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A new meiofauna sample splitter

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A new apparatus is described for splitting meiofauna samples into eight equal subsamples. The method allows even the lightest organisms, with a volume of less than 0.5 nl, to be recovered quantitatively and has advantage of an operating time of only a few minutes.

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1. Introduction

Meiofauna organisms in various types of substrate are known to reach very high numbers (Keynäs & Keynäs 1978, Platt & Warwick 1980) so it is often necessary to split the sample into smaller portions to reduce sorting time (Elmgren 1973, Dybern et al. 1976). Without considering the scientific problems concerned, it is desirable that the subsamples are representative of the original material. The new meiofauna sample splitter described here fulfils this requirement and has been tested against the Askö sample splitter (Elmgren 1973).

This work forms part of a project on the behaviour and ecology of Baltic nematodes carried out at the Tvärminne Zoological Station, Finland and the Marine Biological Laboratory, Helsingør, Denmark.

2. Material and methods

The Askö sample splitter used in this work was constructed according to Elmgren (1973) with one modification: the drain off tube in the wall was replaced by a channel in the centre of the splitter at the same height as the compartments, and which was operated from below. This change was made because the drain tube in the original design is prone to damage during handling. The Askö sample splitter was used according to the method described by Elmgren (1973) and Dybern et al. (1976).

The new meiofauna sample splitter was constructed of PVC. It consists of two cylindrical chambers: a mixing chamber and a splitting chamber (Fig. 1). The base of the mixing chamber is funnel-shaped with a central 7 mm diameter opening leading to the splitting chamber. This hole is closed from above by a rubber stopper and a lid fits tightly onto the top of the chamber. The volume of the mixing

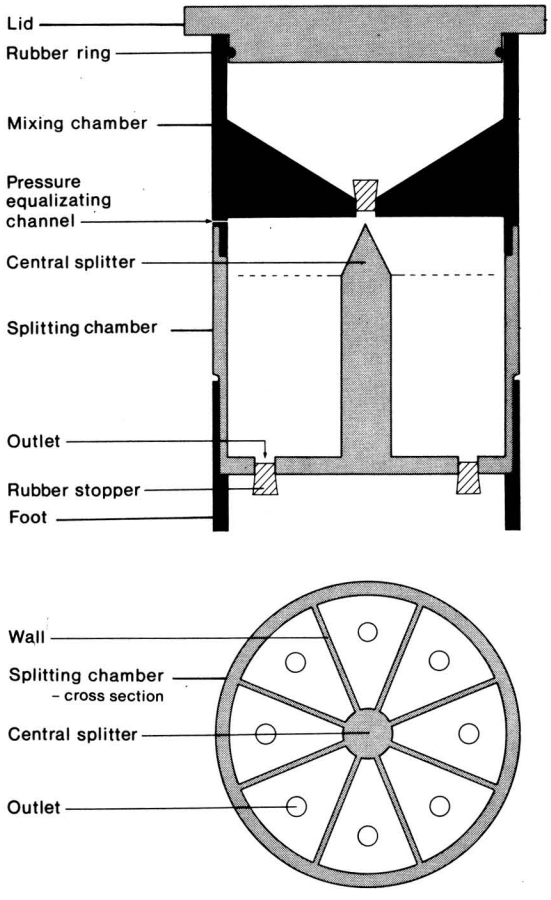

Fig. 1. Diagram of the new meiofauna sample splitter in longitudinal and cross section. Scale 1: 2.7.

Table 1. Actual numbers and calculated 1/8 mean value of meiofauna organisms obtained from 0.142 g dry weight *Pilayella littoralis* at Kvarnskärsgrundet, Tvärminne Zoological Station, Finland and the mean value ($\bar{x}/8$), standard deviation *(SD)* and coefficient of variation *(CV)* of meiofauna organisms from a 1/8 sample from the Askö sample splitter after 1 hour and the new meiofauna sample splitter after 1 min.

	Actual numbers		Askö splitter			New splitter		
	Total	1/8	$\bar{x}/8$	*SD*	*CV*	$\bar{x}/8$	*SD*	*CV*
Nematoda	2034	254.3	234.3	8.9	3.5	254.3	9.4	3.5
Copepoda	102	12.8	12.8	1.2		12.8	1.3	
Isopoda	9	1.1	1.1	0.6		1.1	0.6	
Amphipoda	8	1.0	1.0	0.9		1.0	0.9	

chamber is 350 ml. The mixing chamber is located onto the rim of the splitting chamber by watertight joints. There is a 2 mm pressure equalization channel in the mixing chamber just below the base. The splitting chamber has a central rod which is conical at the top, and eight equal compartments divided by thin, 64 mm high walls. Each compartment has a 7 mm diameter drain hole at the bottom which is closed from below by a rubber stopper. The volume of each compartment is 55 ml. The whole meiofauna sample splitter fits on a circular foot which allows the rubber stoppers to be raised above the laboratory bench.

The new sample splitter is used as follows, starting with an already fixed, extracted and concentrated meiofauna sample:

1. The material is washed from the sieve into the mixing chamber and the volume made up to about 75 % of the total chamber volume.
2. The lid is put on and the contents shaken, although in practise the material may already have been suspended during stage 1.
3. With the mixing chamber in place on the splitting chamber the rubber stopper is removed with forceps.
4. The material runs through onto the cone below and is split evenly into the eight compartments and any remaining organisms on the forceps or walls of the mixing chamber are washed down with a gentle jet of water.
5. The mixing chamber is removed.
6. The compartment(s) to be examined (selected by means of a table of random numbers) is emptied by removing the rubber stopper and the subsamples are collected in a small container, again washing out any remaining organisms with a gentle jet of water.

The whole sequence of events may be completed within a few minutes and the one-eighth sample is now ready for stereomicroscopical observation.

3. Results

Both meiofauna sample splitters gave similar results for meiofaunal organisms of more than 2 nl; such as amphipods, isopods, harpacticoid copepods and some nematodes (Table 1 and Fig. 2). The coefficient of variation of nematode numbers recovered in the compartments of each type of splitter was 3.5 %. However, the total numbers calculated were lower in the Askö sample splitter: 234.3 ind. ± 8.9 vs. 254.3 ind. ± 9.4 (a difference of 7.9 %). This difference was significant at the 0.01 level ($n = 14$, $t = 4.31$) and was due to animals being retained in the drain-water of the Askö sample splitter (Table 2). In subsequent tests of the Askö sample splitter, allowing the animals more time to sink down into the compartments, gave somewhat better results but even after 2 hours there were still 7.1 % of the nematodes left in the drain-water (Table 2). Moreover, another test showed that the nematodes left in the drain-water were not adhering to the water-air interface or sticking to the wall but floating around freely in the water (Table 3).

The nematodes in the drain-water belonged to the lightest fraction and constituted 1—2 % of the total body volume of nematodes tested, but made up 13—80 % of the portion smaller than 0.4 nl (Fig. 2).

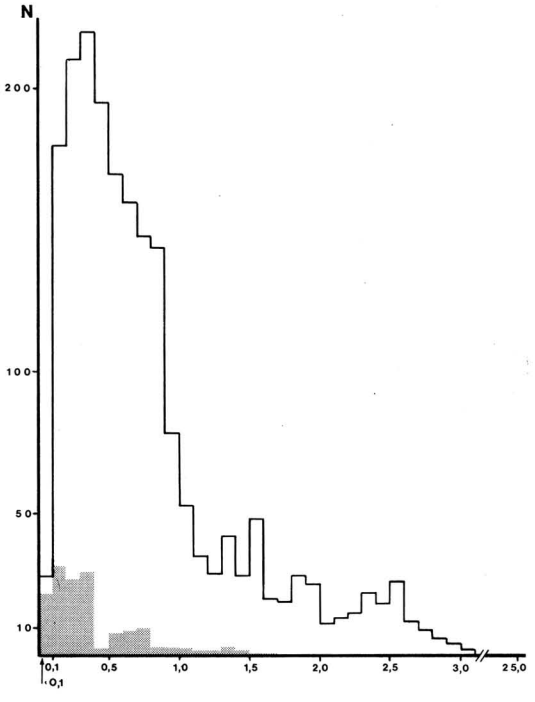

Fig. 2. Body volume distribution in nl of nematode numbers found in the Askö sample splitter after 1 hour, excluding 36 ind. measuring 3.6-25.0 nl. Non-shaded area: in dividing compartments. Shaded area: in draining water.

Table 2. Numbers and percentage of total nematode numbers found in the drain-water of the Askö sample splitter after 1, 2, 3 and 12 hours.

	1 h	2 h	3 h	12 h
Numbers	160	144	120	85
Percent	7.9	7.1	5.9	4.2

Table 3. Nematode numbers found at four equidistant positions in the Askö sample splitter after 1 hour. Splitting compartments are 30 mm in height.

	Position from bottom (mm) 0-30	30-60	60-90	90-120
Number of nematodes	1830	107	59	8

4. Discussion

The reliability of both sample splitters was acceptable from a statistical point of view calculating total abundances and biomass of meiofauna organisms. Olsson (1975) also came to the same conclusion for nematodes when testing the Askö sample splitter but it gave unsatisfactory results for the foraminiferan fauna. This was believed to be caused by their heavier weight and in certain types their tendency to clump together. The foraminiferan fauna was not tested in the present study due to their absence from the substrate.

The biological advantage of the new meiofauna sample splitter is that all those animals belonging to the lightest fraction are also taken into account, i.e. those animals passing a 100 μm sieve but retained on a 40 μm sieve. In my nematode material I am faced with very abundant species covering stages in the range of 0.01-0.5 nl (*Monhystera* and *Diplolaimella* species), 0.03-1.5 nl (*Chromadorita, Neochromadora, Punctodora* and *Theristus* species), 2-30 nl (*Adoncholaimus* species) and 10-65 nl (*Enoplus* species): a volume range of 3-4 orders of magnitude. Studies on the population dynamics of these species, including all their life stages, can only be made accurately for the two latter groups of species with the Askö sample splitter since 13-80 % of the size classes of nematodes less than 0.4 nl (the two former groups of species) remain floating in the supernatant drain-water even after 1 hour. The sub-unit in the new meiofauna sample splitter covers representatives of all size classes present in the orginal extracted and concentrated material. The same method can no doubt also be used for living animals.

Another advantage over the Askö sample splitter is that the new splitter takes only a few minutes to operate, whereas the Askö sample splitter requires more than 1 hour. Even then, the animals retained in the drain-water have to be checked, and this together with the detergent used in the Askö splitting method. The presence of animals in the drain-water is a very disturbing factor, although this may vary in importance depending on the characteristics of the original sample substrate and the specific animals to be analyzed (see review in Uhlig et al. 1973). Benthic nematode faunas from the Baltic are, however, known to consist mainly of small forms (Elmgren 1976 and Keynäs & Keynäs 1978) as are the meiofauna on the submerged vegetation (Fig. 2).

Finally, it is easier to empty the compartments of the new splitter by simply pushing the stoppers out from above using a suitable rod. This is possible because the volume of water in each compartment is only at most two-thirds of the total space, leaving a free space at the top and the whole splitting chamber is only 8.5 cm deep so that the drain-plugs can be reached easily. After draining the water from the Askö sample splitter, the remaining water is at the uppermost level of the compartments and the splitting chamber is so high that the compartments have to emptied from below. This is not easy to accomplish without the danger of animals floating on the top of one compartment being tipped into another.

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