EFFICIENCY OF EXTRACTION OF MEIOFAUNA FROM SANDY AND MUDDY MARINE SEDIMENTS.

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ABSTRACT

The efficiency of sorting of meiofaunal taxa from coarse and muddy marine sediments is assessed. For sandy sediments (mean % Silt + clay (S/C) = 8.5), the manual mixing in tap water and immediate decantation, repeated ten times, offers a 100 % efficiency. For muddy sediments, two sets of random samples (mean % S/C = 39 and 56 respectively) were compared using a flotation technique in a high density sugar solution. There are no differences in the efficiency of extraction using: manual mix in 200 mL flasks (method 1) and machine (vortex) mix in several 50 mL centrifuge tubes (method 2). Method 1 is recommended because of shorter time of processing, reduced potential error of manipulation and efficiency is independent of sediment size particle. For quantitative extraction of nematodes, three repeated extractions are acceptable; however, the sorting of less abundant taxa (e.g. copepods) should demand more processing steps.

Key words: meiofauna, sample processing, sorting animals, flotation technique; ASW, Cuba.

RESUMEN

Se estima la eficiencia de extracción de taxa de la meiofauna de sedimentos arenosos y fangosos. Para sedimentos arenosos (% promedio de fango (S/C) = 8.5), la agitación manual en agua corriente e inmediata decantación, repetida 10 veces, ofrece un 100 % de eficiencia. Para sedimentos fangosos, dos series de muestras aleatorias (% promedio S/C = 39 y 56 respectivamente) fueron comparados usando dos métodos diferentes de flotación en una solución de alta densidad. No existieron diferencias en la eficiencia de extracción usando agitación manual en frascos de 200 mL (método 1) y mezcla en varios tubos de centrifuga de 50 mL en un aparato vortex (método 2). El método 1 se recomienda por su menor tiempo de procesamiento, error potencial de manipulación reducido y la eficiencia es independiente del tamaño de partícula del sedimento. Para la extracción cuantitativa de nemátodos, tres extracciones repetidas son aceptables, sin embargo, la extracción de taxa menos abundantes (ej. Copépodos) demanda más pasos de extracción.

Palabras clave: meiofauna; procesamiento de muestras; técnica de flotación; ASW, Cuba.

The meiofauna are a widespread and diverse group of interstitial or epibenthic animals with key ecological roles in marine ecosystems. The identification and enumeration of members of the group for ecological purposes must be carried out under stereomicroscope due to tiny size of organisms. The sorting of animals from sediment is a strongly recommended routine before observation; particularly, when high number of samples should be processed.

In sandy substrates, simple agitation and decantation in tap water is adequate for an acceptable efficiency in the extraction of animals from sediments (Pfannkuche and Thiel 1988; Danovaro *et al.* 2004). For muddy sediments, flotation techniques using a variety of high density solutions (see Pfannkuche and Thiel 1988 for review) is useful and efficient. However, practical applications of such techniques are limited by the relatively high prices of solutions (e.g. Ludox) or by difficulties which arise in the standardization of a routine process among different laboratories. Particularly, the use of sugar solution in the extraction of meiofauna from sandy sediments has been examined by Esteves and DaSilva (1998).

The critical importance of an accuracy assessment of the efficiency of various methods of extraction in quantitative studies and the necessity of determining a simple and inexpensive protocol which can be used to process a high number of samples lead us to state the following objectives: (1) To evaluate the efficiency of extraction in sandy sediments (sample set # 1) using agitation and decantation in water; (2) To compare the efficiency

of two distinct protocols (sample sets # 2 and 3) of extraction from muddy sediments.

MATERIAL AND METHODS

Samples of meiofauna were collected in a variety of subtidal soft bottom habitats (including seagrass beds, sand flats and mangroves) from the NW shelf of Cuba (Gulf of Mexico) in the 2003 and 2004. The samples were taken with hand held corer (i.e. plastic syringe 60 mL, and 6.16 cm^2 area) to sediment depth of 10 cm and immediately preserved with buffered formalin 4 %; after, in the laboratory each sample was sieved by 500 and 45 µm with filtered tap water. From a complete collection of samples (n = 112), three sets were chosen at random as processed as follow:

Set 1. Ten samples from sandy sediment (average percentage of silt + clay (% S/C): 8.5; range: 1.1 - 12.1%) were selected for the extraction using agitation and decantation in tap water. For each sample, approximately ten parts of water were added to one part of sediment in a container (950 mL) and it was vigorously mixed by movements of rotation during ca. 10 s. The container was allowed to remain immobile by 2 – 3 s permitting the settlement of the sand grains. Immediately after time had passed, the supernatant was decanted onto a 45 µm sieve; the procedure was repeated ten times (i.e. ten extractions for each sample), and finally the material retained in the sieve (all extractions pooled) was stored in a flask and the sediment in another flask.

Set 2. Ten samples of muddy sediment (average % S/C: 39.0; range: 7.0 – 70.4%) were selected for the extraction using it that we named method 1. For each sample, the sediment retained in the 45 µm sieve was passed to a flask (200 mL) and the overlying water was then carefully extracted using a pipette which had a 35 µm mesh fastened at the end. A high density sugar solution was added in a proportion of six parts to one part of sediment. The sugar solution was a supersaturate solution (1.16 – 1.20 g cm^{-3}) of commercial sugar crystals added to filtered tap water. The flask was mixed as in method for sorting sandy sediment (set 1) and allowed to rest for 20-30 minutes in order for the settlement of sediment. Afterwards, the supernatant was carefully decanted onto a 45 µm sieve and the material retained was poured to an independent flask. The procedure was repeated three times for each sample (i.e. three extractions). As result each sample yield three flasks with supernatant, each one containing the result of one extraction, and a fourth flask containing the sediment.

Set 3. Six samples of muddy sediment (average % S/C: 56.3; range: 12.9 – 87.6%) were selected for extraction using method 2. For each sample, the material retained in the 45 µm sieve was transferred to four or five centrifuge tubes (50 mL) maintaining approximately 10 – 12 mL of sediment in each tube. When sediment was completely settle (around 15 – 20 minutes), the overlying water was carefully extracted with a pipette and the high density sugar solution (same as in method 1) was added in proportions of four parts to one part of sediment. Each tube was mixed in a vortex machine (Heidolph Top Mix 94323) for three minutes to high speed, after it was allowed to rest for 20–30 minutes and then the supernatant was poured onto a 45 µm sieve. The procedure was repeated three times for each sample (i.e. three extractions), resulting in four flasks for each sample (as in method 1).

The content in each flask (containing supernatant = sorted meiofauna or containing sediment = non-sorted meiofauna) was preserved with formalin 4%, stained with alcoholic eosin 1% and carefully examined under BMC-9 stereomicroscope (56X maximum); i.e. sediment was observed under microscope, and non-sorted meiofauna quantified . The meiofauna were identified to major taxa (e.g. nematodes, copepods).

The percentage of S/C was determined by first obtaining a 100 g sample of sediment dried at 70°C, and later passed through a 63 µm sieve by wet sieving. The sediment retained in the sieve (sandy fraction) was dried again and weighed. The difference of weight between the whole sample and the sandy fraction was considered as the silt + clay fraction and expressed as a percentage of the whole weight.

The total of meiofauna in a sample was calculated by sum of number of individuals counted in all steps of extraction plus in sediment. The efficiency of extraction was defined as the number of individuals sorted in an event of extraction divided by the total of meiofauna in the correspondent sample. As the number of individuals in each sample was highly variable, for comparative purposes each number of individuals was expressed as percentage of total. The data of efficiency of extraction (in percentage) for three variables (number of nematodes, copepods and other meiofauna) were transformed as ArcSin $(x)^{1/2}$

in order to eliminate the correlation mean – variance; it was done successfully. Nested ANOVAs were applied for test significant differences between methods (two levels: 1 and 2); and among extractions (four levels: 1, 2, 3 and Sediment) nested within method.

RESULTS

Extraction from sandy sediment (set 1)

Copepoda were the dominant taxon (average: 58% of total meiofauna; range: 33 – 78%) and Nematoda ranked second (average: 27%; range: 9 - 60%). No meiofauna were detected in the sediment after decantation, hence resulting in 100 % efficiency for taxa and sample.

Comparison of methods (set 2 vs. 3)

Nematoda were the most abundant taxon accounting for an average of 84% (range: 55 – 99%) of total meiofauna (includes all recorded animals in supernatant and in the sediment in both summed sets), and second in rank were Copepoda (average: 9%; range: 0 – 41%). Other meiofauna included ten major meiofaunal taxa; the mostly of them being annelids or peracarid crustaceans.

A nested ANOVA showed no difference in the percentage of nematodes, copepods and other meiofauna between methods (Table 1). There were significant differences among extractions nested within methods (Table 1) for nematodes and other meiofauna. The first extraction had a higher efficiency (i.e. almost 50% of nematodes were extracted) than the other (Fig. 1). There were no differences among extractions nested within methods for copepods (Table 1).

There was a significant correlation between % S/C and the efficiency for the extraction in method 2, however, no correlation was detected for method 1. There is a greater variance (dispersion) for the efficiency values corresponding to method 1 in comparison with method 2 (Fig. 2).

DISCUSSION

Extraction from sandy sediment (set 1)

The agitation – decantation method used in the extraction of the meiofauna exhibited perfect efficiency, in accordance with the efficiency ranges (98 – 100%) reported by Danovaro *et al.* (2004). The method is useful for samples with a content of S/C of 20 % or less, whereas, for higher contents of S/C the quantity of fine particles of sediment that fallen to the sieve during decantation increases the time of observation under the stereomicroscope and also increases the difficulty of detection and extraction of the animals.

Comparison of methods (set 2 vs. 3)

The use of Ludox – AM as high density solution in combination with centrifugation offers efficiency of 98 – 100 % (Pfannkuche and Thiel 1988); however high prices and toxicity of Ludox – AM solution prevent its use in some laboratories, in this situation the use of sugar solutions is an alternative method.

In a preliminary survey, centrifugation (centrifuge Janetzki T32B) was applied to separate the samples in two phases (supernatant and pellet); however, the efficiency of extraction for any sample was always less than 60%. Three possible causes of low efficiency using centrifugation are suggested: (1) the low centrifugal force applied to the samples (range: 250 – 520 x g), since the intensity of this force directly affects the efficiency (Heip *et al.*, 1974); (2) the narrow diameter of centrifuge tube (2.8 cm), which reduces the area for the exchange of animals between the two phases, however Burgess (2001) reported very high efficiency (> 95% for most taxa) using 50 mL centrifuge tubes; and (3) low volumetric proportion of sugar solution/sediment, which causes a dilution of the solution with the interstitial water. Anyways, if centrifugation is carried out, the centrifugal force should be higher than used in present study and the centrifuge tubes should be the widest possible and, in addition, carry relatively low amounts of sediment.

Method 2 demands more time and greater manipulation of samples (with consequent inherent sources of error) when compared to method 1. The relative higher surface area of the walls of 50 mL tubes increases probabilities of animal adhesion; additional drawbacks of the use of centrifuge tubes are mentioned above. Also, the processes of water extraction with a pipette and the decantation of the supernatant unto the sieves demand extreme care due to small diameter of tube. The use of an automatic device (vortex) for the mechanical mixing of the samples resulted in a high efficiency for nematodes (97% after Burgess 2001) and we obtained only slightly lower values of efficiency (95%).

Table 1. Results of nested ANOVA on transformed data as arcsin $x^{1/2}$ for method and extraction (nested within method). F-values and probabilities (in brackets) are showed.

Source of variation	Nematodes	Copepods	Other
Method	0.12 (0.72)	1.00 (0.32)	0.16 (0.69)
Extraction (method)	**11.7 (<0.001)**	0.80 (0.58)	**2.62 (0.03)**

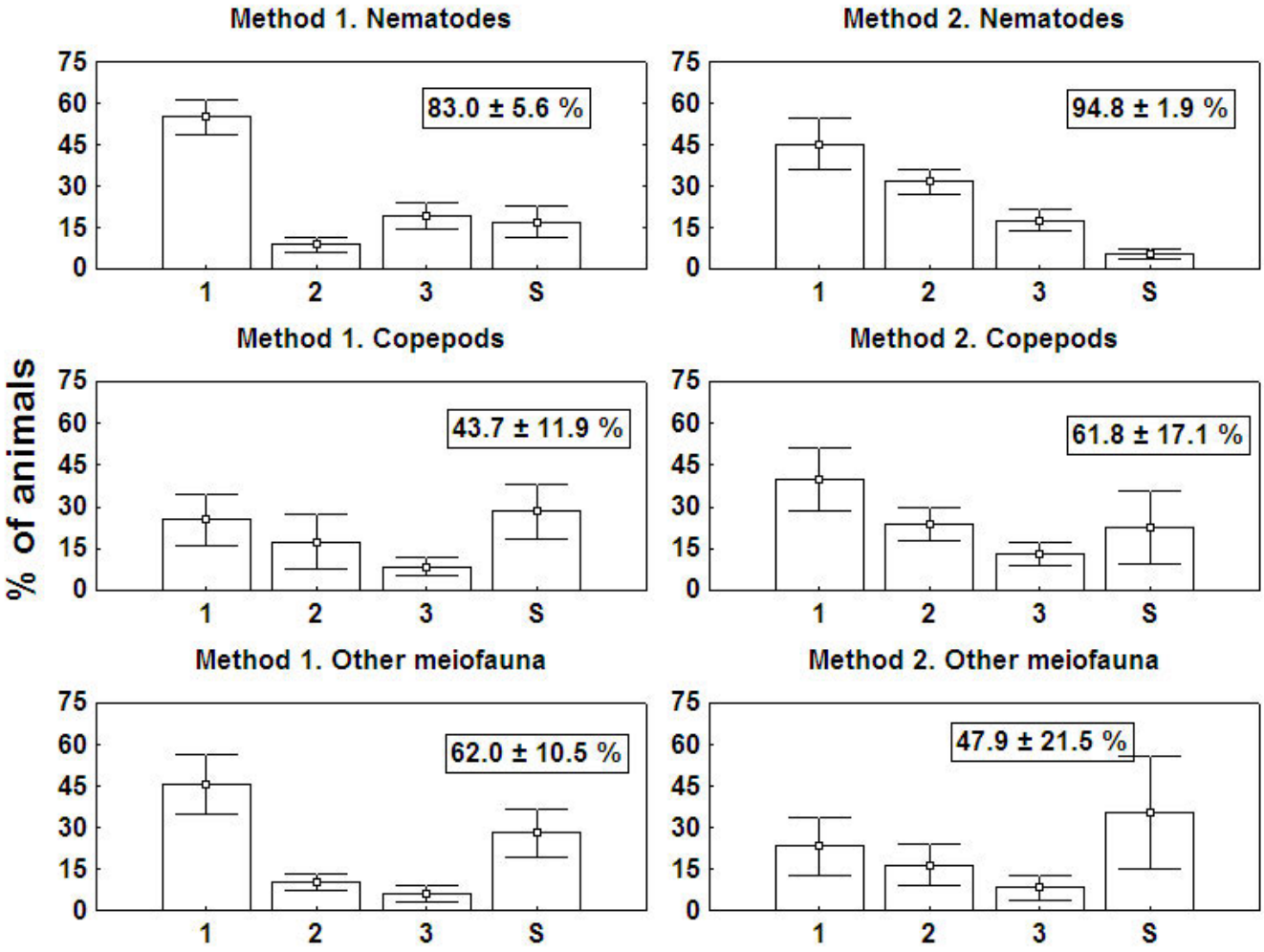

Fig. 1. Mean values ± SE of the percentage of nematodes, copepods and other meiofauna in extractions 1, 2 and 3 and in sediment (S) for two distinct sorting methods. Values inside the boxes represent mean value and SE of total efficiency (three summed extractions). Number of observations: 10 for method 1 and 6 for method 2.

The relative high variance in the efficiency values (Fig. 2) for the samples from set 2 (method 1) would mask the difference between both methods (i.e. due to lack of statistical power). We explained the high variance by differences in specific composition of nematode assemblages in the samples (e.g. some species of Chromadorids attach to sand grains affecting the extraction, after de Jonge and Bouwman 1977); this is particularly possible due to a relative extensive area (ca 200 km^2) from where the samples were obtained.

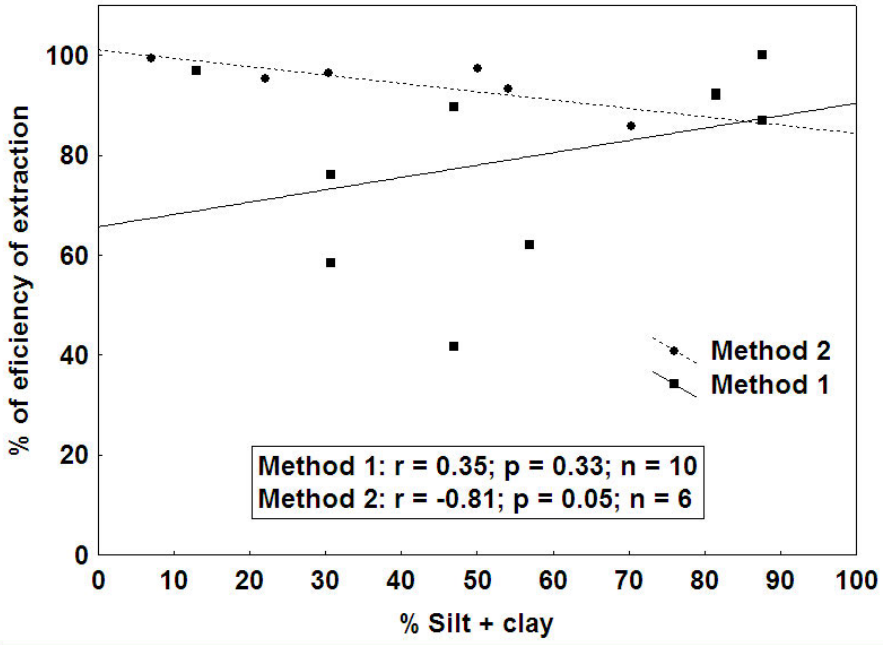

Fig. 2. Relationship between efficiency of meiofaunal extraction (all summed taxa) and size particle (% silt + clay) for two distinct methods. Values of coefficient of correlation of Pearson (r), statistical significance (p), and number of observations (n) are showed.

Another cause for the high variance in method 1 could have been accredited to the presence of very fine sediments (i.e. finer sediments reduce efficiency), however, this reason had to be dismissed due to the fact that samples with high content of silt + clay also show high values of efficiency (Fig. 2).

The efficiency of extraction for method 1 was reasonably independent of particle size, a feature very convenient in quantitative studies dealing with a wide range of particle sizes. The negative correlation between efficiency and % silt + clay in method 2 would be explained by a higher consolidation of sediment due to longer high frequency vibration by the vortex apparatus. Fine sediment tends to compact more than coarse, thus preventing the flotation of animals potentially concealed within interstitial spaces. Burgess (2001) did not detect major trends between efficiency and sediment type using a methodology similar to our method 2, however, higher content of the fine sediment fraction in our study would account for this difference.

In the sorting of nematodes from sediment samples the first extraction is the most important but to guarantee more than an 80% yield, at least two more are necessary. If the goal of the investigation is to assess the diversity of the rarer taxa found in muddy sediments, for example copepods, more than three extraction steps should be done. The compromise between the number of samples to be processed and the accuracy of assessment of meiofaunal community should be carefully evaluated.

In summary, taking method 1 into account: (1) its efficiency being statistically similar compared to method 2; (2) its simplicity of application; and (3) its lack of relationship between size particle and efficiency, we recommend its use over the later. For quantitative surveys of meiofauna, no less than three extractions are necessary.

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