A comparison of two extractors for separating meiobenthic nematodes from fine-grained sediments

J.P. Furstenberg and A.G. de Wet
Department of Zoology, University of Port Elizabeth, Port Elizabeth, and Department of Statistics and Operations Research, Vaal River Branch, Potchefstroom University, Vanderbijlpark

Two methods for the quantitative extraction of meiobenthic nematodes from fine-grained sediments were investigated and compared: (i) Oostenbrink-sugar flotation and (ii) Ludox flotation. The results show that Ludox flotation yielded between approximately 200 and 700% more nematodes. A conversion graph for nematode numbers of the two extracting methods was compiled.

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Twee metodes vir die kwantitatiewe ekstrahering van meiobentiese nematodes uit kleierige grond is ondersoek en vergelyk: (i) Oostenbrink-suikerflottasie en (ii) Ludox-flottasie. Resultate toon dat Ludox tussen ongeveer 200 en 700% meer nematodes ekstraheer. 'n Omsettingsgrafiek vir nematodegetalle van die twee tegnieke is saamgestel.

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J.P. Furstenberg*
Department of Zoology, University of Port Elizabeth,
P.O. Box 1600, Port Elizabeth 6000, Republic of South Africa
A.G. de Wet
Department of Statistics and Operations Research, Vaal River Branch of Potchefstroom University for C.H.E., P.O. Box 1174,
Vanderbijlpark 1900, Republic of South Africa
*To whom correspondence should be addressed

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Since the beginning of the century a number of methods have been developed for the extraction of nematodes from sediments. Techniques involving decantation and elutriation are useful in quantitative separation of nematodes from sandy sediments (Furstenberg, Dye & De Wet 1978). Huling & Gray (1971) recommended that for quantitative work with fine-grained sediments sieving and hand-sorting are necessary. This is an extremely laborious and time-consuming method when dealing with coastal and estuarine muds (Nichols 1979), in comparison with centrifugal flotation of soil suspensions. To date sugar (centrifugal) flotation or a combination of Oostenbrink and sugar flotation has been used by many nematologists for separating meiobenthic nematodes from fine-grained sediments. However, the efficiency of extraction with the popular sugar solutions can be variable (Heip, Smol & Hautekiet 1974) apart from the distortion which it can cause to the nematodes. This makes the technique less acceptable to taxonomists. A substitute for sugar with less harsh effects on animals in sediment samples is a colloidal sol called Ludox which to our knowledge has been used by nematologists for less than a decade.

The aims of this study were to compare Ludox with Oostenbrink-sugar flotation and to compile a conversion graph for their nematode yields per sample.

Material and Methods

Sediment samples were taken at eleven sites selected in the Swartkops River, Port Elizabeth (34°S/26°E) over a distance of 24 km from the mouth. Each site consisted of two mid-tidal locations, *ca.* 5 m apart, and were sampled with a hand corer, 30 cm long and 3,57 cm in diameter. These sites were initially chosen for ecological studies on the meiobenthic fauna of the Swartkops River. For the purpose of this study all coarse sandy stations were excluded.

Samples taken at each location were thoroughly mixed (Furstenberg *et al.* 1978) and subsequently divided into three subsamples of 200 ml each. Two were extracted by Oostenbrink-sugar flotation and Ludox respectively and the third analysed for particle size. Particle size distribution was measured using 50 g of oven-dried substrate by net-sieving through sieves conforming to the Wentworth scale at 1 phi intervals (Morgans 1956).

Extracting techniques

(i) Oostenbrink-sugar flotation. Samples of 200 ml were first extracted by Oostenbrink elutriator (Furstenberg *et al.* 1978). After extraction a silty deposit was collected. This was treated by sugar flotation (Caveness & Jensen 1955) to separate the very fine particles from the nematodes. This method is used by nematologists when only the normal small centrifugal tubes are available.

(ii) Ludox flotation. All samples were fixed prior to centrifugation with 10% hot formalin (Goodey 1963). One hundred millilitres of sample were washed through a very coarse sieve (2 mm) into six 440-ml centrifuge tubes. By adding distilled water the volume in each tube was made up to 10 times the sediment sample volume. Three grams of kaolin powder were added to each tube and stirred thoroughly. A centrifugal speed of 3 000 rpm (relative centrifugal force of 1 800 G) was maintained for 10 min. The supernatant liquid was poured off in one smooth motion without disturbing the deposit at the bottom of the tube and collected on a 38 μm sieve. Ludox HS 40%, a commercial preparation of silica sol (pH 10; viscosity 16 centipoise; density 1,3; particles with a negative loading), and sold by E.I. du Pont de Nemours & Co., was diluted with distilled water to 50% of the commercial concentration. The diluted Ludox was added to each tube to the same volume as previously and the contents were well mixed before centrifugation. After centrifugation the final supernatant was poured on a 38 μm sieve and rinsed thoroughly with distilled water to remove all traces of Ludox. The process was then repeated twice.

Results and Discussion

The percentage of sediment particles (all smaller than 63 μm) varied between 19 and 56,7%; the MdΦ between 1,45 and 4,10; QDΦ between 0,93 and 1,73 and SkqΦ between −0,65 and 0,70.

Figure 1 shows a plot of the logarithm of the Ludox count versus the logarithm of the Oostenbrink-sugar count. Counts were expressed logarithmically to linearize the relationship (Mosteller & Tukey 1977: 91). A linear regression fitted by least squares accounts for 74% of the variation in the logarithm of the observed Ludox count (r^2 = 0,74), enabling one to estimate the expected Ludox count for an Oostenbrink count in the range 15 − 700 nematodes 200 ml^{-1} (Lindley 1947: Sections 5.2 and 5.3). The regression line together with a 90% confidence band (Neter & Wasserman 1974: Section 5.3) is shown in Figure 1. Formulae for computing this line and the confidence band are as follows:

estimated expected $\log_{10}$ (Ludox count) = 1,332 + 0,6443 × $\log_{10}$ (Oostenbrink-sugar count).

Ninety percent confidence band for the regression line:

estimated expected $\log_{10}$ (Ludox count) ± 0,4140 × A, where A^2 = 0,0455 + 0,2206 × ($\log_{10}$ (Oostenbrink-sugar count) − 2,071)^2.

An example of the use of the above-mentioned formulae follows:

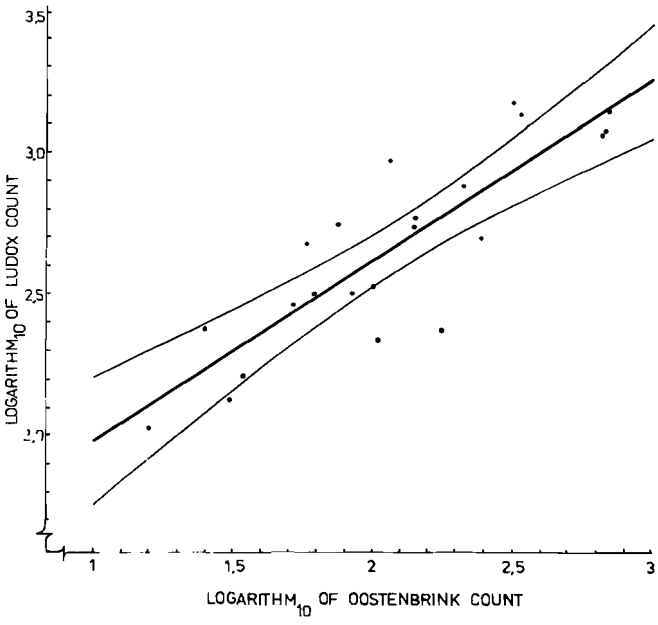

Figure 1 Logarithm of Ludox nematode count versus logarithm of Oostenbrink-sugar nematode count together with the least-squares regression line and a 90% confidence band. (n = 22; r^2 = 0,74),

Suppose that an Oostenbrink count of 250 was obtained. The following calculations show the estimate as well as a 90% confidence interval for the expected Ludox count:

Log_{10} (Oostenbrink count) = 2,398

estimated expected $\log_{10}$ (Ludox count) = 1,332 + (0,6443 × 2,398) = 2,877.

estimated expected Ludox count = $10^{2,877}$ = 753

A^2 = 0,0455 + 0,2206 × (2,398 − 2,071)^2
= 0,0691

Ninety percent confidence interval for the expected $\log_{10}$ (Ludox count):

$2,877 \pm 0,4140 \times (0,0691)^{\frac{1}{2}}$
= (2,768; 2,986)

Ninety percent confidence interval for the expected Ludox count:

$(10^{2,768}; 10^{2,986})$
= (586; 968)

Yield efficiencies vary according to the number of nematodes per sample. Table 1 shows a number of efficiences obtained from the fitted line of Figure 1.

Table 1 Nematode yield efficiencies of Ludox vs. Oostenbrink-sugar

Oostenbrink-sugar counts 200 ml^{-1}	Expected Ludox counts 200 ml^{-1}	Increased percent efficiency
10	95	950
100	417	417
1 000	1 840	184

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