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A Simple Density Separation Technique for Quantitative Isolation of Meiobenthos Using the Colloidal Silica Ludox-TM*

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Abstract

A simple, quantitative density separation method is described. The method is based on differences in specific weight between meiobenthos and sediment. Nematodes and copepods could be separated from sediment and detritus when samples were suspended in Ludox-TM, a colloidal silica. Organisms float at the surface, while sediment particles sink. Results obtained with this new method were compared with the well-known decantation method. For a quantitative isolation of nematodes from sediments, rich in coarse detritus, a maximum volume of 7 cm^3 sample could be used. For copepods this maximum was 13 cm^3. For such sediments the density method is more reliable than the decantation method. The time needed for sorting the meiobenthic organisms is reduced to about 30% compared with the former method. The new method can be used for preserved as well as for fresh sediment samples and can also be applied for the isolation of small polychaetes, small oligochaetes, larvae of some macrofaunal groups and net-zooplankton.

Introduction

In estuaries with sheltered areas, which are usually rich in detritus and fine-grained sediments, the most abundant component of the meiobenthos is generally formed by the nematodes while copepods often rank second. Quantitative isolation and subsequent analysis of such populations are rather difficult, because detritus particles hamper detection of organisms under the dissecting microscope. For the extraction of meiobenthic organisms from sandy sediments several methods are applied. Uhlig *et al.* (1973) described the specific efficiency of methods such as decantation, elutriation and sea water ice treatment. The same methods can be used for quantitative isolation of meiobenthic organisms from muddy sediments, but this is very time-consuming due to the presence of detritus particles which make hand-sorting necessary. Heip *et al.* (1974) modified the method described by Jenkins (1964) to isolate nematodes and copepods from muddy sediments by centrifugation in a saccharose solution. However, they had to repeat the centrifugation procedure several times to harvest the population of nematodes quantitatively. Bowen *et al.* (1972) introduced density gradients built up from the colloidal silica Ludox-AM to separate different groups of marine zooplankton from each other. The method described in this paper is based on the use of Ludox-TM for quantitative separation of meiobenthic organisms from detritus as well as from the fine-grained sediment fraction. The use of Ludox-TM (specific weight 1.39 g cm^{-3}) instead of Ludox-AM (specific weight 1.19 g cm^{-3}) is not essential for the method described in this paper. Ludox-TM was chosen because separation of other, heavier sediment components is under investigation. These results will be published in the near future. Attention was focused on nematodes and copepods, as being the most abundant groups in the localities investigated.

Materials and Methods

Surface sediment from the upper 0.5 cm was collected from tidal mud flats at five different localities in the Ems estuary (Fig. 1). The collected samples were brought to the laboratory, put into

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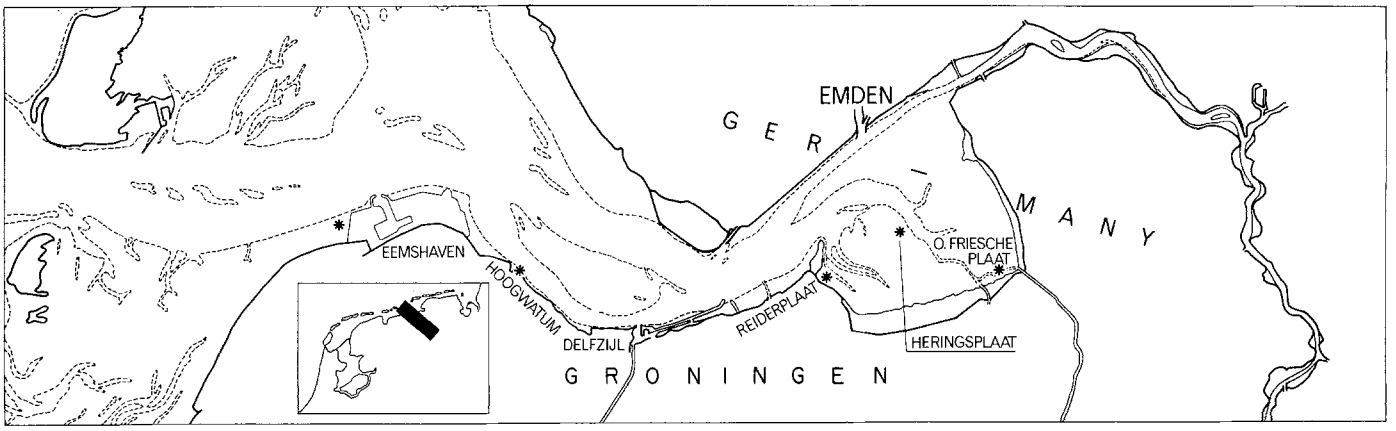

Fig. 1. Map of the sampling stations Eemshaven, Hoogwatum, Reiderplaat, Heringsplaat and Oost Friesche Plaat

a plastic beaker and the sediment diluted by 15 to 20% (v/v) with sea water until a slurry was obtained. The slurry was homogenized by a variable speed IKA-Werk stirring machine (Janke & Kunkel K.G., Staufen, Breisgau, FRG) with a propeller. The number of revs/min of the stirrer was dependent on the kind of sediment. The stirrer was adjusted to between 800 and 1200 revs/min to achieve thorough mixing. A propeller size of 65% of the beaker diameter proved to be effective. To prevent settling of sand grains, a T-piece was attached to the lower side of the propeller. This T-piece rotated just above the bottom of the beaker. After 10 min stirring, subsamples were taken while stirring was continued by a plastic syringe (2.5 cm^3) from which the conical top had been cut off. Other series of subsamples were taken by a plastic syringe (20 cm^3) with a pore of 4 mm at the conical top. The subsamples were divided at random into two series. One series was processed by decantation and sieving, the second series by the method described in this paper.

Decantation Method

Nematodes

Subsamples of 1.5 or 2.0 cm^3 of fresh sediment were distributed between two glass tubes of 16 x 1.5 cm. These were half-filled with sea water and whirled at full speed for 20 sec on a Vortex Genie mixer, type EP 900 (Scientific Industries, Inc., USA). The larger sediment particles were allowed to settle for 8 sec, then the supernatant was poured out through a 35 µm mesh-sieve of nylon gauze. This procedure was repeated at least 5 times. Residues were checked for the presence of nematodes. The meiobenthic organisms were washed out of the sieve with sea water, collected in a Petri dish and counted under a dissecting microscope.

Copepods

Subsamples of 17.5 cm^3 were put in about 100 ml of sea water in 250 ml flasks. The flasks were placed on a magnetic stirrer and the suspensions were stirred vigorously for about 20 sec. The subsequent procedure was the same as described for nematodes.

Density Separation Method

In this procedure, differences in specific weight between meiofauna and other sediment components were used to separate these fractions. For our purpose Ludox-TM was used. Ludox is the trade name of a colloidal silica polymer (Du Pont, 1973). The specific weight of the undiluted product is 1.39 g cm^{-3} and will be considered here as 100%. The product is toxic to all living organisms and may contain insoluble floccules of Ludox in the gel-form, which can be removed by filtering the Ludox over a thick layer of gauze bandages and paper filter (Schleicher & Schüll, nr. 520 b II). Beakers (14 x 9 cm diameter) were filled with 300 ml 25% (v/v) Ludox-TM. Subsamples of 1.5 or 2.0 cm^3 of fresh sediment

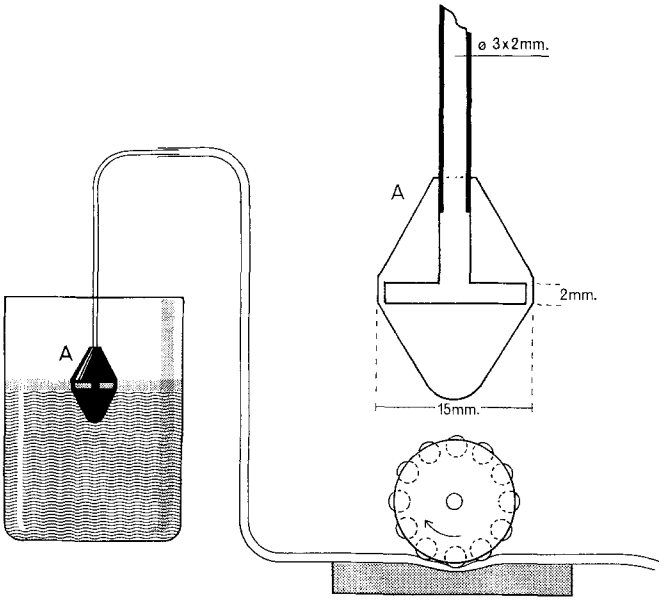

Fig. 2. Arrangement for pumping a water layer upon the Ludox surface. The peristaltic pump is adjusted to a capacity of 15 ml min^{-1}. A: Detail of conical part through which the water is pumped in a horizontal direction upon the Ludox surface. Conical part is made of PVC and tube of stainless steel

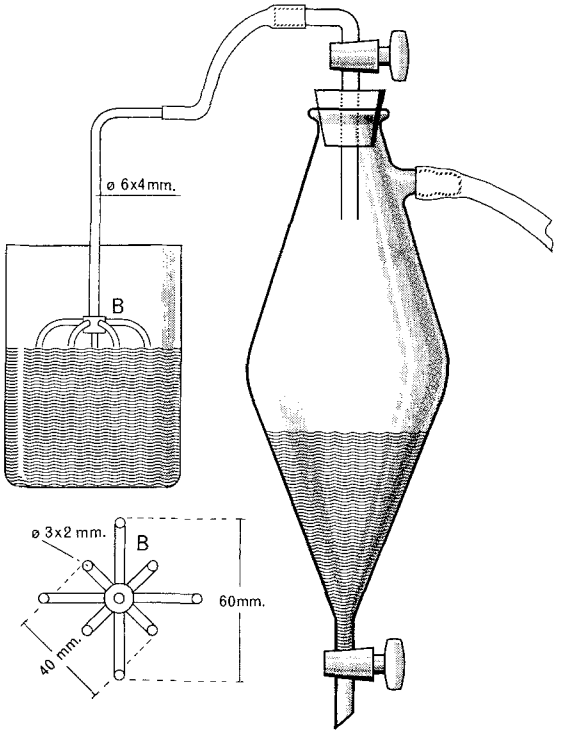

Fig. 3. Arrangement for collecting the floating meiobenthic organisms from the Ludox surface. Organisms are caught in the vacuum flask (volume about 1.8 l) and drawn off through the lower valve. Then the flask is rinsed with distilled water to remove the Ludox as well as the meiobenthic organisms retained. B: Detail of the sucking-apparatus through which water layer and subsequent Ludox top layer are transported to the vacuum flask. Tubes are made of stainless steel and central part of PVC

were put in 25 ml glass tubes and half-filled with 25% (v/v) Ludox-TM. Then 1 ml formaldehyde (37%) was added, the tubes stoppered and vigorously shaken by hand for some seconds. Next, one of the beakers with Ludox was placed on a magnetic stirrer and the sediment was added while stirring vigorously with a teflon-coated bar (5.5 x 1.1 cm). The 25 ml tubes were washed out with Ludox. The stirring was continued for some minutes and then the beaker was removed from the stirrer and used as a flotation chamber. With the apparatus shown in Fig. 2, a 0.5 cm thick layer of distilled water was pumped onto the surface of the Ludox to prevent desiccation, which would have caused transformation of the Ludox into the gel-form, possibly disturbing the procedure at a later stage. After about 16 h, the heavy sediment particles and the bulk of the detritus had sunk to the bottom, while meiobenthic organisms floated near the Ludox surface. The upper 2.5 cm of the liquid containing the organisms were removed by the suction arrangement shown in Fig. 3, using a vacuum pump with a capacity of 5 N m^3 h^{-1} air. The removed suspension was caught in a vacuum flask. Thereafter, the meiobenthic organisms in suspension were poured on a 35 µm mesh-sieve of nylon gauze on which the organisms were rinsed with distilled water to remove the Ludox. The meiobenthic organisms were washed out of the sieve with distilled water, collected in a Petri dish and counted under a dissecting microscope. To check the sediment residues on retained meiobenthic organisms, some series of flotation chambers were filled again with Ludox, stirred and treated according to the procedure described above.

As the sediment of Station Hoogwatum was rich in coarse detritus, it was investigated which sample volume could be separated quantitatively within one run. Subsamples with increasing volume were taken in the usual way.

Results

Tables 1 and 2 present the numbers of counted nematodes and copepods, respectively, isolated according to the two different methods. The statistical evaluation, two residue check series, and sediment qualification are given as well. As the data of each of the series pointed to a normal distribution, Student's *t*-test was applied to investigate differences between the decantation method and the density method. It appeared that no statistically significant differences existed between both series of counts of

Table 1. Data and analysis of counted nematodes obtained with decantation method and density method without preservation and shaking. RC: Residue check

	Station										
	Eemshaven			Hoogwatum		Reiderplaat		Heringsplaat			Oost Friesche Plaat
	Decantation	Density		Decantation	Density	Decantation	Density	Decantation	Density		Decantation	Density
cm^3 sediment sample	1.5	1.5	RC	2.0	2.0	2.0	2.0	1.5	1.5	RC	2.0	2.0
No. of nematodes	84	93	0	244	314	32	13	95	141	0	160	208
	88	97	0	246	288	36	8	127	110	0	147	231
	100	77	0	217	294	26	16	128	142	0	201	181
	91	77	1	241	249	27	19	129	136	0	159	143
	83	102	0	212	309	18	24	124	138	0	193	166
	76	95	0	198	238	29	21	104	133	0	210	156
	97	85	0	245	313	28	17	175	144	0	188	188
	90	83	1	290	298	26	18	139	133	0	173	
	92	84	0	244	248	21	16	133	144	0	230	
	87	97	0		297	32	21	129	139	0	197	
Mean	88.80	89.00		237.44	284.80	27.50	17.30	128.30	136.0		185.80	181.86
Standard deviation	6.94	8.91		26.49	28.82	5.30	4.52	21.18	9.98		25.83	30.46
Student's *t*-test												
Student's *t*	0.056			3.715		4.631		1.040			0.281	
Degrees of freedom	18			17		18		18			15	
Significance level	>0.1			<0.01		<0.001		>0.1			>0.1	
Sediment qualification	Coarse-grained, not much detritus			Coarse-grained, many coarse particles of organic detritus		Fine-grained, many fine particles of organic detritus		Coarse-grained, not much detritus			Practically no sand grains, many very fine particles of organic detritus	

Table 2. Data and analysis of counted copepods obtained with decantation method and density method

	Station Eemshaven		
	Decantation	Density	Residue check
cm^3 sediment sample	17.5	17.5	
No. of copepods	71	79	0
	92	62	0
	103	94	0
	80	86	0
	73	89	0
	84	95	0
	78	89	0
	70	91	0
	74	96	0
	67		
Mean	79.20	86.78	
Standard deviation	11.16	10.65	
Student's *t*-test			
Student's *t*	1.510		
Degrees of freedom	17		
Significance level	>0.1		
Sediment qualification	Coarse-grained, not much detritus		

Table 3. Data and analysis of counted nematodes obtained with decantation method, density method without preservation and shaking, density method without shaking and density method as described in this paper

	Station Reiderplaat			
	Decantation	Density, no shaking, no preservation	Density, no shaking	Density
cm^3 sediment sample	2.0	2.0	2.0	2.0
No. of nematodes	32	13	21	26
	36	8	22	28
	26	16	14	32
	27	19	23	34
	18	24	17	33
	29	21	14	29
	28	17	21	19
	26	18	25	17
	21	16	23	33
	32	21	21	25
Mean	27.50	17.30	20.10	27.60
Standard deviation	5.30	4.52	3.81	5.93
Student's *t*-test				
Student's *t*		4.631	3.585	0.040
Degrees of freedom		18	18	18
Significance level		<0.001	<0.01	>0.1

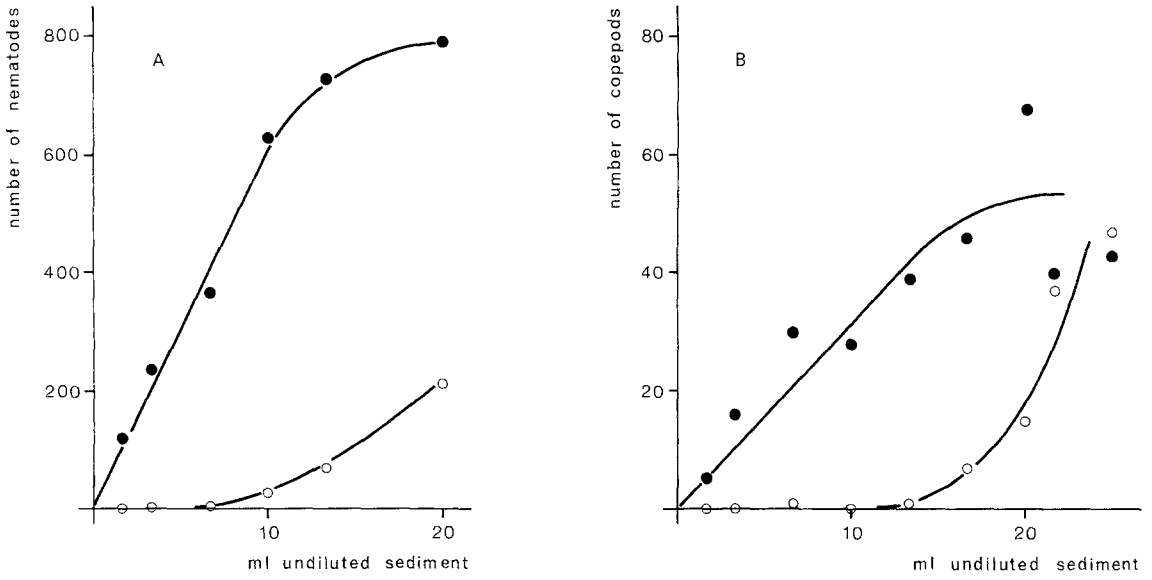

Fig. 4. Relation between increasing sample volume and number of isolated nematodes and copepods obtained with the density separation method. Samples were obtained from Station Hoogwatum, were the sediment was rich in coarse detritus. Filled circles: counted numbers of organisms after the density separation procedure; open circles: counted numbers of organisms after repeated treatment of the residue

nematodes for Stations Eemshaven, Heringsplaat and Oost Friesche Plaat (Table 1) and of counts of copepods for Station Eemshaven (Table 2). For Stations Hoogwatum and Reiderplaat on the other hand, a statistically significant difference was calculated between nematode counts according to the two methods With the density method, on an average, significantly more specimens ($P < 0.01$) were counted for the station Hoogwatum but significantly fewer specimens ($P < 0.001$) for the station Reiderplaat. It should be mentioned that in density-processed residues no specimens normally were found (Tables 1 and 2). Two additional experiments were made to investigate the deviating results of the Reiderplaat series. One experiment was carried out according to the density method with unpreserved samples and without shaking by hand, another was carried out according to the density method with 4% formaldehyde-preserved samples but without shaking by hand and the third series was handled according to the density method procedure as described in this paper. The results are given in Table 3. From Table 3 it is obvious that preservation and shaking by hand before the Ludox treatment improve the recovery. Using the improved procedure, no statistical differences between the two isolation methods could be indicated for the Reiderplaat series.

Fig. 4 presents the relationship between sediment volume and the recovery of nematodes and copepods in the first and in the second Ludox treatment. For the nematode fraction it appeared that sediment samples up to 7 cm^3 could be processed within one density separation run, for copepods the size of this volume was 13 cm^3. If more sediment has to be processed a second Ludox run may be necessary.

Discussion

For Station Hoogwatum, differences in counts between the decantation method and the density method can be explained by many small specimens escaping detection as they were hidden by coarse particles of detritus. The nematode fraction obtained by the density method contained a small amount of detritus, whereas the decantation method failed to separate the bulk of this material from the nematodes. It is suggested here that, especially for small-sized specimens and larvae, the decantation method may lead to inaccurate results. Moreover, repeated hand-sorting after each resuspension of the decanted fraction is very time-consuming.

An investigation of the species composition of the four Reiderplaat series showed that the difference between the

two methods for that station was caused by the presence of great numbers of Chromadoridae which were attached to very small sand grains by their tail gland. These organisms could be separated quantitatively by the decantation method. In the density method, however, those organisms attached to the sand grains were prevented from floating to the surface of the Ludox. Shaking by hand detached the nematodes from the sand grains, while either preservation with 4% formaldehyde or anaesthetization with chloroform prevented renewed adherence. Moreover, preservation with 4% formaldehyde did not affect floating of the organisms and consequently did not lower recovery (Table 3). This is due to the relatively large particle size (21 to 24 nm, Dupon brochure) of the polymer silica which prevents Ludox particles from penetrating dead organisms. Although the organic detritus content is very different at the stations Eemshaven, Heringsplaat and Oost Friesche Plaat (Tables 1 and 2), counting results by the decantation method and the density method did not differ significantly. Usually, however, the average yield for the density method is somewhat higher. Moreover, the Chromadoridae could have been missed since at first the density method was carried out without preservation and shaking by hand.

A certain relation was observed between the diameter of the flotation chamber and the maximum sample volume required to obtain quantitative separation. For sediments which are rich in coarse detritus, relatively large samples can be separated at one run. When the sample concentration is too high (Fig. 4), organisms can be caught by coarse-grained detritus particles and in this way be retained in the residue. This might be one reason why Heip *et al.* (1974) could not separate all nematodes at a single centrifugation.

It was not possible to check the suitability of the density method for small oligochaetes, small polychaetes or larvae of some macrofaunal groups in the way described for nematodes and copepods. This was either due to too small numbers of organisms or to their small size. The long, thin specimens of the oligochaetes and polychaetes were damaged during the mixing procedure. However, we are sure that for these organisms the density method as described here may also be used, if mixing is omitted. In the second run specimens of these groups were never found. The method is not applicable to molluscs and ostracods. The density method was also tested for zooplankton by J.W. Baretta (Netherlands Institute for Sea Research). The method gave a very satisfactory separation between detritus and net-plankton.

Preserved as well as unpreserved nematodes did not show any damage or change of structure and taxonomically important details were in excellent condition for species identification after a Ludox treatment.

To prepare meiobenthic fractions for biomass estimation by a carbon analyzer only some cleaning by hand-sorting of organic detritus particles is necessary after the separation procedure with Ludox.

From current investigations in our laboratory it seems possible to isolate nematodes alive from sediments with dialyzed Ludox-TM. This means that Ludox-TM itself does not influence the viability of these organisms.

A disadvantage of the use of Ludox-TM is its property to dry rapidly and consequently to change into the insoluble gel-form. The main advantages of the method are its simplicity and the possibility to work with large series of chambers, often necessary for ecological purposes. The time necessary for hand-sorting in the density method is reduced to about one third of the time needed in the decantation method.

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