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A Rapid Method of Extracting Meiobenthic Nematodes and Copepods from Mud and Detritus

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Abstract

The extraction of nematodes and harpacticoid copepods from muddy sediments or sediments containing a large amount of organic detritus can be achieved through a series of centrifugations of the material in a saccharose solution of at least 900 g/l. The number of individuals appearing in the supernatant after each centrifugation forms a geometric series, with a ratio which is dependent on the amount of material, the concentration of the saccharose solution, and the acceleration of the centrifuge. Knowledge of this ratio allows an estimation of density after only one centrifugation. More than 90% of the animals present in the sample can be extracted after only two centrifugations.

Introduction

Nematoda and Copepoda Harpacticoida are the most numerous animal groups in the meiobenthos of all marine and brackish-water habitats, from the deep sea to the intertidal. Numbers frequently range between 0.1 and 10 million individuals per m^2. The extraction of these animals from sandy sediments does not represent any serious difficulty (see Hulings and Gray, 1971, for a review of techniques), but much greater problems arise when the sediment is muddy or when it contains large amounts of organic detritus. There exist certain techniques which can be used on living material (Higgins, 1964; Uhlig, 1964; Uhlig *et al.*, 1973), but they have probably more significance in qualitative work (e.g. morphological studies) than in quantitative investigations, which often require the treatment of large numbers of preserved samples. There seems to be no general technique which can be used on preserved material; this is reflected by the fact that Hulings and Gray (1971) still recommend sieving and hand-sorting for quantitative work on muds. In view of the enormous amount of time necessary to extract nematodes and copepods, under the dissecting microscope, from the organic detritus which remained after washing the sandy sediment of a brackish-water locality in northern Belgium, and the possibility of large errors when this extraction is not done very carefully, we looked for another method.

Results

A method was found through the application of the centrifugal-flotation technique of Jenkins (1964), which is used to extract nematodes from terrestrial soils. In its original form, the technique is not applicable on the sediment we studied, but the modification we used is highly successful. In this modification, the sample is elutriated and freed from the sand. It is then placed in a saccharose solution of 900 or 1000 g/l after removing, through sieving, as much water as possible. The sample is then centrifuged at 6000 revs/min for 5 min. The supernatant is removed and immediately placed into a 0.038 mm-mesh sieve, where it is rinsed to remove the saccharose. The remaining material is brought in suspension again and centrifuged a second time. The RCF (relative centrifugal force) of the MSE centrifuge we used is 4000 x g at 6000 revs/min and 18000 x g at 12000 revs/min.

Apart from its rapidity, this method possesses a feature which enables density to be calculated after only one or two centrifugations. Indeed, one of us (Heip, 1974) has demonstrated that the numbers of nematodes appearing in the supernatant after each centrifugation constitute a geometric series since, during each centrifugation, a constant proportion of the animals remains trapped in the material. The total number, N_t, of animals in the sample can be calculated as (Heip, 1974):

$$N_t = \frac{N_1 a - 1}{a - 1} = \frac{N_1^2/N_2 - 1}{N_1/N_2 - 1},$$

where $a = N_1/N_2$ is the ratio of the series, N_1 the number of animals in the first supernatant, and N_2 the number of animals in the second supernatant.

The present paper extends these results to copepods, and investigates the factors influencing the ratio of the series a. This ratio is constant when the procedure is standardized. The important parameters in its determination are the amount of material, the concentration of saccharose, and the acceleration of the centrifuge, more than its centrifugal force.

We used between 2 and 5 ml of detritus-saccharose-solution in 25 to 30 ml of saccharose in an Oak Ridge Type 50-ml polycarbonate bottle

Table 1. Influence of saccharose concentration on ratio a of geometric series. Values of a are obtained from n replicates

Saccharose concentration (g/l)	a ratio	
	Nematoda (n=5)	Copepoda (n=3)
750	2.25 ± 0.05	-
900	3.18 ± 0.07	3.75 ± 0.10
1000	3.77 ± 0.07	4.22 ± 0.11

Table 2. Number of nematodes appearing in first supernatant of the same sample treated at different accelerations and maximum speeds. Mean value of 3 replicates. RCF: relative centrifugal force

Maximum speed (revs/min)	RCF x g	Position A (18000 revs/min)	Position B (15000 revs/min)
3000	1750	264 ± 31	219 ± 22
6000	4000	295 ± 35	240 ± 21
12000	18000	303 ± 27	247 ± 24

(MSE Cat.no. 59466). As the amount of material which can be treated largely depends on the type of centrifuge used, we did not investigate this factor further.

The saccharose concentration has an important and linear effect on the value of a (Table 1). Values lower than 750 g/l were tried, but proved to be inefficient in removing part of the nematodes and nearly all the copepods. Values lower than 900 g/l are inefficient in removing part of the copepods. The regression between saccharose concentration S and the ratio a is: $a = -2.31 + 0.0061\ S$, for nematodes; $a = -0.48 + 0.0047\ S$, for copepods. The higher values for the copepods indicate that they are removed more efficiently than the nematodes in the range 900 to 1000 g/l.

The acceleration of the MSE centrifuge used could not be measured exactly, but it was found that values of a differed significantly according to whether the centrifuge was started at maximum acceleration (Position A = 18000 revs/min) or at an intermediate acceleration (Position B = 15000 revs/min) (Table 2). Moreover, the difference between values of a obtained in this way was greater than the difference obtained with different centrifugal forces. The values of a are virtually the same whether using 6000 or 12000 revs/min as the maximum speed.

Table 3. Accumulated percentages of total number in sample extracted after n centrifugations

n	a= 2.25	a= 3.18	a=3.77	a=4.22
1	55.6	68.6	73.5	76.3
2	80.3	90.1	93.0	94.4
3	91.2	96.9	98.1	98.7
4	96.1	99.0	99.5	99.7
5	98.3	99.7	99.9	99.9

Discussion

We did not consider the factors determining a in more detail, since their influence will depend on the type of centrifuge used. The important thing is that a can be determined in an accurate way when the procedure is standardized. Once determined, the value of a allows an estimation of density after only one centrifugation. This value can also be used to obtain an estimate of the number of centrifugations necessary to obtain a given percentage of the total number of individuals present in the sample. In the first supernatant there are $N_1 = N_t \frac{a-1}{a}$ individuals, so $N_1/N_t = \frac{a-1}{a}$. When standard errors are known, this ratio can be used to obtain an estimate of the precision of these percentages. In each consecutive supernatant, the number of individuals is $N_{n+1} = N_n/a$. These accumulated percentages are shown in Table 3 for the different values of a resulting from the use of different saccharose solutions (Table 1). It is clear that in our study, in the majority of cases more than 90% of the individuals present in the sample are extracted after only 2 centrifugations.

The high concentration of saccharose we used did not appear to damage the specimens obtained. After washing the supernatant immediately after centrifugation, examination showed the specimens to be suitable for determination, even in the case of the nematodes. This method thus allows an accurate and rapid determination of density of the two most important groups of meiobenthic animals.

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