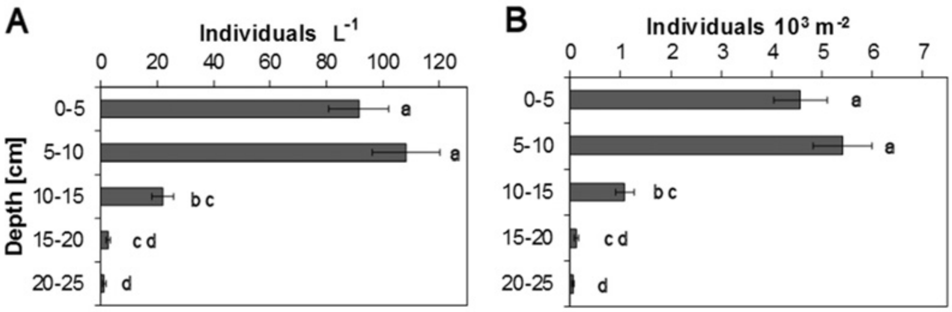

Fig. 1. Vertical distribution of zoobenthos density in sediment sections of equal lengths, calculated per unit sediment volume (A) and per unit sediment surface area (B), as a function of depth from the sediment surface (Whiskers – SEM). The data are means from two sites and three sampling occasions. Sediment sections sharing the same letter are not significantly different (*post-hoc* test for Friedman ANOVA, $P < 0.05$).

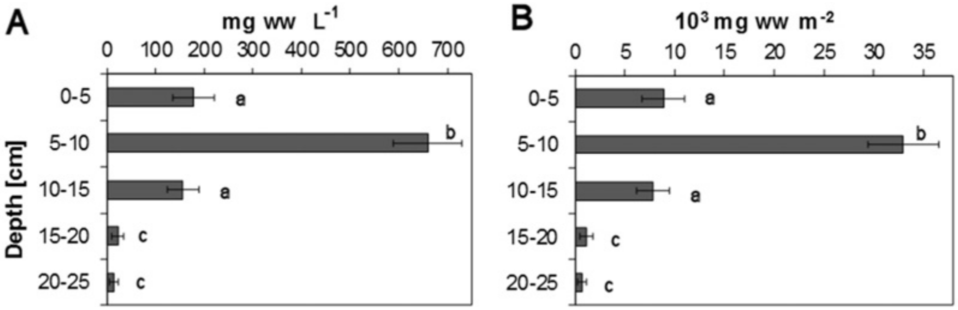

Fig. 2. Vertical distribution of zoobenthos biomass in sediment sections of equal lengths, calculated per unit sediment volume (A) and per unit sediment surface area (B), as a function of depth from the sediment surface (Whiskers – SEM). The data are means from two sites and three sampling occasions. Sediment sections sharing the same letter are not significantly different (*post-hoc* test for Friedman ANOVA, $P < 0.05$).

Vertical distribution in sediment cores divided into sections of unequal lengths

Both the density and biomass of the fauna were significantly affected by the sediment depth, irrespective of the applied method of data calculation (volume or area) (Table 1). However, depending on the manner of density calculation, the differences were statistically significant among the different sections.

Therefore, the pattern of vertical distributions of the fauna was somewhat different. The highest density per unit volume was recorded in the three upper sections down to a depth of 10 cm (Fig. 3(A)). Statistically significant differences were found between benthos density in each of the three shallowest sections and the three deepest ones, as well as between the deepest layer (20–25 cm) and the middle layer (10–15 cm).

Density expressed per unit surface sediment suggested a substantially higher increase with depth down to 10 cm, with a maximum in the 5–10 cm section (Fig. 3(B)). The differences between sections were particularly significant between the middle section 5–10 cm and the remaining ones, and between the shallowest and deepest sections.

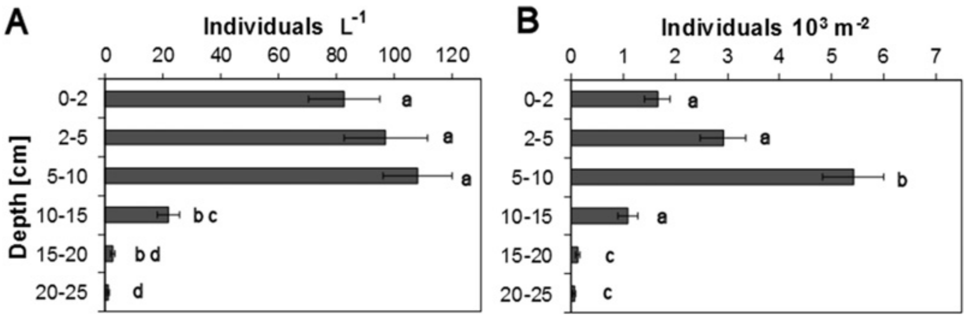

Fig. 3. Vertical distribution of zoobenthos density in sediment sections of unequal lengths, calculated per unit sediment volume (A) and per unit sediment surface area (B), as a function of depth from the sediment surface (Whiskers – SEM). The data are means from two sites and three sampling occasions. Sediment sections sharing the same letter are not significantly different (*post hoc* test for Friedman ANOVA, $P < 0.05$).

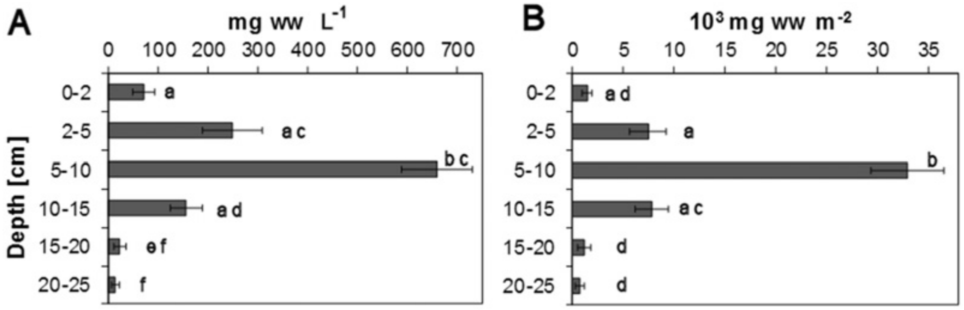

Fig. 4. Vertical distribution of zoobenthos biomass in sediment sections of unequal lengths, calculated per unit sediment volume (A) and per unit sediment surface area (B), as a function of depth from the sediment surface (Whiskers – SEM). The data are means from two sites and three sampling occasions. Sediment sections sharing the same letter are not significantly different (*post hoc* test for Friedman ANOVA, $P < 0.05$).

The distribution patterns of zoobenthos biomass also differed depending on the method of data calculation (Fig. 4).

Discussion

In this study sediment cores were divided into two groups: those with sections varying in length, and those having equal lengths. When the cores were divided into equal sections, irrespective of the applied method of calculating density and biomass (per unit area or per unit volume), the distribution of invertebrates in a sediment profile did not differ (Figs. 1 and 2).

With unequal lengths, the uppermost sections were shorter, and the bottom ones longer, as has very often been the case in the literature (Nalepa and Robertson, 1981; Van de Bund and Groenendijk, 1994; Martin *et al.*, 1999, 2005; Persson and Svensson, 2006; Urban-Malinga *et al.*, 2013, 2014; Kornijów and Pawlikowski, 2015). The choice of what section lengths to use usually depends on greater variability and activity of invertebrates in the uppermost sections (*e.g.*, Martin *et al.*, 2005), but the volume of sediment in sections of unequal lengths is different. Therefore, the density is related to varying volume. As evidenced by the example of the Vistula Lagoon, expressing benthos density or mass per unit surface area results in the overestimation of density and biomass in sections with greater lengths, and the underestimation of the density and biomass in shorter sediment sections (Figs. 3 and 4). The greater the difference in the length of sediment sections, the higher the degree of distortion of the results. Longer sections obviously contain higher volumes of sediment, and can (although not necessarily) contain higher numbers of invertebrates.

The failure to consider the length of sections within a sediment core in the calculation of density/biomass per unit area may result in a spurious reading of the preferred depth of occurrence of invertebrates. The same error is made if density is expressed as the percentage contribution of the numbers/biomass or number of specimens recorded in particular unequal sections. The only way to obtain an accurate estimate of distribution of invertebrates in the sediment core divided into sections varying in length is to express the data per unit volume. An additional advantage of such a conversion is that the values for invertebrates then correspond not to a flat bottom, but to the three-dimensional space which they actually occupy (Kornijów and Pawlikowski, 2015). Calculations per unit area will be distorted, leading to a false determination of the availability of food for fish, for instance, or the response of biomarkers to environmental factors.

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