Ultrasonic treatment of sediment samples for more efficient sorting of meiofauna[1]

Abstract—The ultrasonic treatment of meiofaunal samples breaks up sediment aggregations, permitting more efficient washing of the samples and reducing sorting time. Such treatment also allows recovery of specimens associated with fecal pellets and mud balls, and it cleans Foraminifera, improving their staining properties. Damage to agglutinating Foraminifera and certain other taxa limits the utility of the technique.

The importance of meiofauna in benthic associations is well established (e.g. Gerlach 1971), but most quantitative studies are based on a small number of samples because sorting the animals from the sediment is extremely time consuming. Sorting time depends on the type of sediment, its detritus content, and its suitability for ecological or mechanical presorting methods (Uhlig et al. 1973). Sorting time can be as long as 40 h per 10-ml sample and is a major limiting factor in meiofaunal research.

The sediment at the site of our quantitative investigations of the meiofauna of the San Diego Trough consisted mostly of fecal pellets, ranging in size from that of coarse sand to that of coarse silt and retained by meiofaunal screens. The sorting process was very slow until we found that ultrasonic treatment of samples would fragment the fecal pellets so that a substantial portion of their constituent particles would pass our smallest screen (42 μm). This paper discusses the efficiency of the method in reducing sample volume, together with the advantages and disadvantages of working with ultrasonically treated samples.

Two types of ultrasound generators were used in the experiments: a water bath (Branson automatic cleaner model D-50) and a soniprobe (Branson Sonic Power Co. model J-17A). The sample (ca. 3 ml) to be sonified was washed into a 250-ml beaker and the volume made up to 200 ml. Either the beaker was immersed in the ultrasonic bath or the sonifying tip was submerged in the beaker. The bath had a single intensity; the soniprobe was run on its lowest intensity. Ultrasound was applied from 10 to 60 s, the sample was washed from the beaker into a set of screens, and the residues were concentrated for staining and sorting.

The efficiency of sonification in breaking sediment aggregations, and thereby reducing the volume of the sample to be sorted, varies with the duration of the treatment. A 10-s application of the soniprobe results in about a 40% reduction in sample volume; exposures of 30–60 s give a 60–70% reduction. Ultrasonic treatment can decrease substantially the sample volume to be sorted.

Though sorting efficiency is maximized by long exposure to ultrasound, a shorter duration may be desirable because of the adverse effect of the ultrasound on specimens, discussed below. In our case, 30 s of ultrasound treatment permitted us to sort quantitatively all preservable meiofaunal taxa except agglutinating Foraminifera: selected subsamples were sorted without ultrasound treatment for information on these. This combination of procedures reduced our sorting time by 30%. However, no general formula that would apply for all sediment types can be provided.

Ultrasound treatment damages some organisms exposed for periods as short as 10 s. Alcohol-preserved harpacticoid copepods varied in their condition; some were undamaged but many had pieces, such as parts of appendages, broken off. Fifteen-second exposures resulted in a greater proportion of damaged copepods with some broken in half. Specimens preserved in Formalin appear to be less damaged by ultrasound than living or alcohol-preserved

[1] This research was supported by National Science Foundation grant GA 40322. This study is report No. 8 of the results of Expedition Quagmire. R. R. Hessler read and commented on the manuscript. K. H. Nealson and W. A. Newman kindly permitted the use of their ultrasound generators.

Table 1. Meiofauna removed from an unsorted fraction 100–150 μm after sonification.

Taxa	100-150 μm (% of total)	42-100 μm
Agglutinating Foraminifera	14 (17)	67
Calcareous Foraminifera	42 (37)	72
Nematoda	22 (52)	20
Benthic Copepoda	41 (87)	6
Nauplii	12 (63)	7
Other Metazoa	12 (80)	3
Total	157 (44)	196

material. Agglutinating Foraminifera may be fragmented and lost during 10 s, although some species resist sonification for at least 60 s. Nematodes and calcareous Foraminifera are not damaged.

Ultrasound treatment changes the sieving characteristics of the fauna as well as that of the sediment. In our investigations, each sample is divided into size classes with sieves. This procedure improves the accuracy of sorting under a dissecting microscope because the particles in the subsamples are of similar size. To explore the effect of ultrasound treatment on the size distribution of organisms, an initially unsorted 100–150-μm sample was sonified and washed through 100- and 42-μm screens. After sonifying, only 44% of the meiofauna was retained by the 100-μm sieve. The percentage varied considerably for the various taxa (Table 1). Two processes seemed to be involved: the liberation of animals previously in or attached to fecal pellets or mudballs, and the alteration of sieving characteristics by changed particle size. The former is demonstrated by the fact that more organisms are found in sorted samples after sonifying and re-sorting than can be accounted for by our known sorting error.

Ultrasonic treatment improves the staining characteristics of Foraminifera by cleaning the test, permitting better stain penetration. Since foraminiferans are stained with rose bengal to discriminate animals living at the time of sampling from dead tests, ultrasound treatment increases the accuracy of counts of living Foraminifera. We counted up to 30% of the living foraminiferans in a second sorting after ultrasound treatment. This high proportion cannot be explained by mistakes in the first sorting because, again, it greatly exceeds our known sorting error. These individuals must either have taken up stain after ultrasonic cleaning or have been released from sediment aggregations.

Hjalmar Thiel

Institut für Hydrobiologie
und Fischereiwissenschaft
der Universität Hamburg
2 Hamburg 50, West Germany

David Thistle
George D. Wilson

Scripps Institution of Oceanography
La Jolla, California 92037

References

Gerlach, S. A. 1971. On the importance of marine meiofauna for benthos communities. Oecologia (Berl.) **6**: 176–190.

Uhlig, G., H. Thiel, and J. S. Gray. 1973. The quantitative separation of meiofauna. Helgol. Wiss. Meeresunters. **25**: 173–195.

Submitted: 5 September 1974
Accepted: 20 January 1975