QUANTITATIVE EXTRACTION OF MEIOFAUNA: A COMPARISON OF TWO METHODS

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ABSTRACT

Two methods for the quantitative extraction of meiofauna from natural sandy sediments were investigated and compared: Cobb's decanting and sieving technique and the Oostenbrink elutriator. Both techniques were more efficient with pre-fixed samples than with fresh samples. The results indicated that elutriation is the more reliable method due to its low variability, 7,5% for nematodes and 8,4% for harpacticoids and mystacocarids compared to 11,2% and 29,0% respectively in the case of decantation. However, with samples containing 3 000 to 5 000 animals per 200 cc, decantation yielded 25% more animals than elutriation.

INTRODUCTION

One of the basic requirements of many meiofaunal investigations is the quantitative extraction of the organisms from the substrate. No method yet used successfully extracts all the animals from the substrate (Edwards & Fletcher 1971). Most of the methods, devised in the last few decades, are based on either the decanting and sieving technique (Cobb 1918; Goodey 1949; Heip *et al.* 1974) or Cobb's flotation technique (Cobb 1924). The Oostenbrink elutriator is based on the latter (Oostenbrink 1960).

The decanting and flotation techniques were devised for the extraction of nematodes from agricultural soils but were also found by the authors to be efficient for the extraction of other soil meiofauna. In the present case the elutriator was adapted for use with marine sediments.

The meiofauna studied here is that section of the benthos which passes through a 1,0 mm sieve but is retained on a 0,045 mm sieve.

The aims of the study were to determine the most efficient extractor and whether there was a difference in the extraction between fixed and fresh samples.

METHOD

Description of the apparatus

(i) Oostenbrink elutriator

The Oostenbrink extractor was first made in 1954 (Oostenbrink 1954) but was later modified (Oostenbrink 1960). The extractor used in the present experiments is a further modification utilizing a closed water system as opposed to the open system of the original model.

The apparatus consists of a stainless steel funnel with a trap and water inlet at the bottom through which a controlled flow of water can enter (Figure 1). A 1,0 mm sieve is supported at the top of the funnel by an aluminium frame. This frame also supports an inner plastic funnel connected to an aluminium tube, at the bottom of which is a small horizontal disc of the same material. The outlet, placed between the main funnel and the trap, is closed off during extractions by means of a length of rubber tubing and a clamp. During each extraction water is allowed to rise to the level of the aluminium disc. A sand sample of aproximately 200 cm^3 is washed through the sieve and the inner funnel. The effect is to disperse the sample and to allow the sand to percolate through the water column. The inflow of water through the bottom is kept constant during the addition of the sample and throughout the extraction process. The water flow is maintained by means of a gravity feed system consisting of a feed reservoir and a flow meter (Figure 1). The reservoir is placed some height above the extractor and is supplied with water from a storage tank by means of an automatic electric pump. The automatic control for the pump consists of an electronic controller activated by two reed switches in the feed tank, an "on" switch (M2) near the bottom and an "off" switch (M1) near the top (Figure 2). In case of a malfunction an overflow leading to the storage tank is provided. Between the pump and the feed reservoir is a 5 μm filter which ensures clean water for the extractions. In addition to the automatic filling system a warning system, consisting of a siren, a reed switch (M3) and a float switch was installed. The system gives warning if the pump fails to activate and also when the water reaches the top of the extractor at the end of the extraction process. When this occurs, the clamp on the outlet is released and the water is allowed to flow through one or two 0,045 mm sieves which are supported by a framework attached to a plastic bucket. Water returns from the bucket to the main storage tank via a plastic pipe.

The principle is that heavier sand grains sink to the bottom while the lighter grains and the animals remain in suspension due to the upwelling of the water.

(ii) Cobb's decanting and sieving technique

Essentially the decanting process consists of agitating a sand sample in a volume of water. In the experiments this was done by shaking a 200 cm^3 sample in a 2 litre measuring cylinder together with one litre of sea water. The sample was allowed to settle for 8 seconds to enable the heavier particles to settle before pouring the supernatant through a 1,0 mm and then a 0,045 mm sieve. This procedure was repeated four times.

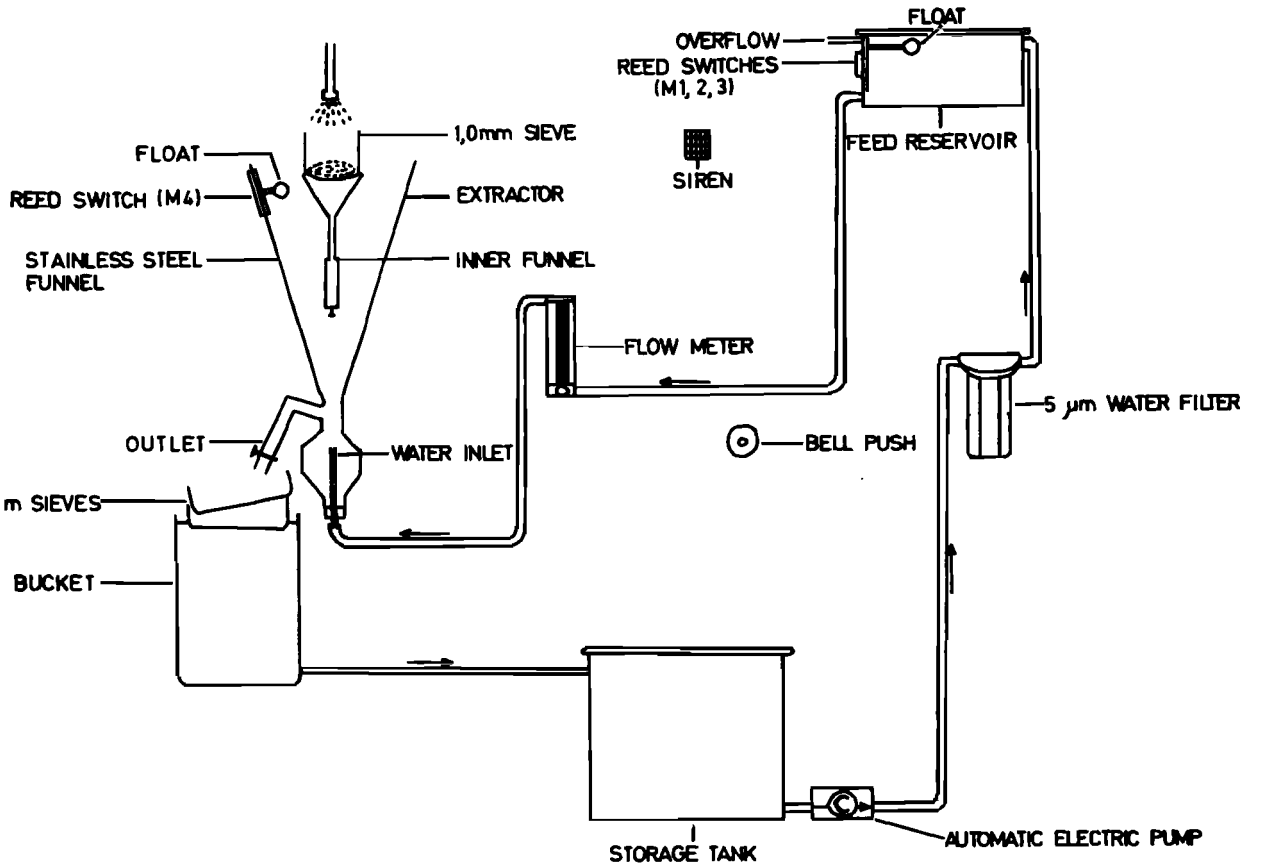

FIGURE 1

The modified Oostenbrink elutriator.

Experimental procedure

Sand was collected for the extraction tests from the mid-tide level at King's Beach, Port Elizabeth, as well as from a sand flat at the mouth of the Swartkops Estuary near Port Elizabeth. These areas have median particle diameters of 200 and 180 µm respectively and a low percentage of particles smaller than 63µm (McLachlan 1977; Dye 1978). A large number of cores from the top 10 cm were mixed and the sand spread in a shallow pan and divided into small squares. Representative 200 cm^3 samples were compiled from six sub-samples taken at random from the pan.

Since the rate of water inflow to the Oostenbrink extractor determines size and number of

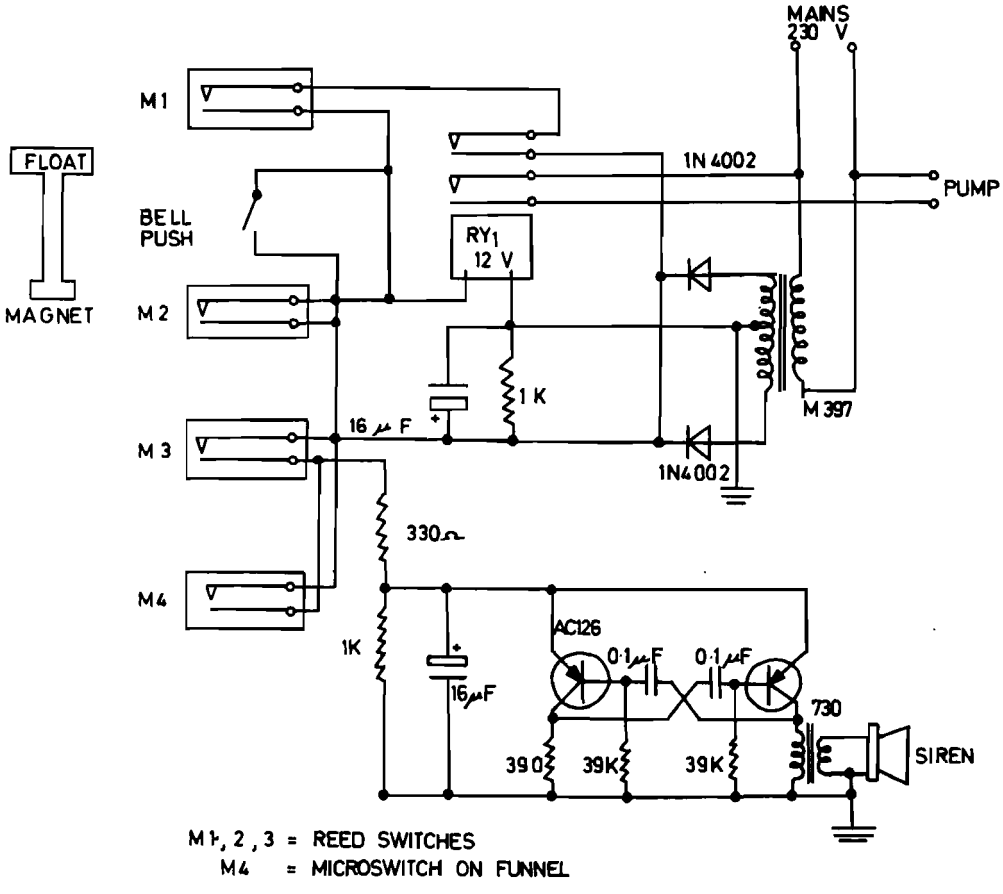

FIGURE 2

Schematic diagram of the electronic controlling system.

particles which will remain in suspension, it is clear that the same flow rate cannot be used for all grades of sand. Oostenbrink (1954) recommended flow rates between 400 and 650 ml/minute. Just as flow rate determines the number of particles remaining in suspension, so will it determine the number of animals in suspension. For this reason it was decided to ascertain the degree to which variation in flow rate affected the extraction for a particular grade of sand. For this purpose three homogeneous 200 cm^3 samples were extracted as described at a flow rate of 400 ml/minute and three at a flow rate of 650 ml/minute.

In addition tests were done to determine whether the extraction was affected by pre-fixing the samples. For both methods six fresh and six fixed samples (8% formalin) were extracted as described and the results compared. Since it is also possible that the number of decantings of a sample may affect the extraction of the decanting process, it was decided to determine the percent extraction per decanting on fresh as well as fixed samples. Six 200 cm^3 samples of each treatment were tested and the yield per decanting recorded.

The efficiency of both methods was determined on sterile sand samples inoculated with known numbers of fixed, stained animals as follows:

TABLE 1

Yield in No./200 cm^3 from extractions carried out by elutriation at flow rates of 400 and 650 ml/minute.

Taxon	Flow rate in ml/minute	
	400	650
Nematodes	319	410
	400	435
	390	495
Mean count*	368	445
Confidence interval†	(298; 453)	(378; 524)
Harpacticoids/Mystacocarids	28	30
	15	15
	10	15
Mean count*	16	19
Confidence interval†	(7; 39)	(10; 37)

*Geometric mean.

†Individual ninety percent confidence interval for the true mean count.

(a) Twelve 200 cm^3 samples were each inoculated with 100 nematodes, 100 harpacticoid copepods and mystacocarids (Crustacea: Mystacocarida). (Elutriator only).
(b) Six 200 cm^3 samples were inoculated with 300 nematodes, 300 harpacticoid copepods and mystacocarids.
(c) Six 200 cm^3 samples were inoculated with 50 nematodes, 50 harpacticoid copepods and mystacocarids.

From the yield in each case the percent efficiency of the method could be determined.

The extraction of the two methods for fixed samples was compared by using the samples mentioned above as well as six additional 200 cm^3 samples—three for each method.

RESULTS AND DISCUSSION

The statistical analyses performed on yield (except for the coefficients of variation of Table 6) were done on the logarithm of yield (Snedecor & Cochran 1967, Section 11.17; Tukey 1977, Section 3F) and the calculations dealing with percentages, on the logit of the percentages (Tukey 1977, Section 15B).

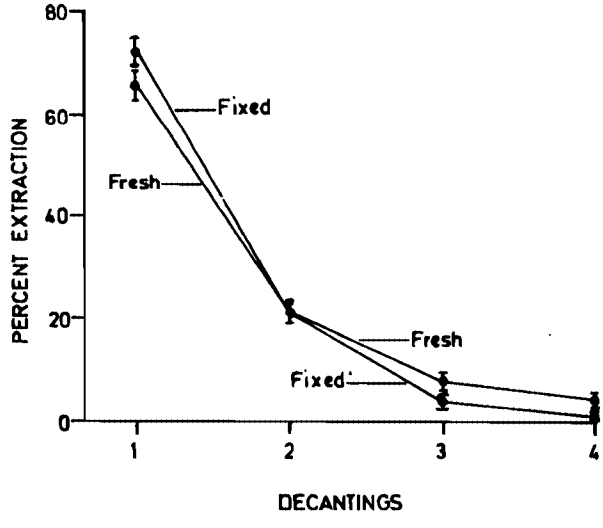

FIGURE 3

Percent extraction of meiofauna after successive decantings of fixed and fresh samples. The results are expressed as average percentages of the total obtained after four decantings. Ninety percent confidence intervals for the true average percent extracted with every decanting are indicated.

Elutriator flow rate

Table 1 gives the total yield in number/200 cm^3 from samples extracted by elutriation at flow rates of 400 ml/minute and 650 ml/minute respectively. The number of nematodes increased on the average by 21% with increasing flow rate (ninety percent confidence interval for the true average percentage increase: 0%; 47%). No significant difference in the yield of harpacticoids and mystacocarids was evident (t-test; $p > 0,5$).

Number of decantings

Figure 3 shows the extraction of meiofauna after successive decantings expressed as average percentages of the total obtained after four decantings. With the fourth extraction only 1,4% of the total is obtained for dead animals and 4,2% for live animals, suggesting that even three

TABLE 2

Yield in No./200 cm^3 from fresh and fixed samples extracted by elutriation and decantation.

Method	Fresh	Fixed
Elutriation	2735	3564
	2158	3296
	2006	3151
	2201	3272
	2083	3528
	2095	3477
Mean Count*	2201	3378
Confidence interval†	(2009; 2412)	(3244; 3517)
Decanting	3175	4671
	2770	4630
	3785	4657
	3283	4552
	3316	3132
	3620	3932
Mean count*	3309	4220
Confidence interval†	(3025; 3619)	(3699; 4814)

*Geometric mean.

†Individual ninety percent confidence interval for the true mean count.

decantings are adequate for quantitative extraction. Also, dead animals are extracted more rapidly, i.e. require fewer decantings than live ones.

Fresh vs. fixed samples

The results of elutriation extractions of fixed and fresh samples at a flow rate of 650 ml/minute, are given in Table 2. The average yield from fixed samples was 53% higher than for fresh samples (ninety percent confidence interval for the true average percentage increase: 39%; 70%). Furthermore, yields obtained from fixed samples were less variable than those from fresh samples (F-test; $p < 0{,}10$).

Table 2 also gives the results of extractions of fixed and fresh samples by decantation. As

TABLE 3

The efficiency of the elutriation and decantation extraction methods expressed as percentage of the original number of animals in each sample.

Taxon	Original number/200 cm³					
	Elutriation				Decantation	
	50	100		300	50	300
Nematodes	90	70	84	79	62	70
	72	74	88	70	70	68
	70	92	82	73	58	74
	72	86	80	81	64	70
	76	84	82	72	78	76
	70	80	82	86	56	78
Mean count*	76,1	82,7		77,4	65,1	72,8
Confidence interval†	(67,4;83,0)	(79,4;85,6)		(71,6;82,3)	(57,7;71,8)	(69,4;76,0)
Harpacticoids/Mystacocarids	74	78	76	77	32	69
	70	90	80	74	68	52
	70	74	82	78	28	58
	74	68	82	74	60	68
	70	76	84	76	62	48
	70	90	82	78	52	59
Mean count*	71,4	81,0		76,2	50,1	59,2
Confidence interval†	(69,6;73,1)	(77,3;84,2)		(74,7;77,7)	(36,1;64,1)	(52,1;66,0)

*Calculated on the logit scale.

†Individual ninety percent confidence interval for the true average percent efficiency.

above, the average yield for fixed samples was 27% higher than those for fresh samples (ninety percent confidence interval for the true average percentage increase: 10%; 46%). However, no significant difference in the variabilities was found (F-test; $p > 0,2$). It is thus concluded that fixed samples are preferable for both methods.

Extraction efficiency

The percent efficiency of the Oostenbrink and decantation methods is given in Table 3. The average efficiency for extraction of nematodes by elutriation, found to be independent of the number of animals in the sample (analysis of variance; $p > 0,10$), was 78,9% (ninety percent confidence interval for true average efficiency: 76,1%; 81,4%). The average efficiency for the harpacticoid/mystacocarid group, however, varied with the number of animals in the sample (analysis of variance; $p < 0,10$).

Using decantation the average efficiency for nematodes was found to vary with the number of animals in the sample (analysis of variance; $p < 0,10$); a higher average efficiency being found for samples containing 300 animals than for samples containing 50 animals. For the harpacticoid/mystacocarid groups the average efficiency was not significantly different

TABLE 4

Yield in No./200 cm^3 from fixed samples extracted by elutriation and decantation.

Method	Nematodes	Harpacticoids/ Mystacocarids
Decanting	440	24
	560	10
	480	10
Mean count*	491	13
Confidence interval†	(400; 603)	(6; 31)
Elutriation	616	16
	544	24
	621	28
Mean count*	593	22
Confidence interval†	(523; 672)	(14; 36)

*Geometric mean.

†Individual ninety percent confidence interval for the true mean count.

TABLE 5

A comparison of the yield of the elutriation and decanting methods using fixed samples.

	Number of nematodes per 200 cm^3			
	25–45	200–250	440–620	3100–4700
Observed average percent increase of Oostenbrink over decanting	116	106	121	80
Confidence interval*	(95; 142)	(94; 119)	(72; 201)	(64; 99)

	Number of Harpacticoids/Mystacocarids per 200 cm^3	
	10–40	140–240
Observed average percent increase of Oostenbrink over decanting	157	130
Confidence interval*	(101; 243)	(110; 154)

*Joint ninety percent confidence intervals for the true average percent increase of Oostenbrink over decanting.

TABLE 6

A comparison of the coefficient of variation (in percent) of the elutriation and decantation methods using fixed samples.

	Number of nematodes per 200 cm^3					
Method	25–45	70–90	200–250	440–620	3100–4700	Mean
Elutriation	10,2	7,1	8,0	7,3	4,9	7,5
Decantation	12,6	—	5,3	12,4	14,6	11,2

	Number of Harpacticoids/Mystacocarids per 200 cm^3			
Method	10–40	70–90	140–240	Mean
Elutriation	15,0	7,9	2,4	8,4
Decantation	44,0	—	14,0	29,0

for samples containing 50 than for samples containing 300 animals (analysis of variance; $p > 0{,}20$). The observed average efficiency was 54,7% (ninety percent confidence interval for the true average efficiency: 40,4%; 68,3%).

Decantation vs. elutriation

The yield and variation of the methods were compared by using the data for fixed samples in Tables 2, 3 and 4.

Table 5 compares the yield of the two methods. For nematodes, elutriation gives a higher observed average yield than decantation (although the true average is not proven higher as a result of wide joint confidence intervals) except when high numbers of nematodes are present, but such high numbers of meiofauna in sandy substrates are very rare. For mystacocarids and harpacticoids elutriation gives a significantly higher yield than decantation over the range covered by the experiment.

Table 6 shows that the coefficients of variation for elutriation are smaller than those for decantation, suggesting that elutriation gives less variable counts. This is supported by comparing a pooled estimate of the variance for elutriation (assumed constant in the logarithmic scale) with a similar estimate for decantation. The ratio of the pooled estimate of variance of the decantation method to that of the elutriator was 2,55 (F-test; $p < 0{,}10$) in the case of nematodes and 10,28 (F-test; $p < 0{,}005$) in the case of harpacticoids/mystacocarids indicating significantly smaller variations for elutriation than for decantation.

It is concluded that the Oostenbrink elutriator is not only more efficient than standard decanting techniques but has an inherently lower variability. Pre-fixing of the samples in 8% formalin increases the yields as well as giving the added advantage that a large number of samples may be taken at one time or from areas where laboratory facilities are not available and some storage time is necessary.

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