Meiofauna in Marine and Freshwater Sediments

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The Role of Meiofauna in Marine and Freshwater Ecosystems

The meiofauna is defined on a methodological basis as all metazoans retained on a sieve of 42 μm (Mare, 1942). Meiofauna occur in freshwater and marine habitats, although most ecological studies on meiofauna have been performed in the marine environment. Meiofauna occur from the splash zone on the beach to the deepest sediments in the sea: they are found in all types of sediments (clay to gravel), are common as epiphytes on seagrasses and algae, in sea ice and in various animal structures (as commensals or parasites). Only members of the free-living interstitial community which live between sediment grains will be described in this chapter.

Twenty-three higher taxa of the thirty-three metazoan phyla have some meiobenthic representatives: *Nematoda, Turbellaria, Oligochaeta, Polychaeta, Copepoda, Ostracoda, Mystacocarida, Halacaroidea, Hydrozoa, Nemertina, Entoprocta, Gastropoda, Aplacophora, Brachiopoda, Holothuroidea, Tunicata, Priapulida, Sipunculida.* The phyla *Gastrotricha, Gnathostomulida, Kinorhyncha, Loricifera* and *Tardigrada* are exclusively meiobenthic (meiobenthos = meiofauna living in sediments). Nematodes, copepods and turbellarians comprise more than 95% of the meiofauna in most sediments. A comprehensive guide on sampling procedures and ecology of the meiofauna is presented in Higgins and Thiel (1988) and in Giere (1993). In this chapter, a general methodology about meiofauna is presented first and then nematodes, copepods, kinorhynchs, gastrotrichs, gnathostomulids, nemertineans, tardigrades and oligochaetes are discussed briefly.

In the last 20 years, much ecological information about meiofauna is illustrating more and more the picture that these small creatures are important in

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marine sediments, both indirectly, by processes such as bioturbation and the stimulation of bacterial metabolism, and also directly, as food sources for organisms in higher trophic levels, such as shrimp and juvenile fish. They also play an important role in pollution research.

On average, 1-10 million individuals of meiofauna are present in 1 m^2 sediment, which represents, however, a biomass of only a few grams. These abundance:biomass ratios vary according to temperature, water depth, tidal exposure, grain size, etc. Meiofaunal productivity, however, is very high with P/B ratios ranging from 6 to 50 (Vranken *et al.*, 1986).

In almost all meiobenthic studies, the majority of the fauna has been found in the upper 2 cm of sediment. Vertical zonation is typically controlled by the depth of the redox potential discontinuity (RPD) layer, i.e. the boundary between aerobic and anaerobic sediments. The primary factor or 'super parameter' responsible for vertical gradients in the RPD is oxygen, which determines the redox potential, as well as the oxidation state of sulphur and various nutrients. When redox potentials drop below +200 mV (measured with microelectrodes, and not always directly by the colour change from the grey-brown of the upper sediment layer to the black of the deeper layers), metazoan meiofauna densities greatly decrease (McLachlan, 1978). Nevertheless, numerous geochemical cycles and microbial processes together with the bioturbative impact of sediment fauna create a complicated three-dimensional 'landscape of the redox potential' (Ott and Novak, 1989) with oxic/anoxic areas often surrounding a vertical redox threshold. The sulphidic ecosystem, dominated by the presence of reduced substances such as dissolved sulphide, methane and ammonium, is much too complex to allow for simple answers to the previous debate about the existence of a 'thiobios' (Giere, 1993 and references therein). Typical representatives of the reduced redox potential layers are specialized nematodes, oligochaetes and turbellarians. Sediment grain size is another key factor affecting the structure of meiobenthic communities (density, biomass and diversity).

Sampling/Extraction Techniques

Sampling strategy has to be adapted to the kind of sediment being examined (Kramer *et al.*, 1992), although we aim at one sampling technique for several types of sediments in order to work with a standardized technique for biodiversity comparison between different regions. Baseline studies are missing for most of the habitats still to be investigated for biodiversity studies, and, therefore, one standardized method is presented in this chapter.

Almost all investigators have considered the problems associated with sampling, and many have arrived at individual solutions to their problems. The more common sampling methods and equipment for use in a wide variety of habitats are described here.

Biodiversity measurements are only relevant if they can be calculated from samples taken on a quantitative basis and so only quantitative sampling methods are discussed (of course, all processing methods are suitable for qualitative methods as well).

Sampling methods, the number of samples, fixation and extraction techniques are mostly the same for all meiofauna groups (certainly for the hard-bodied taxa such as nematodes, copepods, etc.). Microscopical examination and identification will be discussed for the dominant taxa separately.

Sampling methods

Methods for meiofauna in general, as well as for all the individual taxa, have been discussed in detail in Higgins and Thiel (1988).

Coring

In sediments, coring is the best quantitative sampling technique. Corers are devices of known sampling area. Most are cylindrical and can be made from tubing or piping of any rigid material locally available. The diameter chosen depends on the volume and depth of the sample required and whether the reduced layers are being sampled or not. Corers with an inner diameter of 2 cm have been used in many habitats and provide a meiofaunal sample that can be sorted in its entirety. Smaller corers (1 cm diam. or less) are desirable in sediments where densities are usually high, or to determine small-scale distributions. **For biodiversity studies, corers of 2-4 cm diam. up to at least 5 cm sediment depth are recommended.** Kramer *et al.* (1992) mentioned that there is no optimum between surface area (corer diam.) and number of organisms per sample. In practice, experienced scientists have arrived at a procedure that involves somewhat larger size samples in sandy sediments and smaller ones in muddy sediments. However, for the most dominant phyla of the meiofauna, the nematodes, it has been shown by several authors that a standard corer surface of about 10 cm^2 is appropriate for all types of sediment.

In intertidal areas, tubes with the characteristics described above may serve as a sampler for the upper portion of the sediment. A tight-fitting stopper secured in the upper end of the coring tube or the piston of the syringe will provide suction to hold the sediment in place while the corer is removed. Another stopper may secure the lower end of the tube for transport.

In deeper waters, the different types of box corers or multiple corers are to be preferred to grabs (Bett *et al.*, 1995). An ideal corer should penetrate the sediment without a shock wave and as slowly as possible. Out of the box corers, similar sampling corers can be used as for the intertidal conditions.

Number of Samples

The number of samples (as well as sample size) depends on the problem being studied. Variability in density of meiofauna appears to be mostly on a scale of a few centimetres and again on a scale of many km, or even more, depending on the substratum heterogeneity. In studies, aiming at studying small-scale horizontal distribution obviously the largest amount of the smallest possible samples is required, with the important restriction that sample size must be large compared with the individuals being sampled. When the problem consists, as is usually the case, in obtaining an estimate of meiofaunal density and diversity, there may be two alternative solutions. The first is to destroy all small-scale variability in the sample and take a sample as big as possible (e.g. mixing three corers), mix it thoroughly and, if necessary, take subsamples for further analysis. Second, when the intrinsic pattern is not destroyed, aggregation will require that the sample size is as small as possible and the number of samples taken as large as possible. Most statistical analyses are robust when they are based on a large number of error degrees of freedom (Green, 1979) and so, for 'general' biodiversity studies, it is recommended that three replicates of 10 cm^2 are analysed separately.

Fixation

Sediment samples should be stored, after addition of 4% (v/v) neutral formalin, in warm (60°C) seawater solution, in polyethylene bottles prior to analysis. A warm solution of formalin is advised to prevent nematodes rolling up, which will make identification nearly impossible. Formalin may be neutralized with a saturated solution of $LiCO_3$, taking care that no excess buffering solution is added. (Excess $LiCO_3$ causes crystallization of formalin around the animals, which makes them impossible to identify.) Preserved samples may be stored for many years until analysis.

Extraction Techniques

Decantation

The extraction of meiofauna from sediments is easy when the sediment is a sand with low amounts of detritus or silt-clay. Simple decantation on a sieve is often satisfactory and a 38-42 µm sieve is standard for meiofauna work. For decantation:

- put the sample in a vial of 5 litres and stir well
- put the supernatants on a sieve of 38 µm diameter
- repeat this action 10 times on the same sieve.

Density gradient centrifugation

The extraction from mud or detritus (after the sand has been removed by decantation or other methods) is done most efficiently using a density gradient in a centrifugation procedure (Heip *et al.*, 1974, 1985). Three possible liquids with a density larger than the density of meiofauna (i.e. 1.08 for nematodes, but this seems to be workable for all hard-bodied meiofauna) can be used, e.g. 'Ludox HS40', 'Ludox AS' and sugar. 'Ludox' is a silicasol (a colloidal solution of SiO_2), which causes no plasmolysis. 'Ludox HS40' is toxic and so may be used for fixed material. 'Ludox AS' (the most expensive) is not toxic and can be used when living meiofauna has to be separated from sediments. For both types of 'Ludox', a 50% solution in distilled water is used (density of 1.15). A sugar solution is made by adding sugar to 700 ml hot water, until 1000 ml is reached, and is best used when sediments contain a lot of organic detritus (e.g. in salt marsh and mangrove sediments). The method developed in our laboratory consists in the slightly modified procedure of Heip *et al.* (1985).

- Rinse the fixed sample thoroughly with tap water, to prevent flocculation of 'Ludox', over a sieve of 38 μm. Decantation can be done comparable to the sandy sediment sample.
- Bring the sample from the sieve in a centrifugation tube as large as available
- Add the 'Ludox' solution (60% 'Ludox' and 40% water; density = 1.18).
- Centrifuge at 1800 *g* in water for 10 min.
- Repeat the centrifugation three times more.

The supernatant is finally rinsed over a 38 μm sieve for some time with water, because 'Ludox' and formalin react and form a gel which is difficult to wash out. Centrifugation with sugar only takes 5 min, but requires washing with much water afterwards, because the sugar solution causes plasmolysis in nematodes.

After extraction, 4% neutral formalin is added again to the treated sample. Counting is facilitated by staining of the entire sample with rose bengal (1% for 48 h). Preserved samples can be stored until analysis.

Counting

Using a stereomicroscope and a counting box with a grid of 10 × 5 or 10 × 10, the densities of animals are determined either by counting all specimens or by taking out only the first 200 animals of a specific group encountered (e.g. the first 200 nematodes, which will be identified to species level and, therefore, mounted in glycerol).

Microscopic Examination and Identification

Nematodes

After fixation, nematodes must be transferred to anhydrous glycerol. Specimens are transferred from formalin to glycerol through a series of ethanol-glycerol solutions to prevent the animals from collapsing. They are picked out and put into a cavity block (recipient) under a stereoscopic microscope into a solution of 99% formalin (4%) and 1% glycerol. The recipient is then put into a vial containing a bottom of 95% (v/v) ethanol at 35°C for about 12 h.

At 35°C, ethanol is evaporated into the solution of formalin and glycerol. After 12 h (e.g. overnight), the cavity block (with nematodes) is partly covered and put in an oven at 35°C. Every 2 h, some drops of a solution of ethanol with glycerol (95%:5% v/v) is added with a pipette. After about 6 h, some drops of an ethanol and glycerol (50%:50% v/v) solution are added. The cavity block stays partly open at 35°C until all ethanol is evaporated and the nematodes remain in pure glycerol.

Animals may be mounted on glass slides when in glycerol. For this a paraffin ring is put on a slide, within a small droplet of glycerol and 5-10 nematodes of about the same thickness are put into the glycerol drop. A cover glass is put on the droplet and slightly heated at 40°C in order to let the paraffin melt. For permanent slides, the cover glass may be sealed with 'Glyceel', 'Clearseal' or 'Bioseal' (certainly necessary in tropical areas).

Whole preparations are usually satisfactory for species identification. A good-quality microscope with a ×100 oil-immersion lens is required. Comprehensive guides for the identification of nematodes have been available for 10 years, and pictorial keys to genus level are especially useful (e.g. Platt and Warwick, 1983).

Copepods

Sorted specimens should be placed in 70% (v/v) ethanol if prolonged storage is anticipated. Initial identification to species level almost always involves examination of the appendages and setation, and may required observations of the head appendages and body surface spinulation patterns. Such details are seen most clearly in dissected specimens mounted on slides. Once species have been identified, they can be recognized subsequently on gross structures visible with a stereomicroscope. For permanent mounts, whole animals, or dissected parts, should be mounted in a gum-arabic-based medium, or in fluid mountants, such as glycerol or lactic acid. Lactic acid is a powerful clearing agent and is inadvisable for long-term storage, but this problem is substantially reduced if it is mixed with glycerol (at about 1 part of lactic acid in 4 of glycerol). Polyvinyl lactophenol has been widely used, but tends to shrink with time and there is little doubt that the gum arabic mountants give the best long-term results (Wells, 1988).

Kinorhynchs

Sorted specimens should be placed in 70% (v/v) ethanol, if prolonged storage is anticipated. The identification of kinorhynchs demands that the specimens be mounted in a perfectly dorsoventral aspect; specimens mounted otherwise are virtually worthless. Specimens should be moderately cleared in order to observe the details of external morphology (use 62.5% v/v chloral hydrate solution) (Hoyer's medium). Carefully sealing the cover slip is extremely important in producing a permanent mount (Higgins, 1988).

Turbellarians

(See this volume, Chapter 17, for details of this group.)

Gastrotrichs, gnathostomulids and nemertineans

Since gastrotrichs are soft-bodied meiofauna, identification and extraction methods are the same as those described for turbellarians (see this volume, Chapter 17). Since many gastrotrichs are extremely small, only small sediment quantities should be used when applying the $MgCl_2$-decantation method. A fine mesh size of 35 µm is recommended and an Erwin loop is better than a pipette to pick them out. Gnathostomulids are mainly found in larger quantities of sediment.

Tardigrades

Animals are transferred with a drop of 2% (v/v) formalin to microslides and covered with a cover slip (Kristensen and Higgins, 1984). The formalin preparation is infused with a 10% (v/v) solution of glycerol in 96% (v/v) ethyl alcohol and allowed to evaporate to glycerol over a period of several days. The resulting whole mount can be sealed with 'Clearseal'.

Oligochaetes

Identification of aquatic oligochaetes is, in most cases, possible only if sexually mature specimens are available. Most of the taxonomically important features are restricted to the genitalia and a few other internal structures. For permanent mounts, Canada balsam or artificial balsam can be used as a mounting medium. For temporary mounts of fixed animals, 4% formalin:glycerol is an excellent medium as it has a clearing effect, but does not harden the specimen. The use of lactophenol for clearing/mounting should be restricted to studies where deterioration of the material after some time (several months) is permissible, and where the species studied have enough setal characteristics, such as cuticular penis sheaths, to ensure that identification is possible (provided always that

taxonomy of the species studied has already been well established). Soft internal structures are not always visible in lactophenol (Erseus, 1988; see also this volume, Chapter 20).

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