NEIL C. HULINGS
and JOHN S. GRAY

A Manual for the Study of Meiofauna

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Foreword

The term meiofauna has been in use for only a relatively brief period. It was coined by Mare in 1942 to describe metazoans (and Foraminifera) too small to be retained on the 1-mm sieves used by marine biologists for the extraction of bottom fauna from samples of sediment when analyzing benthic communities. Thus, meiofauna refers only to a size class between macrofauna and microfauna. The term is now used for benthos and phytal fauna, but not for planktonic organisms, which are treated traditionally as a separate category and by special methods.

The variation in form and the biological complexity of the meiofauna make it difficult to determine upper and lower limits in terms of body size which can be applied to all taxa. For the time being, however, the lack of a definitive and generally accepted definition is less important than standardized methods of work for the taxonomists and ecologists studying these organisms.

Practically all classes of the Metazoa, including the smaller species of macrofauna, are represented in the meiofauna, while the Protozoa are represented by their largest forms, e.g., Foraminifera and Ciliata. The smaller protozoans are generally considered as microfauna. Gnathostomulida, Gastrotricha, Kinorhyncha, and Tardigrada are some groups of metazoans that are known exclusively from the meiofauna, while Mollusca, Brachiopoda, Echinodermata, and Ascidiacea are examples of groups represented only by a very few species.

McIntyre (1969) uses the term "permanent members" for species belonging to the meiofauna during the whole of their life cycle. The meiofauna also includes a large number of "temporary members," i.e., larvae and juvenile stages of species that, as adults, belong to the macrofauna.

If we refrain from an exact division according to type of biotope but are satisfied to compare the three main groups—mud, interstitial sand, and phytal meiofauna—we find certain morphological and biological features for each of these categories, reflecting the adaptation of the organisms to particular environmental conditions. These adaptations undoubtedly are most marked in the interstitial fauna of sand. Since Remane's pioneering work in the 1920s the interest of psammonologists has been focused on the study of such adaptations. Within several systematic groups a greater proportion of elongated forms is found than in types other than the interstitial environment. The development of various types of adhesive organs is another adaptation found in many groups belonging to the interstitial fauna. Other adaptations are common for the meiofauna as a whole—for example, reproductive adaptations, which are related to the relatively small number of gametes that small animals produce. Copulation, hermaphroditism, and the formation of egg cocoons, as well as brood protection, improve the prospects of development and ensure the survival of the populations.

The discovery of new groups of animals, particularly in the interstitial sand fauna—often of high taxonomic rank (Gnathostomulida, Mystacocarida, Actinulida, Macrodasyoidea, Acochlidiacea)—undoubtedly has been one of the major contributions to systematic zoology during the present century.

Several research workers regard the many aberrant forms, morphological specializations, and appearance of new organs in the meiofauna as having evolved

under the influence of the unique environment. Neoteny seems often to have been one of the reflections of evolution; it has resulted in a simplified organization compared with the presumed ancestral form and may be manifested in the loss of organs. In addition, neoteny has led to diminution of body size. Specializations such as the appearance of new organs, loss of organs, and reduction in body size have emphasized the systematic isolation of the aberrant species. It is difficult, for example, to demonstrate unequivocally the relationship between the Gnathostomulida and any definite class of the other Metazoa, or to show the affinity of the Archiannelida with a definite systematic group within the Polychaeta. The phylogenetic relationships and the taxonomic problems resulting from regressive evolution must remain unsolved until we discover new links in the often rather fragile morphological series. The definition of phylogenetic relationships is today one of the motives behind the work of many meiobenthologists in the fields of systematics and morphology. The discovery during recent years of a large number of new species of Gnathostomulida, Gastrotricha, Nematoda, and Ascidiacea suggests that systematic zoology may still have much to gain from the studies of meiofauna, especially with the opportunities now available to meiobenthologists to take part in the exploration of the deep sea.

Regardless of the increased interest in meiofauna today, the systematics-morphological field is not yet well covered. Most of the studies to date have been in Europe, thereby leaving much of the rest of the world unstudied. Furthermore, there must be much more research, not only to meet the general needs of systematics and morphology but for progress in ecology.

In the sphere of ecology, Remane's early work also has been of great importance. But it has been during the past ten or fifteen years that the meiofauna has attracted widespread attention of ecologists. Important work has been done by Boaden (1962, 1963a), Gray (1965, 1966a, b), Jansson (1962, 1966, 1967a, d, 1968), Renaud-Debyser (1963), and others concerning, for example, the relation of vertical and horizontal migration to temperature, salinity, and other ecological factors. Such studies have been made in the field and experimentally in the laboratory.

In the field of meiofauna ecologists have interesting and important tasks to perform. Our knowledge of the dynamics of populations of meiofauna is very limited at present. The same is true of our knowledge of the part played by the meiofauna in the organic production within the various zones of the sea. The studies already made usually have been concerned with single species, as a rule within the tidal zone. It is also important to increase our knowledge of trophic relations and the position of the meiofauna in the food chains.

Meiobenthology may be expected to become enriched by several new fields of research. One such field may be the use of species in the meiofauna as test organisms for the study of the biological effect of components of water pollution. In ecological biotest laboratories it is important to study the biological effect during the whole life cycle of the test organism. The rapid development of many of the species in the meiofauna and the relative facility with which they can be cultivated make meiofauna excellent test organisms. The very small size of meiofauna, with organs sometimes composed of a very limited number of cells but which nevertheless are capable of coordinated functions fully comparable to that of much larger organisms, should interest immunologists, biochemists, and physiologists.

The idea of this manual resulted from the International Conference on Meiofauna convened in Tunisia, 1–11 July 1969, by the Mediterranean Marine Sorting Center at Salammbo and by the Smithsonian Institution. Twenty-eight meiobenthologists were given opportunities to confer and take part in valuable discussions contributing to this publication. On the basis of their own experiences, the contributors to this manual have recommended certain methods for the study of the meiofauna. We hope that after being utilized for a few years in different laboratories the manual will be improved and augmented. In any case, we hope it will be of value to both specialists and students, and will contribute toward a standardization of methods for the study of the meiofauna.

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Contents

ABSTRACT

Neil C. Hulings and John S. Gray. A Manual for the Study of Meiofauna. *Smithsonian Contributions to Zoology,* number 78, 83 pages, 1971.—Procedures and methods for the study of meiofauna are presented in terms of general description of the habitat, environmental analyses, general collecting and extracting techniques, general taxonomic procedures, comments on selected taxa, and culture and experimental techniques. Biotopes other than sediment are considered briefly and sources of equipment and materials for the study of meiofauna are listed. A total of 361 references dealing with various aspects of meiofauna are given.

Neil C. Hulings and John S. Gray

A Manual for the Study of Meiofauna

An International Conference on Meiofauna was held in Tunis, Tunisia, 1–11 July 1969. Twenty-eight invited participants from seven countries assembled to review the status of the systematics and ecology of meiofaunal taxa, the ecology of meiobenthic communities, and techniques of faunal and environmental analyses. A total of thirty papers covering the above topics was presented during the first three days of the conference, and these papers will appear in a volume of proceedings of the conference. One day was devoted to field and laboratory demonstrations of equipment and techniques.

The last week of the conference was devoted to committee work. Subcommittees were organized to provide an exchange of ideas and information between ecologists and systematists. Two ecological subcommittees, Littoral and Sublittoral, and two systematic subcommittees, "Soft" Fauna and "Hard" Fauna were established (the terms littoral, sublittoral, "soft" fauna, and "hard" fauna are defined below). The memberships of these subcommittees follow. Littoral subcommittee: B. O. Jansson (chairman), J. S. Gray, W. D. Hummon, S. Husmann, H. Kühl, L. W. Pollock, R. Williams. Sublittoral subcommittee: A. D. McIntyre (chairman), M. T. Gomoiu, R. R. Hessler, O. H. Pilkey, J. B. J. Wells. "Soft" Fauna subcommittee: W. Sterrer (chairman), C. Clausen, C. Jouin, E. Kirsteuer, P. Lasserre, B. Swedmark, G. Uhlig, W. Westheide. "Hard" Fauna subcommittee: W. D. Hope (chairman), R. P. Higgins, N. C. Hulings, F. Monniot, I. M. Newell, W. Noodt, J. Renaud-Mornant, F. Riemann. The subcommittees met separately and then they met collectively to integrate the results of their efforts and incorporate them into this "Manual for the Study of Meiofauna."

This manual is intended to be multipurpose. First of all, it is intended as a guide for both the beginning and the established researcher, whether ecologist or systematist, in the study of meiofauna. It is of primary importance that the ecologist be aware of the problems of the systematist, and vice versa, if studies of meiofauna are to be meaningful. The systematist may find the treatment of his particular taxon rather narrow in scope since emphasis has been placed on techniques of sampling, extracting, fixing, and preserving rather than on detailed systematics. Such information is important, since it is not always realized that samples and specimens must be dealt with in different ways depending upon the taxon or taxa of greatest concern. The ecologist, by the same token, must be aware of the problems of all systematists, especially if he is studying the total meiofauna.

The manual is also intended to provide a standardization for various techniques so that one can compare data from one geographic area with that from another and data collected by one investigator with that by another. At present, one finds great heterogenity in sampling, reporting of data, etc., so that meaningful comparisons are difficult.

Though the manual is brief, the techniques are described in detail and pertinent references to the literature are given. Many of the techniques were developed by the contributors reporting on them. Most have been extensively tested and all have been found to be successful.

No attempt has been made to produce a rigid definition of "meiofauna." The term generally is

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used to refer to animals, mostly metazoans, that can pass a 1.0-mm to 0.5-mm screen. The term "littoral" is here defined as intermittently submerged sedimentary environments (tidal and atidal), including beaches and mud flats; "sublittoral" is defined as continuously submerged sedimentary environments regardless of depth. Thus, the emphasis of the manual is on sedimentary meiofauna, whether it be sand or mud or mixtures thereof regardless of depth of water. It is hoped that a revision of the manual, anticipated within two to three years, will include biotopes other than sediment.

The distinction between "soft" fauna and "hard" fauna is made subjectively on the basis of the resistance of the integument to mechanical damage. The "soft" fauna includes taxa with a soft integument and usually a great ability to change shape and to contract considerably. The taxa grouped under "soft" include Ciliata, Cnidaria, Turbellaria, Gnathostomulida, Nemertina, Gastrotricha, Archiannelida, Polychaeta, Oligochaeta, Mollusca, and Echinodermata. The "hard" fauna consists of taxa whose representatives contract only slightly, if at all, and possess a shell or an inelastic cuticle. The "hard" fauna taxa include Foraminifera, Kinorhyncha, Priapulida, Nematoda, Bryozoa, Brachiopoda, Mystacocarida, Ostracoda, Copepoda, Palpigradida, Halacaridae, Tardigrada, and Tunicata. Though this division may seem artificial, it fits quite well into methodological groupings. Systematists and ecologists will find that, in general certain methods can be applied to "soft" fauna and others to "hard" fauna.

The literature on meiofauna has increased severalfold within the last few years but it is beyond the scope of this manual to include all the pertinent references. There are, however, several published works that are considered basic references on the ecology and systematics of meiofauna, including Ax (1966, 1969), Delamare Deboutteville (1960), McIntyre (1969), Remane (1933, 1940, 1952), and Swedmark (1964). Taken together, these works provide a comprehensive bibliography on meiofauna and should be consulted as basic sources of information for all phases of the study of meiofauna. An excellent source of current literature on meiofauna is *Psammonalia,* a newsletter of the Association of Meiobenthologists.[1] Past issues of *Psammonalia* have included comprehensive bibliographies on several meiofaunal taxa. The "Proceedings of the First International Conference on Meiofauna" (Hulings, 1971a) summarizes the present status of our knowledge of meiofauna, especially systematics and ecology. "The Study of Marine Benthos" (Holme and McIntyre, in press), *International Biological Program Handbook, No. 16,* deals with meiofauna in general terms. A survey of the literature on meiofauna shows that most of our present knowledge has come from the studies of European investigators. Thus, there remains much to be done in both systematics and ecology of meiofauna throughout the rest of the world.

We are indebted to many persons and organizations for the success of the conference. The Government of Tunisia, including the National Scientific and Technical Institute for Oceanography and Fisheries, the Department of Higher Education and Scientific Research, and the Ministry of Foreign Affairs, endorsed the conference and assisted in many ways. The American Embassy, Tunis, was most helpful on numerous occasions. The staff of the Mediterranean Marine Sorting Center—especially Dr. Hédia Baccar, Mrs. Geneviève Dargouth, and Mr. Mohammed Shili—freely provided assistance in making local arrangements and in the initial preparation of this manual. Funding for the conference was made possible through the Office of International Activities of the Smithsonian Institution, Washington, D.C. The management of the Hilton Hotel, Tunis, assisted in many aspects of the local arrangements. Many persons in the Departments of Invertebrate Zoology and Paleobiology of the National Museum of Natural History of the Smithsonian Institution have been extremely helpful in many ways. Special gratitude is owed to Dr. Ray Manning and his staff for encouragement and review of the manual and to Mrs. Roberta K. Smith who prepared the section on Foraminifera. To these organizations and all of the persons involved, we express our sincere appreciation.

[1] Inquiries for information on *Psammonalia* should be addressed to the editor, currently Dr. John S. Gray, Wellcome Marine Laboratory, Robin Hood's Bay, Yorkshire, England.

1. General Description of Habitat

A careful definition of the study area in ecological terms should precede every investigation. For the systematist this means that localities can be found again without difficulty. Simple collecting of specimens, if accompanied by sufficient data, can be of great interest to the ecologist. Finally, the ecologist should record all the data available in order to obtain a complete analysis of the correlation between fauna and parameters. The following should be noted.

1.1. Climate.—Season, date, illumination and insulation, and water temperature. In coastal areas, weather conditions prior to sampling, noting precipitation, air temperature, and wind speed and direction.

1.2. Geography.—Latitude and longitude, local name of site, distance and direction to nearest town or physiographic landmark appearing on maps or charts, means of determining location, and general nature of the physiographic province (e.g., coastal, continental margin or shelf, abyssal plain, midocean ridge). Littoral characterizations should include extent of beach and continuity with adjacent areas.

1.3. Geology.—Substratum, nature of shoreline (deposition or erosion), sediment source and availability, type and structure of rock in adjacent outcrops.

1.4. Hydrography.—*Littoral:* tidal amplitude, wave height, pattern of daily tides. *Littoral and sublittoral:* frequency, intensity and direction of storms, current direction and rate of flow, effect of each on beach or bottom sediments. *Sublittoral:* all available on-station water measurements.

1.5. Physiography.—*Littoral:* beach width, contour pattern, profile, direction and exposure, and presence of features such as berms, tide pools, rock ledges, offshore islands, bars and kelp beds. *Sublittoral:* bottom irregularity, contour patterns, profile, presence of seamounts, trenches, ripple marks, ridges, canyons, and other characters.

2. Environmental Analyses

In most cases the same environmental features can be considered in littoral and sublittoral studies. These include physiography, hydrodynamic regime, temperature, salinity, oxygen availability, granulometry, mineralogy, and sediment porosity. Regional primary productivity, organic matter, bacteria, and other biological constituents of the environment are important for community analysis. In the littoral, water saturation and permeability are of prime importance. Amount of light and benthic chlorophyll should be determined in both environments. In the sublittoral, depth and amount of particulate organic matter in the water column may be significant. In the sublittoral suitable for SCUBA, littoral equipment can be used with suitable modification, thus giving continuity with littoral studies. In deeper water remote gear must be used.

If the objective of the study is synecological—such as a comparison of polluted and unpolluted, arctic and tropical areas—faunal and environmental analyses can be restricted to periods of extremes, i.e., winter and summer or rainy and dry seasons. If the objective is autecological, detailed seasonal measurements should be made. Any study employing only a short period of environmental analysis runs the constant danger of ignoring limitations which occur only during other times

of the year. In addition, it may not be the absolute value of the environmental parameter which is important to the organism, but the degree of its stability and rate of change. Most of the environmental features mentioned are stable in the deep sea; however, changes in temperature, oxygen, currents, and particulate organic matter, with depth, in the water column should be measured.

Wherever possible it is best to make measurements directly in the field. Whether in field or laboratory, the size of the probes used or of the samples taken must always be related to the size of the animals being investigated in order to determine the environmental conditions that immediately surround the organism. This is especially important when vertical gradients are studied because of the variations that often exist.

The general littoral environment should be studied at high tide as well as low tide. In both littoral and sublittoral, attention should be paid to the characteristics of the water column. Many features of the sublittoral are difficult to study due to the lack of suitable instruments that provide the desired data. Gomoiu (1971), Husmann (1971), Jansson (1971), McIntyre (1971) and Pollock (1971) have reviewed various aspects of environmental and faunal analysis in the littoral and sublittoral.

2.1. Interstitial Water Samples.—When collecting interstitial water samples care must be taken to prevent contamination from areas other than those to be sampled. For example, large samplers disturb the sediment, and water from surface layers may run down the side of the sampler and mix with the sample being taken. Thus, a sampler such as that of Brafield (1964) and Tilzer (1967), or a microsyringe is most suitable, as mixing of different water levels is avoided. If a microsyringe is used, the tip should be modified to include a sintered glass screen which prevents particles from blocking the syringe. Extraction of water samples must be slow to avoid the entrance of air bubbles and samples should be stored in clean glass containers or plastic containers (see Section 2.15). The accuracy of some chemical analyses, however, may be impaired by storage. Samples of the adjacent seawater (at low tide) or the overlying seawater (at high tide) should also be taken.

2.2. Temperature.—A number of portable thermistor-type temperature probes accommodating up to twelve small probes simultaneously are available (see Appendix, Section 10.1.1). A tubular metal probe with a small sensitive area is best for this work (e.g., Spembly Technical Products probes). Varying cable lengths can be obtained. Periodic checks of the probe should be made to test for age deterioration.

2.2.1. Instantaneous Readings.—These can be made by inserting the probe vertically into the sediment or horizontally into the side of a hole dug in the sediment.

2.2.2. Continuous Recordings.—Most telethermistors can be used for continuous recordings in conjunction with a chart recorder (see Appendix, Section 10.1.2). Probes are buried in the sediment, and up to 20 readings can be monitored simultaneously. Suitable instruments are listed in the Appendix (Section 10.1.1).

2.2.3. Sublittoral Measurements.—It is not necessary to make the measurement in the sediment; readings from the adjacent water column are sufficient. Probes or reversing thermometers can be employed.

2.3. Salinity.—As the international standard for salinity determinations is based on conductivity, this method is preferable to others.

2.3.1. Wheatstone Bridge.—This method of determining conductivity is

cheap and simple and requires only 0.5 to 1 ml of interstitial water. Water samples should be collected following the methods in Section 2.1.

The smallest type of conductivity cells are equipped with a bulb pipette through which a water sample can be drawn into the cell. The cell is capped and immersed in a water bath. As temperature control is of the utmost importance, all solutions should be kept in a constant-temperature bath. The electrodes should be washed in distilled water, dried, and immersed in a spare portion of the sample before being placed in the aliquot to be sampled.

The International Oceanographic Tables, prepared by UNESCO (see Appendix, Section 10.1.3), recommend a conductivity standard for salinity determinations. The best method of making salinity estimations would, therefore, be the use of these tables. The conductivity of standard seawater (obtained from the Laboratoire Océanographique, Charlottenlund, Denmark) should be determined first, followed by the solution to be tested. The ratio of these two conductivities is translated to salinity at the appropriate temperature in the UNESCO tables. A suitable and relatively cheap commercial instrument is available (see Appendix, Section 10.1.4.3). A circuit diagram is shown in Figure 12. A list of manufacturers of components for personal assemblage is given in the Appendix (Section 10.1.4).

2.3.2. Salinity-Temperature Bridge.—A small portable salinometer (see Appendix, Section 10.1.5) designed primarily for estuarine and coastal hydrographic use is suitable for determinations on 25 to 50 ml of water. Salinity can be read from 0 to 38 ppt and temperature from 0° to 35° C. Samples of interstitial water (obtained by the methods outlined in Section 2.1), are placed in the chamber of the probe head and values are read from the scale. The major disadvantage of this technique is the large volume of interstitial water needed for each determination.

2.3.3. Other Techniques.—Chemical methods are cumbersome and may not be suitable due to insufficient data on the ionic concentration of interstitial water. Specific gravity methods are less preferable due to the large volume of water needed for determinations (see Barnes, 1959, for methods). A new but relatively untested field method, the Goldberg refractometer (see Appendix, Section 10.1.6), requires only a few drops of interstitial water.

2.3.4 Sublittoral.—As with temperature, it is sufficient in most cases to measure the salinity of the adjacent water column. It should be stressed, however, that although the salinity of the interstitial water might be equal to that of the overlying water, the relative ionic composition is likely to be different.

2.4. pH and Redox.—The pH has not been studied in sufficient detail to evaluate its direct effect on meiofauna. The redox potential (Eh), however, is of biological importance, particularly in sheltered or atidal beaches. On well-sorted oceanic beaches there is rarely a change in Eh (and pH) with depth. Low Eh (and possibly low pH) values may indicate the presence of H_2S which may be toxic, and the Eh may indicate limiting biological processes (e.g., sulfur reduction occurs at an Eh of below +100 mv).

Eh readings should always be accompanied by pH readings. Thus, meters that measure both simultaneously are preferable. The instruments (see Appendix, 10.1.7) must be modified to use small probes; otherwise, sediment displacement will give erroneous readings (see Fenchel, 1969, and Whitfield, 1969, for a full discussion of the significance of pH and Eh).

2.5. Oxygen.—Two types of oxygen measurements are common—total oxygen concentration and oxygen availability. Total oxygen concentration can be de-

termined by titrimetric methods, the use of polarographic or exposed electrodes. If a polarographic electrode is used, determination of oxygen is dependent on diffusion of oxygen through a teflon membrane; it requires a temperature compensation as well as water-flow rate in excess of 1 cm/sec around the electrode. Total oxygen also can be measured by a variety of exposed electrodes such as platinum and thallium, but these have not been evaluated by the participants.

Oxygen availability is measured by a stationary, naked platinum electrode reducing the oxygen diffusing to it. The latter measurement incorporates ambient temperatures, water-flow rates, and other parameters affecting oxygen diffusion. In this way the electrode can be considered to be functioning in a manner comparable to an interstitial animal.

2.5.1. Oxygen Concentration.—Oxygen concentration has been estimated by chemical titration techniques and by polarographic electrodes. Both macroprocedures and microprocedures for chemical determination of oxygen are described in detail by Barnes (1959). Membrane-covered polarographic electrodes measure oxygen content (in ppm) and include temperature compensation. Carey and Teal (1965) discuss the construction and reliability of these instruments.

2.5.2. Oxygen Availability.—This technique, pioneered by Lemon and Erickson (1952) for soil, has been applied to the marine environment by Jansson (1966, 1967b) and Fenchel, Jansson, and von Thun (1967). Oxygen diffusion rates are measured by an exposed platinum electrode at a constant voltage of 0.8 v. Values are measured as $\mu A/cm^2$ electrode surface/min. The source, a description of the circuitry used in this instrument, and the procedure for calculation of oxygen are given in the Appendix (Sections 10.1.8, 10.3 and Figure 13).

Care must be taken when using this technique. The entire electrode surface must be covered by a water film, otherwise oxygen availability will be underestimated. If multiple recordings at the same depth close to the sediment surface give highly variable results, it is likely that water content is inadequate to allow measurements of oxygen availability. "Poisoning" of the electrode may occur when positively charged particles or colloids have an isoelectric point higher than the ambient pH precipitate on the electrode surface, thus decreasing its sensitivity. Detection of this phenomenon may be based on sensitivity tests in water of high oxygen availability following exposure of the electrode to an area of potential poisoning. Since electrodes are inexpensive and easily made, it is wise to include several of them in field supplies. As the diffusion is highly time-dependent, readings must be made when equilibrium is attained; e.g., after 3 minutes.

Enckell (1968) and others have shown, however, that above a certain current velocity the availability of oxygen does not increase with increased velocity. The sensitivity to current velocity also may be dependent on the initial oxygen concentration. Caution should be exercised, therefore, in interpreting the data derived from the technique described above.

In the littoral, measurements are easily made by digging a hole and inserting the electrodes to the desired depths in the water. As the effect of length of the wires or distance between the electrodes is negligible, sand beneath the water table can be reached by attaching the sensor to a stick. A diver can employ this technique in the sublittoral by means of an instrument kept on a boat or the shore.

2.5.3. Sublittoral.—Where remote gear is used, particularly in the deep sea, the main difficulty is obtaining the sample or measurement from the water just above the bottom or in just the top layer of sediment. For this reason, water-

sample analysis is unsatisfactory. The oxygen availability electrode offers a promising solution.

2.6. Water Saturation.—Water saturation varies continuously throughout the tidal cycle; thus, the percentage saturation should be quoted for the time at which faunal samples are collected.

2.6.1. Speedy Moisture Tester.—The most accurate field technique is the Speedy Moisture Tester (see Appendix, Section 10.1.9). This technique, which requires 1.5 or 3 g of sediment, measures gas pressure produced on shaking the sample with an absorbent. The gas pressure is proportional to the moisture content.

2.6.2. Displacement Technique.—A less-accurate method is the displacement technique of Hummon (1969). To determine water saturation of pore space, 5 cm^3 of water is first placed in a 10 cm^3 graduated cylinder. Sand is then allowed to settle slowly through the water until the height of the sand column reaches the 5 cm^3 level. The cylinder is agitated frequently to increase packing. Five cm^3 of fully saturated sand displaces the water surface 5 cm^3 upward to the 10 cm^3 level, whereas 5 cm^3 of dry sand having 60 percent grain and 40 percent pore volume would only displace the water surface 3 cm^3 upward to the 8 cm^3 level. Porosities of field samples can be tested by oven-drying the sand and then determining the minimum displacement value. This minimum final value represents 0 percent water saturation of the pores and the upper value 100 percent saturation. Intermediate values measure water saturation of the pores proportionately in percent, and readings to the nearest 2 percent saturation can be obtained.

2.7. Hydrogen Sulfide.—The presence of hydrogen sulfide in sediments indicates areas of high organic decomposition and usually is associated with a lack of oxygen. Nevertheless, sediments containing hydrogen sulfide may have a characteristic and rich meiofauna which hitherto has been largely ignored. Thus, measurements of hydrogen sulfide concentration may indicate the extent of this habitat.

A simple and rapid measure of the hydrogen sulfide content of a sediment can be made using a Hach H_2S test (see Appendix, Section 10.1.10). This test will detect quantities as little as 0.1 ppm and can be carried out in the field. More sophisticated techniques are being developed; e.g., the sulfide electrode of Berner (1963) and the potentiometric titration technique of Fenchel (1969).

2.8. Granulometry.

2.8.1. Grain Size.—As a general guide, a sieve 3.75 cm in diameter can process 15 g of sediment, a sieve 7.5 cm in diameter can process 30 g, and a sieve 15 cm in diameter can process 60 g.

Because of rapid horizontal and vertical changes of grain size in the microhabitat, it is desirable to use the same sample for both faunal and grain size analysis. In order to retain the physical characteristics of the sample and to make the results comparable with other investigations, the total sample must be studied. The coarse fraction should not be omitted from consideration.

Initially the sediment sample should be wet-sieved through a 62-μ sieve using only water of the same salinity as the sample area. This procedure separates the "fines" (silt and clay sizes) which, if sufficiently abundant, must be analyzed by procedures different from those used for the sand sizes. Care should be taken during wet-sieving to prevent crushing of fragile, small calcareous skeletons or the breakdown of fecal pellets. This is a particularly serious problem with deep-sea biogenic sediments.

The fine sediment fraction (passing the 62-μ sieve) needs special treatment

TABLE 1.—*Phi-mm equivalents and suggested sieve series.*

U.S. Standard Sieve Mesh No.	*A.S.T.M. Sieve No.*	*Opening in mm*	*Phi Scale*	*Size Class (Wentworth)*
5	5	4.00	−2.0	Granule
7		2.83	−1.5	Granule
10	10	2.00	−0.9	Very coarse sand
14		1.41	−0.5	Very coarse sand
18	18	1.00	0.0	Very coarse sand
25		0.71	0.5	Coarse sand
35	35	0.500	1.0	Coarse sand
45		0.350	1.5	Medium sand
60	60	0.250	2.0	Medium sand
80		0.177	2.5	Fine sand
120	120	0.125	3.0	Fine sand
170		0.088	3.5	Very fine sand
230	230	0.0625	4.0	Very fine sand

using the pipette method (see Folk, 1968, or Griffiths, 1967, for methodology). In many sediments the fine fraction may be important in blocking pore spaces.

Mud samples may include abundant fecal pellets. Such particulate material is delicate, and an estimate of its relative quantity is required before dry-sieving. Dillon (1964) has given a technique for separating fecal pellets and organisms from sand.

Samples dominated by biogenic materials usually are analyzed in toto on the assumption that calcareous skeletal material is a granulometric component of the bottom sediment, just as are quartz grains or fecal pellets. For example, *Globigerina* tests in Foraminifera ooze are sand-size grains.

The sand fraction remaining in the 62-μ sieve should be washed with many changes of fresh water to remove the salt and then dried at 105° C. The sample is sieved through a series of screens at 0.5 phi (ϕ) intervals. Phi-mm equivalents are shown in Table 1. Ideally, sieves should correspond to the A.S.T.M. series to avoid confusion. For example, the British Standard sieve 8 is equivalent to the A.S.T.M. 10. A mechanical shaker should be used where possible for 15 minutes. Longer sieving times may damage the particle structure. It is important that all samples be sieved for the same length of time. Table 1 shows the suggested sieve series that should be used for sand analysis. If an unusually detailed analysis is desired, 0.25-phi sieve intervals can be used (Renaud-Debyser and Salvat, 1963). Cumulative dry-weight percentages should be plotted on probability paper. The graphic mean, Mz (Folk, 1968), rather than the often-used median should be plotted for detailed analysis. It is defined as:

$$Mz = \frac{(\phi\ 16 + \phi\ 50 + \phi\ 84)}{3}.$$

Similarly, the best measure of uniformity of sorting is the inclusive graphic standard deviation, $\sigma\ I$ (Folk, 1968):

$$\sigma\ I = \frac{\phi\ 84 - \phi\ 16}{4} + \frac{\phi\ 95 - \phi\ 5}{6.6}$$

The asymmetry or skewness of the grain-size distribution pattern can be measured by the inclusive graphic skewness, SK_1 (Folk, 1968).

$$SK_1 = \frac{\phi\ 16 + \phi\ 84 - 2\ \phi\ 50}{2\ (\phi\ 84 - \phi\ 16)} + \frac{\phi\ 5 + \phi\ 95 - 2\ \phi\ 50}{2\ (\phi\ 95 - \phi\ 5)}$$

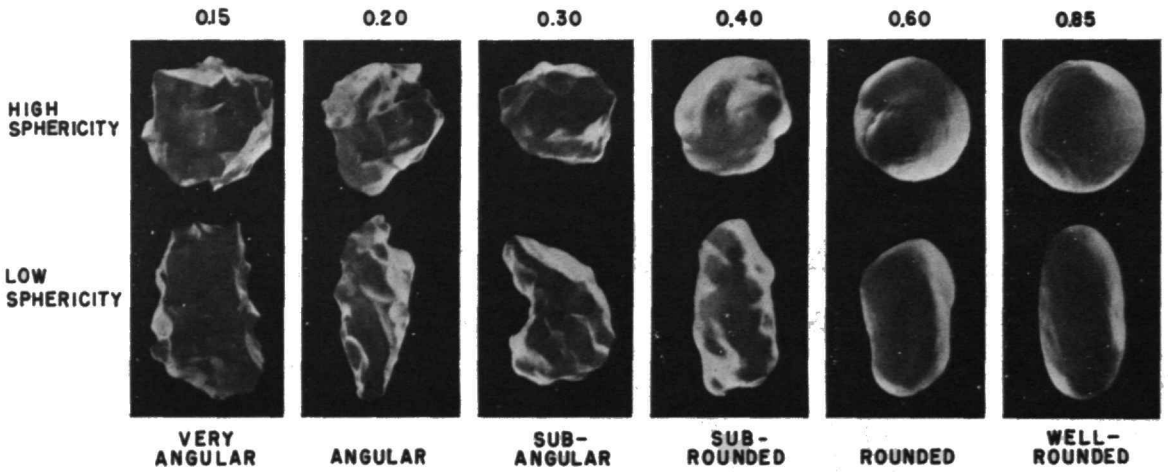

FIGURE 1.—Scale of roundness for quartz grains. (From Powers, 1953.)

All the points used in the above equations are obtained from the cumulative frequency curve plotted with phi scale intervals.

2.8.2. Roundness.—This parameter is of importance in pore space determinations and also in determining the distribution of attached organisms and organic material on grains (Meadows and Anderson, 1966; Webb, 1969).

The scale of grain roundness of Powers (1953) for quartz grains is shown in Figure 1. To determine the roundness of a sample, fifty or more grains are assigned to one of the classes (very angular, angular, etc.) by comparison with the photographs. Average roundness of the sample is determined by multiplying the number of grains in each class by the geometric mean of that class (0.15 for very angular, 0.20 for angular, etc.) and dividing the sum of the products by the total number of grains analyzed from each sample.

Calcium carbonate particle roundness can be classified according to the scale of Pilkey, Morton, and Luternauer (1967), shown in Figure 2. Five roundness classes are recognized. Class 0 (not shown) includes whole shells. Class 1 fragments exhibit no apparent rounding of edges and corners and appear as though freshly broken. Classes 2 and 3 are gradations between Class 1, fresh fragments, and Class 4, highly rounded, usually polished and essentially cornerless fragments. The particles are classified by visual comparison (usually under a microscope) with the figures in the scale.

2.9. Mineralogy.

2.9.1. Calcium Carbonate Content.—The technique of Gomoiu and Gomoiu (1968) can be used for a precise determination of $CaCO_3$ content. A known weight of sand can be digested with a measured excess amount of dilute acid. The reduction in strength of the acid, which is a measure of the quantity of $CaCO_3$, is then measured by titration with NaOH. A less-accurate technique is to weigh a sample, add 10 percent HCl, and weigh again after dissolution of the $CaCO_3$.

2.9.2. Mineralogical Analysis.—The silicate mineral fraction and the calcium carbonate fraction usually can be distinguished quickly and easily. Sand-size particles are mineralogically studied by observation of thin sections of the grains

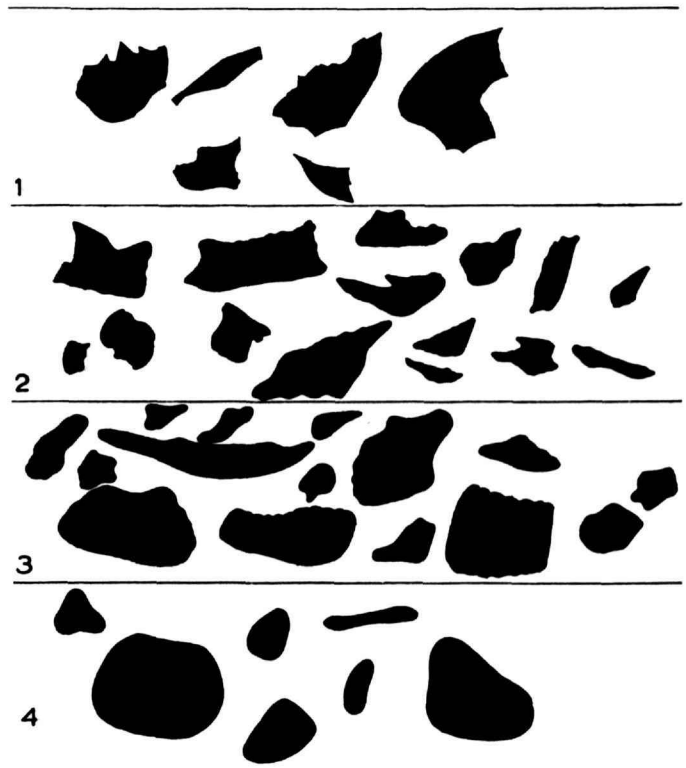

FIGURE 2.—Scale of roundness for calcium carbonate particles. (From Pilkey, Morton, and Luternauer, 1967.)

and by staining techniques. In either case, study under the polarizing petrographic microscope is necessary. In deep-sea muds, coarser components sometimes may be identified under the microscope, but generally X-ray diffraction techniques are needed to determine mineralogy. Identification of major deep-sea sediment types (pelagic oozes, red clay, etc.) is based mainly on microscopic identification of coarse components and overall sediment color rather than by detailed mineralogic work. The coarse fraction is isolated for study by standard wet-sieving techniques (section 2.8.1).

2.10. Porosity.—The porosity of a sediment is defined as the volume of water needed to saturate a given weight of dry sand (usually 100 g) at a given temperature. Fraser (1935), Graton and Fraser (1935), and Webb (1958, 1969) should be consulted for a detailed discussion of porosity.

2.10.1. Pore Space.—Porosity varies slightly with each tidal cycle due to particle reassortment by wave action. It is, therefore, important to note the tidal height, wave action, prevailing weather conditions, and grain size distribution. The range of porosity values due to various packing arrangements can be determined as follows.

Two samples are needed, and each is dried at 105° C. One sample is placed in a measuring cylinder, producing loose packing. The other sample is treated similarly but is agitated so that the sediment is compacted. Water is then added

under a vacuum (which eliminates trapped air bubbles) until the sediment sample is totally saturated. The volume of water needed to saturate the first sample indicates maximal porosity, while the second value indicates minimal porosity. Porosity also can be measured by weight as long as the sediment is reasonably homogenous and the specific gravity is determined.

2.10.2. Spatial Relationship.—The epoxy resin embedding technique of Williams (1971) is useful in this context. Grain-size distribution patterns, porosity, and pore space can be estimated by this procedure.

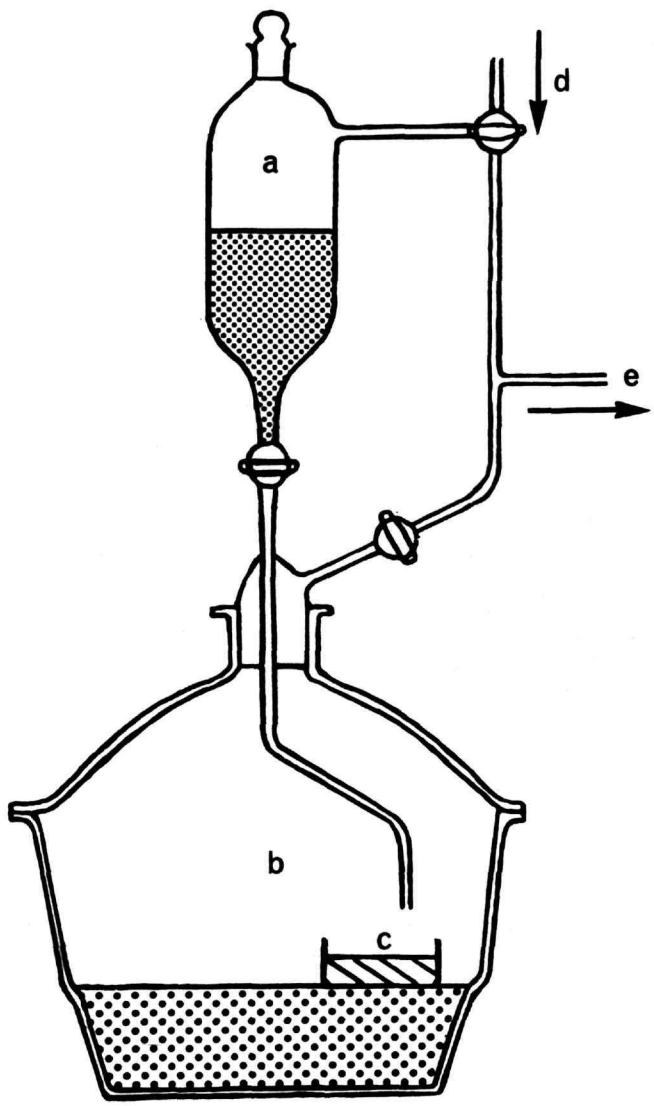

FIGURE 3.—Impregnation apparatus: *a*, vessel containing resin; *b*, dessicator containing anhydrous $CaCl_2$ and the sample (*c*) for impregnation; *d*, inlet for air into *a* and *b*; *e*, suction by water pump via a silica gel vessel inserted into the vacuum line. (From Williams, 1971.)

Undisturbed samples of sediment are collected with the use of a brass rectangular sampler (10 by 5 by 2.5 cm) with double sliding lids. The sampler is pressed into the surface of the sediment, and the lower lid is slid into place after the sediment around the sampler has been cut away. The sampler is then marked with the orientation, tidal height, and date. The sampler is allowed to drain, leaving a minimum amount of water, air-dried, and impregnated with resin. Crystallized salt in the air-dried sample does not hinder impregnation but tends to maintain the structure of the sediment. The resins used for impregnation are Crystic 28C and Araldite MY750 (see Appendix, Sections 10.1.11, 10.1.12). When using Araldite, the inner surface of the sampler should be treated with Araldite Release Agent QZ12 to aid the release of the hardened impregnated sample from the sampler. A fluorescent dye, Uvitex SWN, is mixed with the resin following a technique outlined by Werner (1962). Under ultraviolet light the dye causes the pore system to fluoresce reddish pink, the quartz fraction red, and the calcium carbonate blue. The vacuum apparatus for impregnation, a modification of the method used by Wells (1962), is shown in Figure 3. This apparatus can easily be constructed using "Quick-fit" glassware. Sufficient vacuum for complete impregnation of cohesionless sediments can be obtained with the use of a water pump. Water vapor is prevented from entering the vacuum apparatus by a calcium chloride or silica gel vessel introduced into the vacuum line.

Thin sections are prepared parallel to and at right angles to the surface of the impregnated sample following the procedure of Dalrymple (1957) and Jongerius and Heintzberger (1963). The impregnated samples are sawed, ground, polished, and mounted. Sand can be ground to a thickness of about 50 μ without cracking the grains. Grinding is finished by hand, using varying grades of carborundum powder. Coverslips are then placed over the thin sections, using Araldite AY103 as a mountant and taking care to exclude all air bubbles.

For the measurement of the percent volume of pore space in thin sections, a technique for undisturbed soil developed by Rosiwal and adapted by Swanson and Peterson (1940) is used. The microscope is focused on the surface of the section and measurements are taken only in this plane. The slide is traversed on a mechanical stage parallel to the direction of a graduated scale in the eyepiece, and the distance between the intercepts made by each successive void and each successive particle is measured. This procedure is repeated in two directions—parallel to and at right angles to the slide. The total void measurement is then expressed as percent of the total measurement of void and solid. The method of measurement is shown in Figure 4. Porosity (P) is determined as follows:

$$P = \frac{a+b+c+d}{A+a+B+b+C+c+D+d}$$

The measurements of particle and void dimensions are determined systematically by use of a grid system. The grid is formed by traversing the mechanical stage through a specified number of divisions between each series of measurements. The maximum dimensions in two directions at right angles of each particle, and including the void, are measured. This is possible because the resin matrix is transparent and a three dimensional view of the voids and particles can be obtained. The method of measurement is illustrated in Figure 5. The grain measurements are given by A_1A_2, B_1B_2, etc., and the void measurements by a_1a_2, b_1b_2, etc. The procedure is carried out for the sections which are taken parallel and vertical to the surface of the deposit. Thus obtained are four sets of

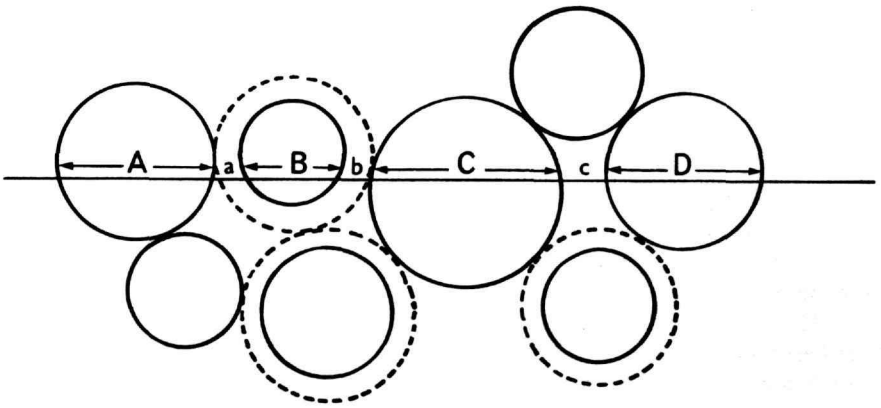

FIGURE 4.—Cross section of a model spherical system showing the method of measuring porosity. Solid line represents the plane of focus. Measurements are random along the straight grid line. (From Williams, 1971.)

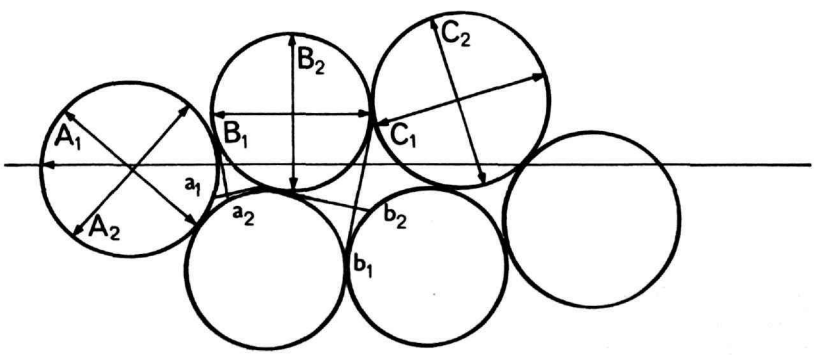

FIGURE 5.—Cross section of a model spherical system showing the method of measuring voids and particles. (From Williams, 1971.)

values, representing the maximum dimensions of the voids in three directions in the deposit, with a duplicate measurement of one of the directions parallel to the surface. From these measurements the possibility of directional orientation of the pores or the particles can be checked by analyzing the data statistically. The measurements of particle and void dimensions can then be plotted against a Log_{10} or Log_2 scale and the relevant parameters obtained.

2.10.3. Permeability.—Porosity values indicate the volume of pore space available to the animals, but for meiofauna the permeability (or flow of water through the sediment) may be of greater significance. For example, fine sands, in general, hold more water than coarse sands but are less permeable due to the smaller sizes of the pores.

Permeability can be measured by filling a glass tube (1.5 cm diameter, 70 to 80 cm long) with sand to a depth of 10 cm. Gauze is fitted over the bottom of the tube. The tube is held vertically and tapped gently to settle the grains. The time required for a head of 65 cm of water to drain to 15 cm from the

bottom of the tube (i.e., 50 cm of water through 10 cm of sand) is the permeability (Webb, 1958, 1969). Fraser (1935) used a similar method. Techniques such as those of Webb and Theodor (1969) for measuring flow rates of interstitial water in situ may give useful information.

2.11. Organic Matter.—Most current techniques for measuring organic matter are unable to separate the fauna from the analysis, and, thus, are inadequate. Tietjen (1969), however, by estimating the weight of the meiofauna, made corrections for determinations of the organic content of sediment alone. Whether what is measured has relevance to the organism remains unknown. In the deep sea it is likely that most of the organic matter is refractory and unavailable to the organism, or perhaps even to bacteria.

The Kjeldahl method with ninhydrin finish of Strickland and Parsons (1968) for determining particulate nitrogen gives reproducible results. The particulate matter is filtered on a 25-mm Whatman GF/C glass filter. The filtrate (particulate matter and fauna) is converted to ammonia by the Kjeldahl treatment with sulphuric acid. The resulting ammonia is determined absorptiometrically using ninhydrin-hydrindatin reagent without prior distillation. The detailed methodology is outlined in Strickland and Parsons (1968).

Steele and Baird (1968) determined particulate organic carbon by wet oxidation. They used 0.2 to 1.0 g of sand, removing the particulate matter by filtration as is done for water samples (Steele and Baird, 1961). Strickland and Parsons (1968) give methods for the dichromate wet oxidation technique. A similar method of Maciolek (1962) has been used by Tietjen (1969).

Kühl (1971) has shown the changes that occur in interstitial water following decomposition of organic matter (including the production of ammonia, nitrites and nitrates) and related this to sediment characteristics, especially grain size.

2.12. Chlorophyll *a*.—Diatoms and other microalgae are important constituents in sediments. Although simple techniques are available for their study, this phytal component has received little attention. One of the techniques of Strickland and Parsons (1968) does not estimate phaeophytin; therefore, it is preferable to other techniques.

The sediment sample is ground in a porcelain mortar for 5 to 10 minutes and the chlorophyll pigments are extracted by use of 10 ml of 90 percent reagent grade acetone with the addition of 1 ml $MgCO_3$ suspension (1 g reagent grade $MgCO_3$/100 ml distilled water). The pigments are extracted for 20 hours in complete darkness and the extinction values read in a spectrophotometer cell having a path length of 10 cm but holding 10 ml or less of solution. The concentration of chlorophyll *a* (mg/liter) is determined by using the formula of Strickland and Parsons (1968): $11.6E_{6650\text{Å}} - 1.31E_{6540\text{Å}} - 0.14E_{6300\text{Å}}$ (E = extinction at indicated wavelengths *after* correction for extinction at 7500Å). Care should be taken to check the extraction efficiency of the acetone. Where chlorophyll values fall below 0.2 mg/liter, the method is less sensitive. Strickland and Parsons (1968) should be consulted for detailed methodology. Pamatmat (1968) used the technique described above.

Steele and Baird (1968) estimated chlorophyll *a* by grinding 1 to 7 g of vacuum-dried sand in a mortar. The chlorophyll *a*, extracted with 90 percent acetone, was estimated by the difference between optical densities at 6300Å and 7500Å. The value of 89.31 liter/g cm was used as the coefficient of extinction

(SCOR-UNESCO, 1964). The SCOR-UNESCO procedure is also given in Strickland and Parsons (1968). Later, Steele and Baird (1968) determined chlorophyll *a* by using the Turner fluorometer (Yentsch and Menzel, 1963) and obtained greater sensitivity for low chlorophyll values. This technique also was used by Tietjen (1968) and is detailed in Strickland and Parsons (1968) as "Fluorometric Determination of Chlorophylls."

2.13. Bacteria.—Quantitative assessments of bacteria in sediments using the standard agar plate techniques underestimate numbers because the medium is highly selective. Possibly only 10 percent of the bacteria are counted in this method. Westheide (1968) has studied seasonal variations in bacteria and fungi by using solid media. Anderson and Meadows (1969) have used a similar technique. Sediment (0.5 to 1.0 g) collected by means of a sterile pipette is added to 10 ml of sterile seawater. Glass beads (Appendix, Section 10.1.20) are added and the sand is shaken vigorously for 10 minutes. From this suspension 1 ml of solution is taken and added to 9 ml of sterile seawater. This dilution is repeated three or four times. From each dilution, 0.05 or 0.1 ml are added to sterile Petri dishes containing a solid medium (1.2 percent agar, 0.3 percent peptone in aged sterile seawater or three parts seawater, one part distilled water). The Petri dishes are incubated at 18° to 22° C for one to three weeks and the number of colonies forming from each bacterium present on the original sand sample are counted. The works of Ferguson-Wood (1965), Oppenheimer (1963), and Zobell (1946) should be consulted for more detailed information.

Respiration rates of sediment samples recently have been shown to give more reliable estimates of microorganism abundance. Hargrave (1969) used darkened and transparent cylinders placed on sediment and compared chemically the changes in oxygen levels in each cylinder to derive a measure of algal respiration. He used oxygen consumption rate of antibiotic treated and untreated sediment cores to estimate bacterial respiration. Pamatmat (1968) and Pamatmat and Banse (1969), using belljars placed in situ, have recorded sediment respiration rates with a lead-silver oxygen electrode both intertidally and subtidally.

Meadows and Anderson (1968) used a staining technique to determine the abundance, distribution, and types of microorganisms (bacteria, blue-green algae, and diatoms) on sand grains. The samples (1 to 3 ml) are stained within a few minutes after collection. Each sample is fixed in 2 percent osmic acid for three minutes, followed by two minutes in Bouin's solution. The sample is then rinsed in dionized water and stained for six minutes with carbol fuchsin. The samples can be stored in 10 percent formalin at 0° C. The stained sand grains are then examined under low-power microscope.

The species of bacteria present may prove to be as significant as their numbers, as shown by Gray and Johnson (1970) in evaluating bacteria as an environmental factor controlling microdistribution of meiofauna.

2.14. Light.—Light extinction in sediments is a function of sediment grain size, and it decreases with a decrease in grain size. Light-extinction rates for various sediments have been studied by Gomoiu (1967), Gray (1966b), and Taylor (1964). A simple method is to hold a photocell under a flat-bottomed glass dish with a strong light source illuminating from above. Sediment is added slowly, and the change in light intensity is noted with increasing depth of sediment. Care must be taken to ensure that the photocell is sensitive to the wavelengths of light being measured. The wavelength of the light source should be noted in reporting on this technique.

The light source should be sufficiently bright to simulate daylight conditions (not less than 10,000 lux), and readings should be quoted in lux.

2.15. Nutrient Analyses.—Nitrate, phosphate, and silicate are probably the most important nutrients present in interstitial water. Since all nutrient analyses need large volumes of water, in excess of 50 ml, they are not particularly suitable for the analyses of interstitial samples unless auto-analyzers are used. For nitrate, 80 to 90 ml are needed, for phosphate 50 ml, and for silicate 50 ml. The silicate and phosphate samples cannot be stored in a glass bottle and should be determined immediately. They can, however, be stored in a deep freeze at −20° C. All the determinations are made using a spectrophotometer. Strickland and Parsons (1968) give a detailed methodology which should be followed.

Recently, auto-analyzers have been used routinely for nutrient analysis of seawater (Brewer and Riley, 1965, 1966; Chan and Riley, 1966; Strickland and Parsons, 1968). These instruments are expensive and require a full-time technician. Many public health and water pollution laboratories have such equipment and may make these facilities available. This technique has the great advantage of requiring only small volumes of sample for analysis.

3. General Collection Techniques

Sampling is one of the most difficult problems in meiofaunal investigations. This is especially true for quantitative sampling, in determining the gear to be used and the number of samples to be taken. Gray (1971b) and Vitiello (1968) have approached this problem from the viewpoint of determining the most suitable core size and the optimal frequency of sampling for meiobenthos. Jones (1961), using a coring device, collected 30 samples at four stations at six-week intervals to determine fluctuations in density and dispersion pattern of benthic animals, and he compared the results of multiple-sampling versus one large sample. McIntyre (1971) has evaluated the effects of sampling techniques, especially coring devices. The results of these investigators and the statistical books of Cochran (1963), Greig-Smith (1964), and Williams (1964) should be consulted prior to attempting quantitative sampling.

3.1. Preliminary Survey. —Before any quantitative sampling program is designed it is essential that a preliminary survey be made to define the faunal components and to establish the dimensions of the ecosystem. In the deeper sublittoral where remote gear has to be used, and exact replication of samples is impossible, data can be derived only from previously published information on the area.

3.2. Littoral Sampling.—Quantitative sampling can be accomplished by sampling along a transect. A reference line is established perpendicular to the low-water line along the surface of the sand from a fixed marker at the top of the beach. The line should extend at least from mean spring low water to mean spring high water. Samples from two or more stations along the surface are taken at intervals of 0.5 to 2 m on nontidal beaches and no more than 3 m on tidal beaches and at various depths from surface down to the level of the low tide water table. For a given sampling effort a continuum of sampling designs can be drawn up, ranging from nonsubsampled stations (Section 3.2.1) to multisubsampled grid stations (Section 3.2.2). The former possess broad extension with little sample reliability, and the latter narrow extension with great sample

reliability. The two sampling designs given below include these features in slightly different ways. In any case, sampling should be repeated at different tidal levels.

3.2.1. Distribution Sampling.—In this case the broadest extension which is consistent with estimated sample reliability is sought. Placement of the samples can be made according to a stratified or random procedure, as suits the investigation. The samples are widely spaced along the transect. Such a sample scheme is well-adapted for determining distribution limits of species for beach surveys and areas where there is great faunal diversity.

3.2.2. Density Sampling.—In this case extension is less of a consideration than sample reliability. Six to eight random 1/16 m^2 subsamples are taken from a 1/4 m^2 grid. This grid should be used at selected intervals along the transect, e.g., low-, mid-, and high-tide levels, to obtain maximum contrast. Such a sample scheme is well-adapted for determining density where a precise estimation of numbers is more important than the distributional limits of species and where there is little faunal diversity.

3.2.3. Corers.—For quantitative sampling, coring is the most suitable method. Almost any type of tubing may be used, but transparent plastic tubing is preferable to metal since it allows visual recognition of core compression so that precautions can be taken. In sand, samples should be taken from as deep as possible into the sediment if the total fauna is required (see Section 3.2.4 for methods of sampling the deeper layers of littoral sand), but on mud most of the organisms are confined to the top few centimeters and cores of 8 to 10 cm in length usually contain most of the fauna. On both substrates, however, an initial examination of a long core will indicate the vertical distribution and suggest how later sampling should be done. In all cases, if vertical zonation is to be studied by sectioning cores, the sectioning must be done immediately upon collection, or as soon as possible afterward, to avoid errors due to subsequent redistribution of the fauna.

Ideally, the diameter of the corer should be such that it provides enough animals so that processing can be done in a reasonable time without recourse to subsampling, which can introduce errors. The most appropriate core size for most meiofaunal studies seems to be within the diameter range of 3 to 4 cm. Narrower cores may not sample the fauna effectively; wider corers may lead to extraction difficulties. Core and sample size, however, must be related to the size and relative abundance of the animals to be studied. In narrow cores there may be the problem of core compression, and where this cannot be avoided the investigator should note the length of cores that can be taken without this occurring. In the sublittoral, other factors may be revelant. The presence of a surface flock or a deposit which may be disturbed and inadequately sampled by narrow cores may necessitate the use of a corer as wide as 10 cm from which subsamples can be taken after removal of most of the supernatant water.

3.2.3.1. Simple Tubular Corers.—Simple tubes of plastic or metal are quite suitable, but the problem of core extraction needs careful thought. Pushing out the core with a plunger inserted into one end disturbs the surface and will almost certainly cause core compression. In moist sediments the core will tend to slide out of the tube under its own weight when it is removed from the substrate, and this tendency may be utilized to achieve an undisturbed sample. One method is to insert a rubber bung into the top of the tube (when it has been inserted to the required depth into the substrate) and then remove the corer

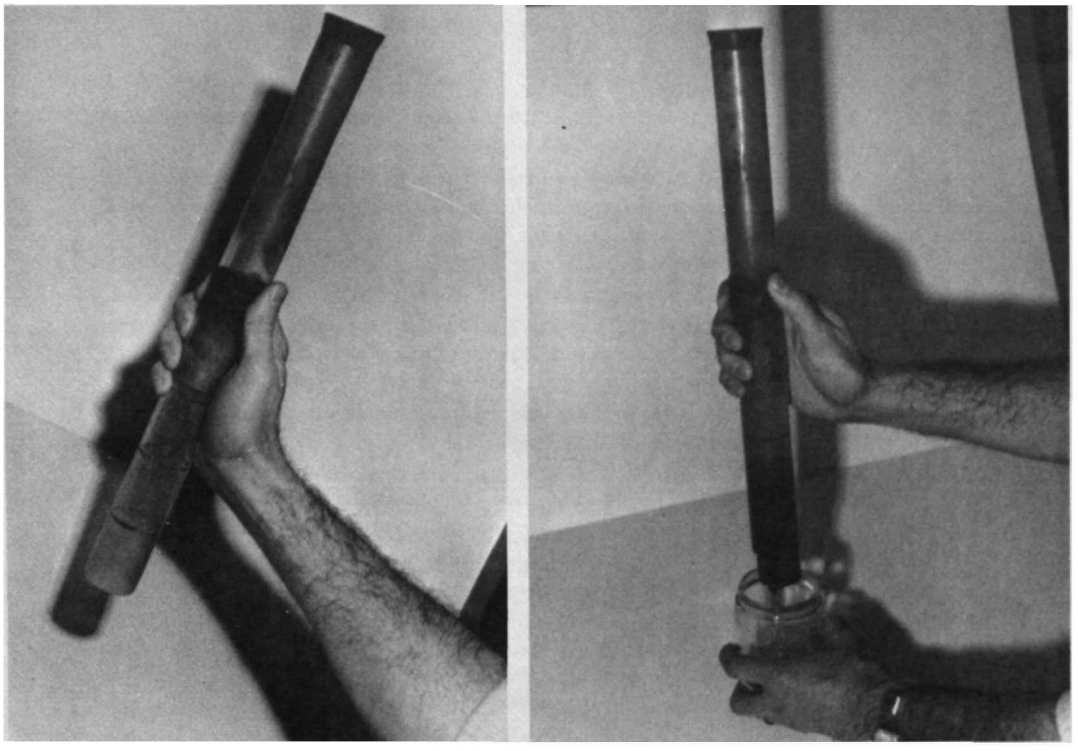

FIGURE 6.—Hope corer.

from the substrate. Gentle pressure on the side of the rubber bung allows air into the top of the tube, and the core slides out. The admission of air can be controlled by this simple method, and when a suitable length of core has emerged it can be sliced off into a container.

A variation of this technique is the use of a corer devised by Hope. This device (Figure 6) is made from a lucite tube approximately 40 cm long with an outside diameter of 37 mm and a wall thickness of approximately 1 mm. Lines are inscribed around what is to be the lower end of the tube to provide a scale for measuring length or volume of core samples. The opposite or upper end of the tube is fitted with a rubber bung. An air-intake hole 1 cm in diameter is drilled through the wall approximately 15 cm below the upper end and then covered with a rubber sleeve 3 cm wide. A section of bicycle inner tube serves well for the sleeve.

Sediment samples are taken by pushing the lower end of the coring tube into sediment, closing the upper end with the bung, and then withdrawing the coring tube and sample from the substrate. The sample is then removed from the coring tube by using the thumb of the hand in which the tube is being held to roll down the rubber sleeve. As the air intake hole is uncovered, air enters and the core slides out of the lower end of the tube. By using the scale inscribed on the coring tube, the sample can be lowered from the coring tube by measured amounts and each portion may be sliced off for vertical distribution studies. This

device has the disadvantage of having to be used in submerged sediment so that the space in the corer above the sediment sample is filled with water.

In drier or very course sand or gravel the techniques given above work less effectively and experimentation may be necessary to find a suitable solution.

3.2.3.2. "Ring-lined" Corers.—Jansson (1967a) has developed a corer which is lined with a series of rings. The core and rings are pushed out together by a plunger, and as each ring emerges it can be removed with a segment of core. This method does not cause core compression as the plunger acts against the rings and not the sediment.

3.2.3.3. Demountable Corers.—This type of corer can be split open and the core cut into sections while still within the tube. The Renaud-Debyser (1957) sampler is of this type and has additional advantages (Section 3.2.4). These samplers depend on cohesion of the sediment and are difficult to use in drier sand.

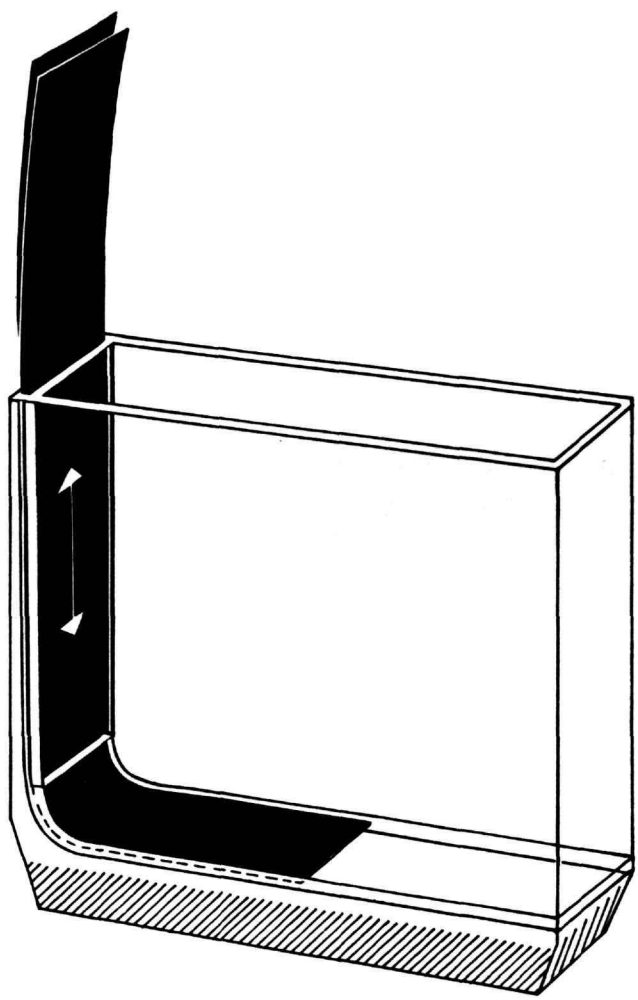

FIGURE 7.—Thiel's "Meiostecher." (From Gerlach, 1968.)

3.2.3.4. "Meiostecher".—The "Meiostecher" (Thiel, 1966) is a modified coring device (see Figure 7 and Appendix, Section 10.1.13). The plexiglass box, 10 by 2.5 cm, is open at the lower end and has beveled cutting edges. It is pressed into the sediment to the desired depth and is then closed by pushing a thin copper blade or flexible plastic sheet through the groove (Gerlach, 1968). The apparatus can be used in flat beaches and also for subsampling (see Section 3.3.2).

3.2.4. Sampling the Deep Layers of Beach Sand.—Although most of the fauna of beach sands will occur in the top 25 to 30 cm, it is necessary to take samples down to the level of the permanent water table, which may be as deep as 2 m or more. Ax (1969) has described and illustrated the various techniques of sampling above and below the water table.

A tubular corer about 30 cm in length can be inserted into the sand to its maximum depth. The sand is then dug away to the level of the bottom of the corer, and the corer is removed. Another corer is then pushed into the sand at the point reached by the first, and this process is repeated until the water table is reached.

A long demountable corer of the Renaud-Debyser type (Renaud-Debyser, 1957) can be used. This apparatus can obtain cores of up to 1 m in length.

A pit can be dug into the sand to the depth of the water table and a series of cores taken horizontally, one below the other, into the wall of the pit (Zinn, 1942, 1968). The disadvantages of this method are the rigidity that it imposes on the vertical subsampling of the sand column and the unknown effects of the prolonged presampling disturbance of the sediment on the more motile elements of the fauna. Hummon (1969) and Pollock (1969) have modified this method by taking horizontal cores from the base of the pit as progressive depths are reached. In this way, presampling disturbance is minimized and it is possible to localize the fauna. Vertical subsampling is resticted, however, since a continuous core is not obtained.

3.3. Sublittoral Sampling.

3.3.1. SCUBA.—The most satisfactory samples from the sublittoral are without doubt those collected by divers. Many of the littoral sampling techniques are workable by SCUBA and the diver can select the exact spot from which to take the sample. The size of the sample can be controlled, sampling irregularities can be reported, and notes can be made on the extent to which the sample is representative of the area as a whole. A diver can also sample areas of the shallow sea inaccessible to remote gear, such as narrow submarine valleys, submarine caves, small patches of substrate, and the transition zone between two adjacent sediment types. For qualitative work large volumes of sediment can be collected easily in bags or containers of any convenient size or shape. For quantitative work, coring techniques similar to those used for littoral sampling can be used, perhaps with some modifications. Sections 3.2.3 through 3.2.3.4 should be consulted.

While SCUBA techniques provide the best sublittoral samples, they have certain obvious limitations, one being depth. The operational limit is 30 to 50 m, and even towards the extreme of this range complex operations are difficult and the time available to the diver on the bottom is limited by problems of providing subsequent decompression facilities. The depth range can be extended only by the use of complex underwater facilities. In collecting samples the diver must take care, especially on muddy grounds, that his own activities do not disturb

the substrate and interfere with further sampling. Finally, it must be emphasized that SCUBA operations can be dangerous; the necessary precautions and restrictions are adequately covered in the handbooks of national subaqua organizations. It is imperative that SCUBA be used only by suitably trained personnel.

3.3.2. Remote Sampling.—For qualitative work, the best sampler is the one which provides the maximum volume of sediment that can be handled by the available facilities. For such work a dredge (defined as an instrument which is towed and which samples horizontally) is probably better than a grab (an instrument lowered on a vertical warp to take a single bite of sediment), but where it is suspected that several sediment types may exist side by side the tow should be of short duration. Several suitable instruments are available; sledges, which are fitted with skids, skim off the surface layer and are particularly recommended. An example is the Ockelmann (1964) sledge. This instrument was designed to collect only the larger species, and it requires modification to obtain the smaller forms.

For quantitative work in shallow water the best remote gear is probably an open-tube gravity corer. Hopkins (1964) has reviewed the various types of gravity corers used up to 1964. The instrument devised by Moore and Neill (1930), or some modification of it, is frequently used. On sand, however, a core retainer is usually required, and the type described by Mills (1961) is suitable. More recently corers have been described by Craib (1965) and Willemöes (1964), the former taking cores of 15 cm in sand and having a built-in retainer. The box corer of Reineck (1958, 1963) has proved very useful (see Appendix, Section 10.1.14). Drzycimski (1967) described a corer that uses the same principle as a cutting knife for core retention; such a corer is much smaller and lighter than Reineck's, and may be very useful in shallow water. A more detailed review of this type of gear is given by Wells (1971). The same difficulties noted for diver-collected cores occur with remote gear (loss of superficial deposit and core compression), but they are more serious with remote gear since they pass undetected. Because of this, all remote gear must be tested in shallow water with a diver observing its mode of action, so that these problems can be eliminated or reduced to the lowest level possible. Other instruments currently in use for sublittoral meiobenthos sampling include the "mouse trap" sampler of Muus (1964), which collects a superficial area of 150 cm^2 to a depth of 2 to 3 cm, and the sampler of Corey and Craib (1966), which takes a similar small sample of the surface layer of the sediment.

Multiple-tube corers that provide a variable number of samples depending on the instrument have been used successfully on sublittoral mud bottoms. The Krogh-Spärck multiple corer (Krogh and Spärck, 1936) employs six tubes, each 28 mm in diameter and 50 cm long and having a rubber check valve at the top. M. L. Jones (Smithsonian Institution, Washington, D. C., personal communication) has developed a "centuple-cell sampler" consisting of 100 square brass tubes each measuring 2.54 cm (Figure 8). The device is so constructed that the individual tubes can be disassembled. In the assembled state, the sampler covers an area of 0.64 m^2. Other multiple corers include those of Buzas (1968) and Willemoës (1964).

Another frequently used method in sublittoral work is the collection of a core sample from a grab haul. On sandy ground the gear should be as heavy as is practicable to produce a good sample, but on mud a sampler that is too heavy will sink into the deposit and disturb the surface layer. As with corers,

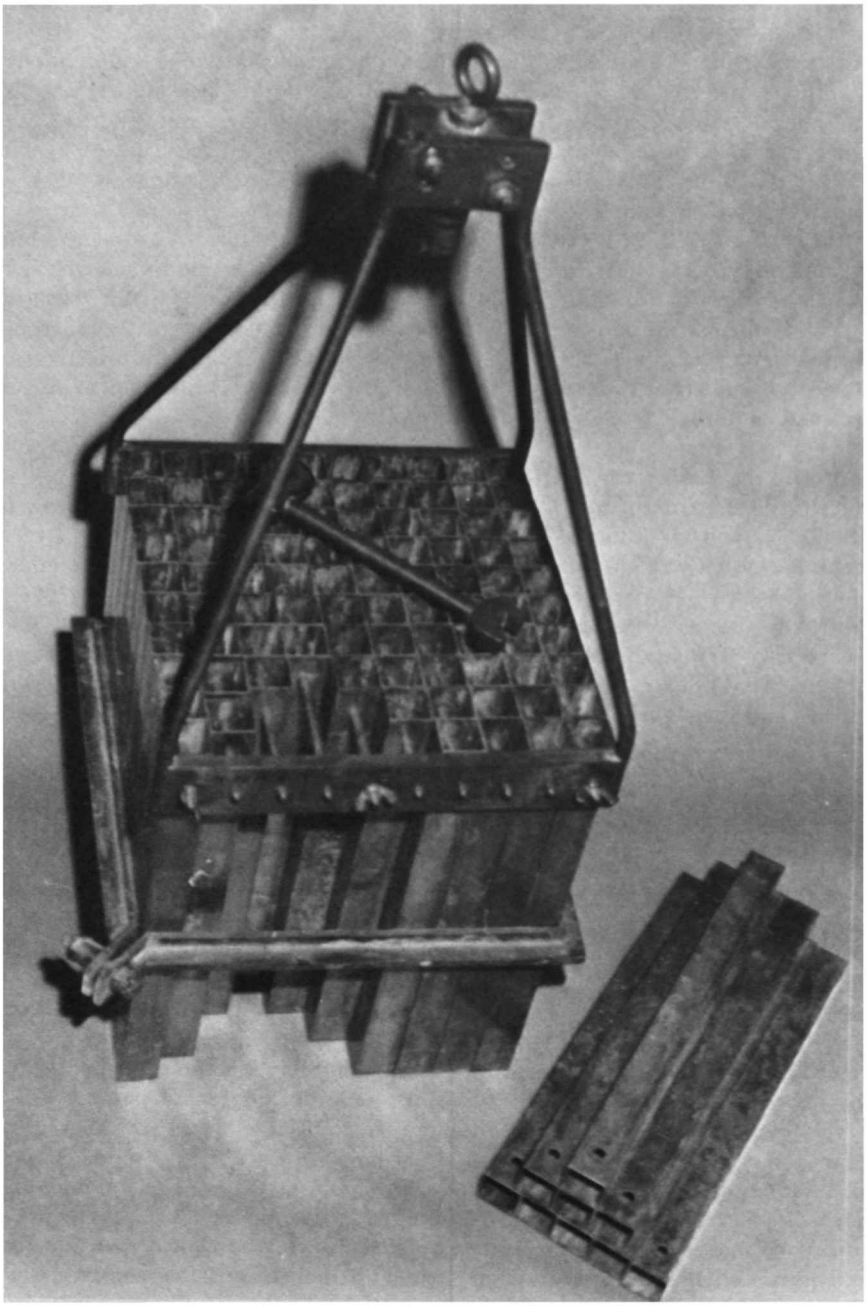

FIGURE 8.—Jones's "centuple-cell sampler."

the downwash of the instrument may disperse some of the superficial flock, and if subsampling is to be done from a grab haul an additional problem is the possible redistribution of the top of the sample during hauling and the possible loss of all the surface flock.

Deep-sea sampling produces a number of additional problems (Hessler, 1971a). Because of the time involved in lowering and raising the gear and because of the high proportion of partial or complete failures, careful planning is required to make the best of each haul. Experience gained from work with the macrofauna has shown that the standing crop tends to be greatly reduced in the deep sea, so that quantitative sampling devices, with their comparatively small volumes of sediment, have been less satisfactory for the systematist than they have been for the ecologist. Consequently, deep-sea programs tend to include both large-volume qualitative samples as well as quantitative methods wherever possible. The generally much higher density of meiofauna may make this unnecessary for some taxa (Thiel, 1966), but until more information is available it is probably still valid to take samples with as large a volume as possible.

There is no single instrument that can be recommended as a quantitative tool. Several grabs including the Petersen, Van Veen, Smith-McIntyre, Campbell, and Ocean (see Hopkins, 1964 and Holme, 1964, for a review of grab samplers) have been used with variable success in deep water. Box corers such as the United States Naval Electronics Laboratory (USNEL) spade corer (Rosfelder and Marshall, 1967) and the Reineck (1958) "Kastengreifer" have only recently been used at great depths, but they offer great promise because of their high success ratio and their ability to take large, apparently undisturbed samples. Hessler (personal communication) has recently succeeded in obtaining a series of undisturbed 0.25-m^2 biological bottom samples from a depth of 5,600 m using the USNEL spade corer.

At shallower depths, both grabs and box corers may be subsampled to give replicate quantitative samples of manageable size. Possibly the Hope corer (Section 3.2.3.1) and Thiel's (1966) "Meiostecher" (Section 3.2.3.4) can be used for subsampling. Clusters of small diameter cores also may be useful. The replicates can be divided horizontally to give information on the vertical distribution of the fauna.

All these instruments suffer from the disadvantage—already discussed—that they produce a "bow wave" which will disperse the light superficial sediment and its associated organisms. This effect can at least be reduced by making every effort to ventilate the sampler by valves and mesh screens and thereby reduce the downwash, and by attempting to lower the gear gently to the bottom. The use of a gauze cover on the grab bucket, as in the Smith-McIntyre grab, is an example of such a ventilation method (Smith and McIntyre, 1954; Wigley and McIntyre, 1964). Soutar (personal communication) has modified this grab so that the approach to and penetration of the bottom is very gentle, thus solving the bow-wave problem. Gear should be hauled in at the maximum possible speed so that winnowing of the sample is reduced, and this speed should be maintained until the sampler is clear of the water in order to minimize the damage caused by the turbulent surface waters. The sampler should be hauled inboard and the sample processed with a minimum of delay, thus avoiding final winnowing and damage to surface and near-surface dwellers caused by high-temperature shock that may result in exposure to air having a much higher temperature than the seawater.

One practical problem in deep-sea sampling is in knowing when the gear has reached the bottom. Wherever possible the use of a pinger attached to the wire a known distance above the sampler and monitored by a precision depth recorder is recommended. A recording tensiometer also is useful. The greatest problem, however, is winnowing during hauling. Few samplers are able to close completely, and this may not represent a serious problem at shallow stations; but in the deep-sea, where the gear is being hauled for a considerable time and through a long column of water, even slight leakage can result in the loss of most of the sample.

These quantitative devices cover a surface area 0.1 to 0.5 m^2, depending on the type. As already suggested, samples of this size may be too small for the investigator's requirements in the deep sea and, in particular, they will not satisfy the systematist. In this case, qualitative samples must be utilized. A suitable qualitative sampler should collect only the top few centimeters of the deposit over a fairly extensive area and should not be subject to winnowing. Suitable devices of this type have not yet been designed for deep-sea meiofauna work, but some of the meiofauna samplers presently in use could be modified appropriately, such as the epibenthic sledges designed at the Woods Hole Oceanographic Institute (Hessler and Sanders, 1967). As used for larger organisms the epibenthic sledge is fitted with a 0.5-mm mesh bag, but for meiofauna sampling the mesh size could be reduced. Also, for depths greater than 2,000 m the mouth should be fitted with a postsampling closing device. In sampling with the sledge a weight should be attached to the wires to reduce the amount of warp required, and for quantitative sampling a pinger and tensiometer are extremely valuable. The latter is particularly useful in that it gives a warning when the gear encounters an obstruction. In trawling, speeds of 1 knot or less are necessary.

Finally, it should be noted that while the use of deep-sea submersibles is not yet widespread, this facility will permit the extension of well-tested shallow-water techniques to great depths.

4. General Extraction Techniques

This section deals with techniques for the whole or at least the major part of the fauna in a sample. Reference is made to techniques used by the ecologist for strictly quantitative work as well as those which treat large volumes of sediment. The latter would produce specimens which, in some cases at least, would satisfy the minimum requirements of the systematists.

For living fauna there are two main divisions of the techniques: those which depend on the natural movements of the animals without the use of anaesthetics, and these techniques are favored by those requiring material for physiological experiments; and those which involve removal of the animals from the deposit by physical disturbance such as sieving or elutriation, when an anaesthetic is required. For preserved material, staining of the total sample (for example, by Rose Bengal) usually is required.

4.1. Field Techniques.—These are usually less than quantitative, but are designed to provide some degree of extraction. Often it is impossible to extract the meiofauna immediately after collection. Though often it is stated that it is desirable to store the samples at low temperature (around 5° C), J. B. J. Wells (personal communication) has had good results in the tropics by storing samples

at or near ambient room or seawater temperature. He found that samples stored at 10° to 15° C deteriorated within 12 hours with all taxa dead, whereas samples stored at ambient temperature in tightly sealed containers for 36 to 48 hours, and in some cases up to four days, were in good condition (the samples did not fill the containers and there was air above the sample). For those animals having short generation time, such as Ciliata, this procedure would not be satisfactory for quantitative studies. Nor would this procedure be satisfactory for highly predaceous organisms such as certain Nematoda.

4.1.1. Sand.—The following method is recommended. Seawater is filtered through a fine mesh (62 μ) net into a clean bucket, and a relaxant (chloroform, alcohol, formalin, $MgCl_2$, propylene phenoxotol) may or may not be added. The sand sample is then added to the bucket and left for 10 to 20 minutes to let the relaxant act. It is then stirred, and after momentary settling the water is decanted through a 62-μ net; stirring and decanting are repeated until the lighter sediments are almost entirely removed. The meiofauna is finally washed down into the cone of the net and the everted net washed into a container. The sample is then divided into two nearly equal fractions. One of these is preserved in formalin and the other in alcohol, by graded increments. For formalin preservation, add 4 percent formalin, a few drops at a time, over a period of 40 minutes. For alcohol preservation add 70 percent alcohol in the same way. At the end of the process, decant the liquid and replace with fresh 4 percent formalin or 70 percent alcohol. The well-known method of Chappuis (1942) also is relevant to this section.

4.1.2. Mud.—The bubbling technique of Higgins (1964) is described in Figure 9. This technique is suitable, but it may be detrimental to certain taxa such as Gastrotricha, Gnathostomulida, and Turbellaria, and generally it is ineffective for removing Nematoda.

4.1.3. Shipboard Techniques.—If operating from a ship which is at sea for only one or two days, it may be possible to maintain the samples in sufficiently good condition for laboratory extraction by storing them in a refrigerator. But on cruises lasting more than a few days it usually is necessary to accept the less-than-ideal extraction method, which can be done at sea, or to preserve the unprocessed sample.

If extraction techniques are to be employed on the open sea, it should be remembered that such techniques must be simple, since the ship's motion and vibration as well as the shortage of space and time on most cruises make complicated techniques impractical. It may be possible to use the Boisseau technique (Section 4.2.1.4) in good weather, but in general, for small samples of living material, shaking with $MgCl_2$ and decanting is the most practical procedure.

At the qualitative or semiquantitative level the techniques described for sand (Section 4.1.1) and mud (Section 4.1.2) can be used on board for collections of surface deposit obtained with gear such as the epibenthic sledge.

For larger volumes of material from deep-sea samples the elutriation technique used for macrofauna (Sanders, Hessler, and Hampson, 1965) can be applied to meiofauna work by substituting appropriately smaller sieves. This technique involves pumping large volumes of seawater at low velocities through the sediment and allowing the supernatant liquid to flow through selected sieves. The flow of water should be sufficient to prevent even the light but coarse particles, such as *Globigerina* tests, from flowing over onto the screens, since these make later sorting unnecessarily difficult. It is important, however, to accept the fact that

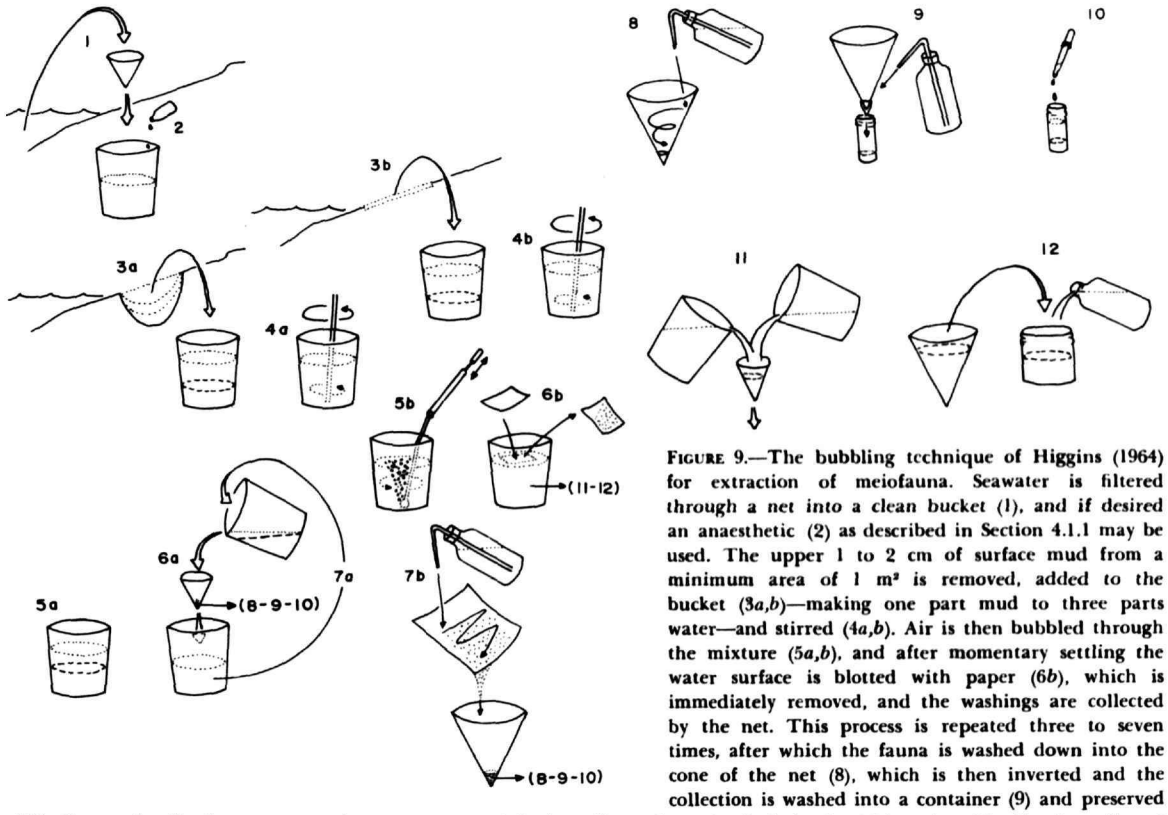

FIGURE 9.—The bubbling technique of Higgins (1964) for extraction of meiofauna. Seawater is filtered through a net into a clean bucket (1), and if desired an anaesthetic (2) as described in Section 4.1.1 may be used. The upper 1 to 2 cm of surface mud from a minimum area of 1 m^2 is removed, added to the bucket (3*a*,*b*)—making one part mud to three parts water—and stirred (4*a*,*b*). Air is then bubbled through the mixture (5*a*,*b*), and after momentary settling the water surface is blotted with paper (6*b*), which is immediately removed, and the washings are collected by the net. This process is repeated three to seven times, after which the fauna is washed down into the cone of the net (8), which is then inverted and the collection is washed into a container (9) and preserved (10). For sand collections, two samples—one preserved in formalin and one in alcohol—should be taken. Finally, the sediment in the bucket is washed through a 62-μ sieve (6*a*) and accumulated in a separate bucket (7*a*). This sediment then is divided into two parts, which are preserved in alcohol and formalin (11, 12) as described in Section 4.1.1.

the stenotopic deep-sea animals are unlikely to survive manipulation on deck. Death is almost certain as a result of thermal shock, which accompanies the transfer of the fauna from the cold deep to the warmer surface conditions. The animals may still be alive on reaching the surface because of the insulating effect of the large volume of sediment, and if adequate refrigeration facilities are available it may be possible to keep them alive at least long enough to apply anaesthetics before preservation. If extensive manipulation of the sample is required in the living state it is best to bring large refrigerated samples ashore for processing, and this may be practical where a laboratory is close to the deep-sea areas, such as at Bermuda.

4.2. Laboratory Techniques.

4.2.1. Sand.—A combination of techniques must be used to extract small motile organisms (e.g., Ciliata) and larger thigmotactic forms (e.g., Nematoda). The motile forms can be extracted with the Uhlig Seawater Ice Technique (Uhlig, 1964, 1966).

4.2.1.1. Uhlig Seawater Ice Technique.—Seawater from the vicinity of the sediment sample is frozen. The sediment sample is placed on a nylon gauze (140-μ pore diameter) at the bottom of a tube. A thin layer of cotton wool is

placed on top of the sediment, followed by the crushed seawater ice. As the ice melts, small motile organisms are carried through the gauze and can be collected in a Petri dish. The technique also has been successfully used with 40° C water instead of the ice, and this might be preferable in tropical regions.

J. B. J. Wells (personal communication) has found that the Uhlig technique works as well in the tropics as in cold climates, based on his studies in Ernakulam, India. Melting time of the ice is, of course, shorter, but he found that this caused no problems in extraction. He found that cold water (temperatures of 4° to 6° C) is quite effective, but that it is essential to place a fine-pore filter over the sand to slow down the filtrate. Very small Ciliata were extracted less well with cold water, however, than with ice. Wells also found that water at 50° to 60° C extracted more animals than water at 40° C. The extraction efficiency for Ciliata was, however, much less, and many animals were killed and washed out passively. He did find that water of higher temperature had a relaxing effect on many animals.

Fenchel (1967, 1969), Thane-Fenchel (1968), and Uhlig (1964, 1966) should be consulted for extraction efficiency of the Uhlig technique. Extraction efficiencies of 75 percent have been reported for Ciliata, Flagellata, Turbellaria, Gastrotricha, and Archiannelida. Lower efficiencies are reported for Nematoda, Rotifera, Harpacticoida, and Tardigrada. Prolonged extraction and the use of larger extraction tubes appear to increase the extraction efficiency of some taxa.

4.2.1.2. Anaesthetization Technique.—After treatment to remove the motile organisms the sediment is placed in a beaker, and an equal volume of 6 percent $MgCl_2$ (i.e., 73.2 g/liter, which is isotonic to 34 ppt seawater) is added. After allowing 10 minutes for anaesthetization the sediment is stirred thoroughly and the supernatant poured through a fine mesh sieve or plankton net (62-μ pore diameter). This is followed by two washes of seawater and a wash of 4 percent formalin or 10 percent ethyl alcohol. The sieve is inverted in a Petri dish and the animals are washed off with a jet of filtered seawater.

The sample must be examined periodically after the washing and the extraction efficiency noted. This is necessary when a new locality is sampled. The accuracy of the method must be quoted in any publication.

4.2.1.3. Deterioration Technique.—Although not quantitative, this technique concentrates the vagile fauna. Large volumes of sediment can be used, but when mud is used the layer should not be too deep. The sediment is placed in a bucket and allowed to deteriorate at a temperature slightly above that in the field. The animals migrate to the sediment surface, provided that deterioration does not proceed too quickly. Surface layers of sediment are removed and animals are extracted following the techniques described in Sections 4.2.1.1 or 4.2.1.2. A wide variety of substrata, including sponges and algae, can be used with this technique.

4.2.1.4. Boisseau Technique.—The apparatus for the Boisseau technique (Boisseau, 1957) is shown in Figure 10. Sediment is placed in the separation funnel (A) and an equal volume of 6 percent $MgCl_2$ is added. After allowing 10 minutes for anaesthetization, a continuous stream of seawater is introduced through the tap on the separation funnel. The pressure is adjusted so that a balance is achieved between vigorous agitation of the sand and only a small amount of sediment is carried over to tube C and the sieve (D). After 15 minutes of elutriation the tap on tube C is opened and the water allowed to drain through the sieve. The sieve is inverted in a Petri dish and the animals are washed off with a jet of filtered seawater.

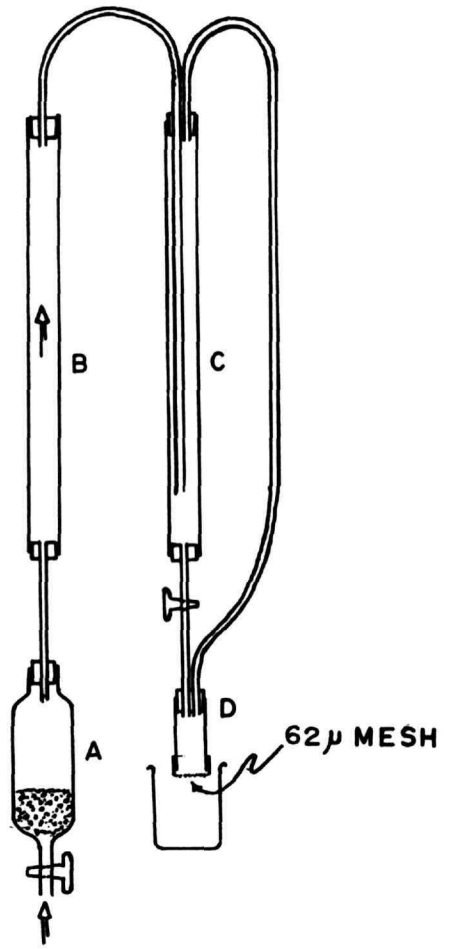

FIGURE 10.—Boisseau apparatus for extraction of meiofauna. (Modified from Boisseau, 1957.)

This technique is not recommended for coarse sands, as the efficiency is reduced. Further, while it can be used as above for live fauna, several groups (Tardigrada, some Nematoda) are difficult to detach from the sand grains, and others, such as Turbellaria, quickly recover from the anaesthetic and attach firmly to the walls of the tube. This difficulty can be overcome by setting up the equipment as a closed system and maintaining an adequate concentration of $MgCl_2$ in the water pumped through the apparatus. The Boisseau technique, however, is highly satisfactory with preserved material, at least for the "hard" taxa, and the apparatus has been modified by Lasserre to operate with one or two liters of sand. The principal parts of the Lasserre modification are one 5-liter glass flask with two stirring systems. The sand is agitated by a flow of seawater entering the tubes, which are divided into four elements, and by a mechanical stirrer used once or twice during the process. A glass column contains a large mesh net which retains most of the very coarse particles and fragments of sea grass and algae. The column is easily drained via the tap on tube C (Figure 10). The animals and fine organic particles are retained on a tightly fitting nylon gauze, the mesh size of which is

62 μ. From 94 to 95 percent of the fauna contained in one to two liters of sand can be extracted in 15 minutes of washing.

4.2.2. **Mud.**

4.2.2.1. **Quantitative Extraction of Total Fauna.**—In deposits without a good pore structure the Uhlig technique cannot be used, and when even moderate quantities of fine material are present the Boisseau or decantation procedures are not applicable, so that sieving and hand extraction are required.

The usual procedure for living meiobenthos is to divide the sample into two fractions, using a fine sieve (30 to 60 μ). The coarser fraction is sorted, using a binocular microscope. The fine fraction, which contains the silt and clay, is stirred into uniform suspension and the liquid is subsampled. The deposit in the subsamples is allowed to settle out in a thin layer on a Petri dish, and the animals can be seen and counted by their movements. A similar technique can be used for preserved material, but the sample is first stained with Rose Bengal (0.1 gm/

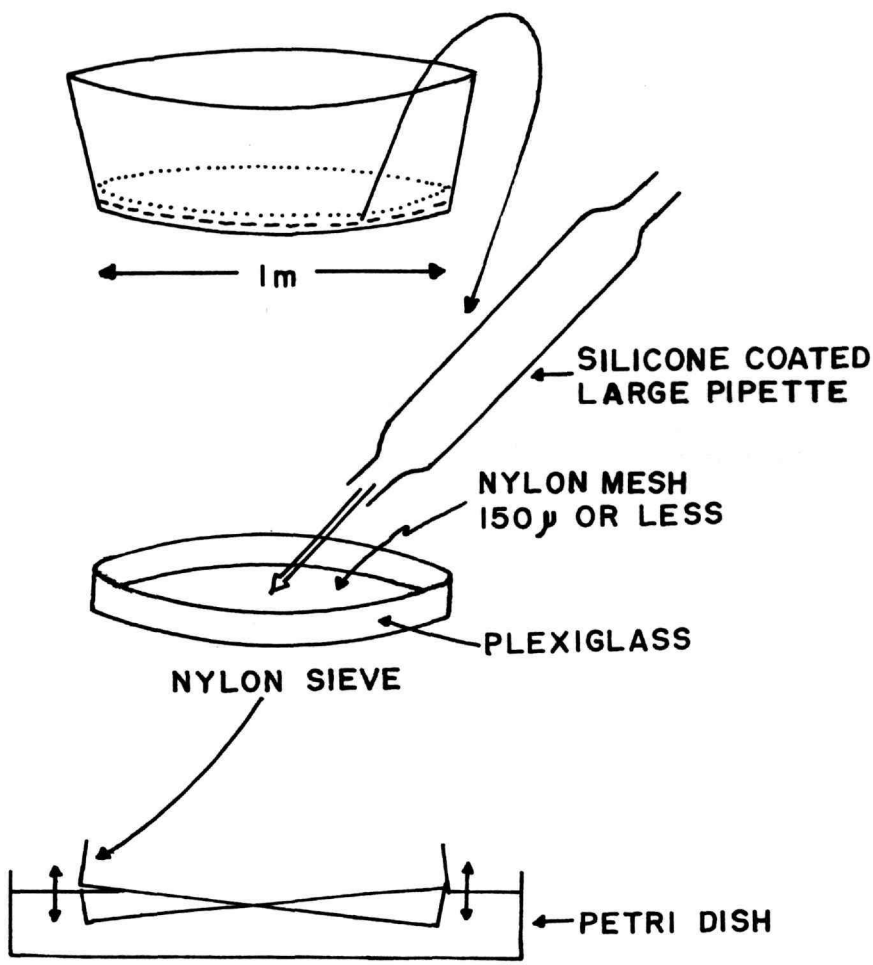

FIGURE 11.—Swedmark technique for extraction of meiofauna.

100–200 ml of 5 percent formalin or 70 percent alcohol), and because of the difficulties of hand-sorting dead specimens from silt and clay material, the finest fraction is usually discarded. Although laborious, the method used for live material is completely quantitative. With preserved material, it is quantitative within the limits of sieve used, and it can be made fully quantitative if an occasional live sample is available to give estimates of what proportion of the fauna passes the sieve.

4.2.2.2. Swedmark Technique.—This technique (Figure 11) can be used for fine sand as well as muds. The deposit, stirred to break up lumps, is placed in a large vessel and covered with 1 or 2 cm of seawater. The mud surface is then pumped into suspension, using a large silicone-coated pipette (for preparation of the pipette see Appendix, Sections 10.4 and 10.1.15), and transferred to a nylon sieve or series of sieves (250 μ, 62 μ), the largest having a diameter slightly less than the normal size of the Petri dish used. Sieving is done by gently rocking the sieve in seawater either in another vessel or in the original one so that the filtrate is returned to the original sample. When sieving is complete the sieve is placed in seawater in a Petri dish so that the fauna can be first examined under a binocular microscope without the damage that later may be inflicted by transferring from the sieves. This procedure can continue for as long as the animals remain alive—for several days if desirable. At this stage, the process does approach a quantitative method as long as the exact size of the original sample is known.

4.2.2.3. Bubbling Technique.—This technique is discussed in Section 4.1.2, for field studies. It can be suitably modified for laboratory use.

4.3. Counting and Observation Efficiency.—Although the extraction efficiency may reach 80 to 90 percent, observational efficiency should be determined periodically by reexamination of previously counted samples. Otherwise this may be a source of error that is frequently ignored. Provided that extraction and observation efficiencies have been estimated, quantitative comparisons of fauna can be made. Frequently, however, raw data cannot be used for comparative purposes. Transformation procedures are necessary for the statistical validity of the data (see Greig-Smith, 1964; Williams, 1964).

5. General Taxonomic Procedures

This section covers procedures for the examination of live material, for fixation, preservation, and curation methods, and for data presentation. There are several publications that provide information on preservation and other techniques for marine animals: British Museum (Natural History), 1954; Guyer, 1953; Lo Bianco, 1899; Russell, 1963; Wagstaff and Fidler, 1955. Most of these publications, however, deal with larger marine invertebrates and they provide little or no information for meiofauna.

5.1. Examination of Live Material.—Examination of live material is highly desirable in all meiofauna groups, and it is indispensable in the "soft" taxa. A wide range of biological data, from locomotion to feeding, sexual behavior, and even life cycles often can be obtained by direct observation, and such data should be incorporated into systematic descriptions whenever possible. In many "soft" taxa, furthermore, determination, as well as description, based on preserved material alone is impossible. The lack of live data can only partly be compensated by time-consuming histological techniques.

5.1.1. Techniques.—Transmitted light usually is preferable, although incident light should be used for color observations. Single "soft" specimens can be placed on a slide with a pipette, the end diameter of which should exceed only slightly the diameter of the specimen. The inside of the pipette should be coated with silicone to reduce adhesion of animals. Specimens with a cuticle can be placed on a slide with an Irwin loop (see Appendix, Section 10.1.16). Specimens should be observed in seawater of the same habitat, using a slide and coverslip. For "soft" taxa, the coverslip must be supported to prevent damage by weight and capillary pressure. The edges of the coverslip can be scraped over bee's wax or plastiline, thus providing the necessary support. Most "hard" taxa do not require support, although the same procedure may be employed. Observations on behavior, color, body outline, and external structures should be carried out immediately.

Anaesthetics can be added from one side of the coverslip following initial observations. Observations on body dimensions and position of internal organs can readily be made. The specimen can be rotated along the longitudinal axis by applying pressure to the coverslip. Drawings at various magnifications can be made under these conditions.

Squeezing of the animal can be accomplished by gradually removing all of the liquid and applying pressure to the coverslip. The animal becomes more transparent, permitting a detailed analysis of the internal organization. Should it be desirable to keep the specimen for histological purposes, squeezing should be kept to a minimum. If histological studies are not required, further squeezing often will reveal more delicate details, such as cuticular structures, gametes, etc.

Although the squeezing method does not present major problems, it can be much simplified by using a "Roto-Compressor," described by Heunert and Uhlig (1966). Use of such an instrument is especially advisable where the animal should be kept in a constant situation over a longer period of time (e.g., for time-lapse filming).

Excellent optical equipment is mandatory for critical observation. Phase contrast is absolutely indispensable for most meiofauna research, and the new interference contrast (Nachet, Reichert, Zeiss) already is adding a new dimension to the analysis of small organisms. Whereas the use of electron and scanning microscopes is not necessarily required for taxonomical investigation, good microphotographic equipment and scale drawing apparatus (Wild, Zeiss, Leitz) should be part of the standard optical equipment. Where a drawing apparatus is lacking, at least photographs should be made to complement the drawings. If both are lacking, drawings should be made with the help of a measuring eyepiece, and whole mounts of specimens should be kept for a check of meristic data until better equipment is available.

In order to obtain maximum information from every specimen examined, a "count-down" of characters should be set up for every taxon. This means a complete listing of all characters to be observed, arranged according to their optimal visibility and/or logical progression during the different stages of examination.

5.2. Fixation.—Meiofauna may be fixed while still in the sediment, as a mixed aggregate of organisms after extraction or as individual taxa or individual specimens after extraction and sorting. Fixation of unsorted meiofauna in sediment is recommended only as a last resort, since there is no single method for optimally fixing both "hard" and "soft" fauna, nor of simultaneously fixing all forms of "hard" fauna. The preferred method is to separate the "hard" fauna;

single specimen fixation is preferable since it allows appropriate techniques for both whole mounts and further histological (light and electron microscopy) studies. It is emphasized that in the case of "soft" fauna any type of fixation without live examination is acceptable only under special conditions.

Unfortunately, it is often impractical or impossible to extract and sort meiofauna before fixation because of the time involved and lack of help or facilities. Under these circumstances the most satisfactory compromise is to anaesthetize (Section 4.2.1.2) the organisms, whether extracted or not, and then add sufficient full-strength buffered formalin to the sample to bring the final concentration to approximately 5 percent for at least 24 hours.

5.2.1. Fixation of "Soft" Fauna.—For unsorted "soft" fauna samples, the use of warm Bouin's fixative for two to four hours following anaesthetization is recommended. Individual fixation can be for whole mounts or for histological purposes. Whole mounts are preferable where hard structures are important for determination and for reference collections and type specimens.

5.2.1.1. Whole Mount Fixation of "Soft" Fauna.—Often it is necessary to fix representatives of "soft" fauna while they are compressed, especially when particular characters appear under this circumstance. A drop of 10 percent formalin is added on one side of the coverslip, and the original medium is removed with filter paper on the other side. A mixture of glycerol and formalin (3:1) is a satisfactory combined medium for fixation and preservation.

5.2.1.2. Fixation of "Soft" Fauna for Histology.—For general histological purpose, Bouin's fixative, preferably at 60° C, is recommended. Following anaesthetization the animals are pipetted from the seawater and then rapidly placed into the warm fixative. The animals should be left in the Bouin's solution for at least two hours, but preservation in the fluid for days or weeks often has no effect on the quality of the preparations.

For electron microscopy, primary fixation in glutaraldehyde in seawater seems to be widely used. Methods using this technique, however, are still in a stage of experimentation.

5.2.2. Fixation of "Hard" Fauna.—There appears to be no single fixative that is optimal for all taxa of "hard" fauna. Formalin makes the internal structure of mites resistant to pepsin digestion (see Section 6.21), and unbuffered formalin erodes calcareous structures which often are important taxonomic features. Alcohol fixation, on the other hand, causes Nematoda to collapse, and internal organs, which are of taxonomic importance, may be badly distorted. The best fixation for most kinds of "hard" fauna, however, is 5 percent buffered formalin in seawater for at least 24 hours.

5.2.2.1. Fixation of "Hard" Fauna for Histology.—Usually it is not necessary to make histological preparations for taxonomic studies of most forms of "hard" fauna since external body features are used extensively for this purpose. However, in instances where anatomical studies necessitate such preparation, especially in the case of Nematoda, it is desirable to avoid alcohol fixatives which extract water faster than alcohol can pass inward through the body wall, thus causing the organism to collapse. In addition, tissues may tend to shrink, causing the tissue components of the body wall to pull away from the extracellular component, or cuticle. The need to preserve water-soluble inclusions of cells or nuclear structures may override the disadvantages of alcohol fixation in certain studies.

Formalin (5 percent) fixation is recommended for histological study of Nematoda, and Bouin's fixative for most other hard fauna.

5.3. Preservation.—The preservative selected must be compatible with the fixatives used, as well as with the nature of the preparations that must be employed in subsequent studies of the specimen.

5.3.1. Preservation of "Soft" Fauna.—As in the case of fixation, it is preferred that as much sorting as possible be accomplished before the specimens are preserved. If sorting cannot precede preservation, the specimens should be preserved in 70 percent alcohol, and it is further recommended that this concentration of alcohol be reached gradually, i.e., over a period of one hour or two hours by using graded concentrations of alcohol.

5.3.2. Mounting of "Soft" Fauna.—Two types of whole-mount media can be distinguished: those which mix with water and fixatives and, therefore, can be applied directly to the fixed specimen; and those where preservation has to be preceded by dehydration techniques.

It is important that whole mounts be permanent and made with great care, especially where type material is involved.

5.3.2.1. Water Mounting.—Glycerol is generally accepted as a good medium. It is drawn under the coverslip where a squeezed and fixed animal can be maintained in a particular position, or added to the animal on the slide before the coverslip is put in place. The coverslip is then ringed carefully, avoiding air bubbles, with a commercial varnish such as Eukitt or Murrayite (see Appendix, Sections 10.1.17, 10.1.18). Other aqueous media are more applicable to different taxa and are dealt with accordingly.

5.3.2.2. Mounting after Dehydration.—Fixed animals are washed in 70 percent ethyl alcohol for 10 minutes; then placed in 95 percent ethyl alcohol (three changes, 5 to 15 minutes each); and then placed in absolute ethyl alcohol or butyl alcohol (three changes, 15 minutes each). After three subsequent 5-minute washings in xylol the animals can be mounted in Canada balsam.

5.3.2.3. Histological Preservation of "Soft" Fauna.—After fixation in Bouin's solution, specimens are rinsed in increasing concentrations of ethyl alcohol. The optimum is 10 minutes with 40 percent ethyl alcohol followed by several hours in 70 percent ethyl alcohol. The specimens then are transferred into 80 percent ethyl alcohol.

5.3.3. Preservation of "Hard" Fauna.—Formalin often is recommended as both a fixative and preservative, but when used as a preservative it should be buffered and the pH checked periodically. Preferred preservation of Nematoda and Harpacticoida is in buffered formalin or anhydrous glycerin. Ostracoda and other fauna that may be degraded in inadequately buffered formalin should be preserved in alcohol.

5.4. Curation.—Frequently, large collections of specimens must be held in bulk storage before sorting and indentification. Since this may involve a considerable lapse of time, it is imperative that specimens be stored in suitable containers with durable labels bearing collection data. Records with detailed collection notes and ecological data, when available, also should be retained in permanent files.

If only selected taxa are sorted from general collections and studied, measures should be taken to bring the remaining organisms to the attention of other qualified systematists, especially when the collections originate from remote localities and/or are accompanied by considerable ecological data.

Finally, it is most important that after specimens are set aside as type material they should be deposited in museums or other institutions where facilities and

personnel exist to assure their proper preservation. Catalog numbers or identifying numbers must be included in descriptions, thus making the specimens readily available to other qualified systematists.

5.5. Presentation of Data.—Recently, Mayr (1969) comprehensively summarized our present conception of taxonomic descriptions. Since all of his statements apply to meiofauna, the following subsection deals only with problems that are more relevant to or are most often neglected in studies of meiofauna. Most of the following statements refer to original species descriptions, but they also are true for complementary descriptions and identification. In the past, descriptions often have been kept to that minimum necessary to distinguish them from species described previously. A modern description, however, should be aimed to the future and include all characters available.

5.5.1. Minimum Requirements.—Whereas it is impossible to recommend a minimum number of specimens on which a description should be based, the series should be as abundant as possible or manageable. Complete and undamaged adult specimens of both sexes are required; descriptions based on juveniles can be accepted only under exceptional circumstances. Observation of the living animals is absolutely indispensable in "soft" fauna except in the few cases where valuable material is available only in preserved state; optimally preserved specimens are required in the "hard" fauna. Descriptions without illustrations must be rejected, and it is recommended that illustrations be made with a drawing apparatus, or checked with photographs, or otherwise carefully constructed. Collection data must be given as completely as possible; and types must be deposited in competent institutions. If the species is not known, it can be designated as "sp." or by placing "cf." (confer, compare with) before the species name wherever there is the slightest doubt about species identity or in case the determination was made by a person who does not consider himself fully competent in the particular taxon. The danger of using "cf." is that this designation is often omitted in subsequent publications, thus leading to erroneous reporting of the species. It is important to note any differences from the original description. The inclusion of all observable morphological characters will provide more useful comparative data.

5.5.2. Descriptions.—It is imperative to distinguish between documentation (presentation of data) and speculation (discussion of systematic relationships, phylogeny, etc.). A good description should yield the following data, preferably in a consistently uniform arrangement.

(a) A detailed statement should be given on number of specimens, developmental stage (juvenile, adult), and state (live, whole mount, or sectioned) of the material examined.

(b) Data on locality, depth, substratum, associated fauna, and as many ecological parameters as possible should be included.

(c) Names of new genera and new species should be etymologically explained.

(d) Numbers, state of preservation, and location of type material should be given.

(e) The description (sensu stricto) should follow the same order within a given taxon (e.g., external features anterior to posterior, then internal organs). It also is desirable to number the specimens available during examination and to do the same in the description ("one specimen was exceptionally long" would then read "Specimen 17 has the exceptional length of 870 μ," and a caption such as "anterior end" would be better as "anterior end of specimen 10"). The concept

of the specimen as the lowest taxonomical entity facilitates revisions (as the material for a description later on sometimes turns out to comprise several species) and makes the material more available for statistical studies.

For relative body dimensions and position of organs, it is advisable to take the body length as 100 Units (U 0 to U 100), e.g., "Ovary from U 60–U 72"; or "maximal width 33 μ = 7 U at U 65"), or as percents.

(f) Drawings should be given of all taxonomically important features and of features that are remotely suspected to be important. Photographs are complementary in view of the original optical impression and should not be used without proper line drawings. All illustrations should be accompanied by scales.

(g) Meristic data, from as many specimens as possible, are ideally presented in the form of tables.

(h) As many biological observations, including behavior, feeding, reproduction, etc., as are available should be included.

(i) Every new description must be accompanied by a diagnosis. Where new facts have to be incorporated in an earlier diagnosis, the modified diagnosis has to be given. For new genera, the type-species must be designated.

(j) A discussion should contain the reason (s) for placing the species in a particular genus, further considerations of the taxonomic value of characters and of systematic relationships and, if necessary, explanations for biological or ecological features observed.

6. Comments on Selected Taxa

This section deals with problems concerning individual taxa as compiled by the specialists. It is concerned with optimal sample size, extraction, examination, fixation, and preservation procedures.

6.1. Foraminifera.—Foraminifera are abundant in most types of sediment and at all depths. Any dredge, corer, or grab sampler that will retain fine sediment is adequate for qualitative sampling. For more accurate relative and absolute abundance data, specific volumes, which are preferable (Walton, 1955), or weights often suffice. For very careful numerical analyses, short cores (or tops of long cores) should be used, and mixing in cores should be avoided. Core diameter should be 3.5 cm (Phleger, 1960); Foraminifera tend to be dense enough that the top centimeter of this diameter usually yields hundreds of specimens. If not, the top 1 to 4 cm can be analyzed, centimeter by centimeter. The number of samples depends on the particular problem. Quantitative studies require several samples or replicates (Walton, 1955; Brooks, 1967; Buzas, 1969).

Five percent buffered formalin is a suitable preservative for whole sediment samples, but the samples should be washed over a 62-μ sieve and stored in 70 percent alcohol as soon as possible. Organic material may become acidic, reducing the buffered formalin which destroys calcareous tests and cements. With samples kept in formalin for any time, the pH should be checked frequently and more buffer (hexamine or Na_2CO_3) added as necessary.

To determine living specimens, the sample can be stained with Rose Bengal, a protoplasmic stain, following a procedure modified below from Walton (1952). The Rose Bengal solution is prepared by the addition of 0.1 g/100–200 ml of 5 percent formalin or 70 percent alcohol. It is important to avoid a precipitate caused by using an excess of powdered Rose Bengal. The stain can be added at

collecting time, or, better, a few hours before study. The sample should be mixed well to insure stain distribution; and this should be done a few hours before study because, with samples fixed in formalin, the pH cannot be checked with pH paper, and, less conveniently, a pH meter must be used. In addition the stain fades with time, although fading can be retarded by storing samples in the dark or in amber glass jars. Stained preserved specimens should be sorted from water-covered samples.

If extraction of specimens from preserved samples is necessary, samples should be washed with water on a 62-μ sieve. This is followed by drying and separation, using heavy liquids. Liquids used include carbon tetrachloride, bromoform, tetrabaromethane, tetracholorethane, Clerici solution, and others (Gibson and Walker, 1967). Bromoform seems preferable. Some undesirable organic debris can be dissolved with NaOCl or H_2O_2, and, even better, by subsequent ignition in a muffle furnace (Smith, 1967). These techniques should *not* be used if one wishes to recognize live specimens and if arenaceous specimens are present or are of interest.

After separation or concentration, the foraminiferal concentrates are examined microscopically. If numbers of specimens are very high, a suitable aliquot of about 300 specimens can be obtained by using a microsplitter, with a dry sample. Usually, specimens are picked out of examination dishes or picking trays (preferably black with a grid) with a wet 00 or 000 red sable brush and placed in 18-to-48-ply assemblage slides. Slides should be precoated lightly with water-soluble gum tragacanth so that specimens will adhere.

If live specimens are desired, they may or may not be extracted upon collection. Arnold (1954) gives a method for extracting very shallow-water specimens associated with plants. Extraction is generally by microscopic picking of unwashed sediment or sediment water-washed over a 62-μ sieve. Otherwise, the entire sample may be placed in aquaria or other suitable receptacles. Natural seawater or distilled water, to which a compound such as RILA marine mix is added to the desired salinity, may be used and aerated. Arnold (1964) and Lee, Pierce, Tentchoff, and McLaughlin (1961) should be consulted for culturing and study of living and preserved specimens and for histology of Foraminifera.

There are several keys and studies of systematics, including Cushman (1948), Galloway (1933), Glaessner (1945), Le Calvez (1953), Orlov (1959), and Loeblich and Tappan (1964). Cushman has been the most widely used in North America. Of the above references, those previous to Loeblich and Tappan (1964) reflect the earlier concepts of the systematics of Foraminifera, and considerable latitude is allowed the individual in taxonomic placement.

6.2. Ciliata.—Ciliata are found in almost all sediments. Their abundance, however, shows great fluctuations. Because of the small size and usually great abundance of Ciliata, small-diameter core samplers can be used. The Reineck (1958) box corer has been used successfully in the sublittoral (Section 3.3.2).

Most species are fragile and are easily damaged. Indeed, some will be destroyed immediately upon coming into contact with the water surface. Nearly all Ciliata must be collected fresh, studied alive, and specially prepared for identification. Only a few can be identified under low magnification. Bright field phase contrast microscopy usually is necessary for identification.

Qualitatively, Ciliata can be extracted from the sediment by the deterioration technique (Section 4.2.1.3). For quantitative studies the Uhlig Seawater Ice Technique is considered the most efficient (Section 4.2.1.1) since over 90 percent extraction can be achieved in 30 minutes. Isolated Ciliata are best preserved after silver

impregnation. As in many "soft" taxa, there is no suitable technique available for preserving Ciliata within the sediment. Fenchel (1967, 1968a,b, 1969) should be consulted for recent methodology.

No general keys are available, but the extensive reviews of Dragesco (1960) and Kahl (1930–1935) should be consulted.

6.3. Cnidaria.—The classes Hydrozoa, Scyphozoa, and Anthozoa are all represented in the meiofauna. In all, 15 genera are known, and most of these comprise only one or two species; however, some species are described from only a polyp or medusa and this constitutes an important taxonomic problem.

Most species may be restricted to localities of only a few meters in extent. Usually the animals are rare, although in some areas they may be locally common. Most Cnidaria are robust and taxonomic characters are not likely to be easily damaged.

Between 5 and 40 liters of sediment, obtained with a grab or dredge, is considered a suitable sample for Cnidaria. Quantitative samples can be obtained by using corers or subsampling with cores from a grab. Live animals can be obtained by using the Uhlig Seawater Ice Technique (Section 4.2.1.1) or by $MgCl_2$ anaesthetization (Section 4.2.1.2). For mud samples, the Swedmark technique (Section 4.2.2.2) is successful. Whole sediment samples can be preserved in 5 percent buffered formalin and animals extracted later by the decantation technique (Section 4.1.1).

Live animals are best studied after anaesthetization with $MgCl_2$ under phase contrast optics. Neutral red should be added to aid identification of the nematocysts. Astomocnides retain their color after discharge, whereas stomocnides lose theirs. The addition of acetic acid accelerates discharge.

For histological purposes 5 percent formalin or Bouin's fixative is recommended. Whole mounts can be stained with carmine and mounted in Canada balsam. Buffered gluteraldehyde and epoxy resin embedding is recommended for thin sections.

Alcohol preserves larger forms best, whereas small forms are best preserved as whole mounts. For publication purposes nematocysts should be drawn in both discharged and undischarged states with measurements given for the different parts.

No keys are as yet available. Clausen (1971), Kramp (1961), Salvini Plawen (1966), and Swedmark (1964) provide the most useful review articles.

6.4. Turbellaria.—This fairly large group (about 6,000 species to date) is represented in all marine and freshwater biotopes, often as one of the most abundant taxa. Except for many polyclads (which are common on deep mud bottoms), marine Turbellaria can be grouped as a whole in the meiofauna.

Determination requires live material, although in some groups, such as the Acoela, sectioning is necessary. For comparison with earlier descriptions, which were based mostly on preserved specimens, sectioning also is necessary. For original descriptions, examination of live and sectioned material should be interrelated.

For qualitative studies, samples of sediment to the order of 10 liters should be used. Quantitatively, 100 ml to 1 liter samples are recommended. Core samplers of most common types can be used effectively (Section 3.2.3.1). Preserved sediment will not yield identifiable specimens.

Animals can be extracted by the anaesthetization technique (Section 4.2.1.2)

or Uhlig Seawater Ice Technique (Section 4.2.1.1); both give quantitative data. Deep-sea Turbellaria should not be anaesthetized. For mud samples the Swedmark technique is effective (Section 4.2.2.2). Most Turbellaria can be extracted using the deterioration technique (Section 4.2.1.3). Examination of live material after anaesthetization with $MgCl_2$ (except for deep-sea forms) is recommended.

Fixation can be preceded by anaesthetization, but for many forms contraction during fixation is an advantage as many minute organs (e.g., canals) become more compacted and easy to see. Warm Bouin's fixative or a warm corrosive sublimate solution (acidulated with acetic acid after killing) is excellent. For electron microscopy (or 1-μ to 5-μ sections) phosphate-buffered glutaraldehyde is recommended. For fixation of live material, especially for type collections, the addition of a drop of glycerin:formalin (1:1) followed by a slide sealer such as Murrayite (see Appendix, Section 10.1.18) is recommended.

No modern comprehensive monograph is available; for smaller groups see Ax (1955, Otoplanidae), Dörjes (1968, Acoela) and Luther (1955, Dalyelliidae). Excellent starting aids are Karling's (1963) and Luther's (1960, 1961, 1962, 1963) investigations of the Fennoscandian fauna.

6.5. Gnathostomulida.—The Gnathostomulida, although only recently described, have been shown to be cosmopolitan and very abundant. They occur in all types of sand with a high organic content from high-tide level to 100 m deep. About 18 genera with 80 species are known so far.

Sampling procedures must take into account the fact that the Gnathostomulida prefer deeper sand layers that require sampling with a spade or corer in the littoral (Section 3.2.4) and with a deep-scraping dredge in the sublittoral (Section 3.3.2). Samples of 5 to 10 liters are usually sufficient for qualitative studies; and samples of 100 ml are suitable for quantitative studies.

The deterioration technique (Section 4.2.1.3) is best for extraction of animals from qualitative samples. It is important, however, to let the sample deteriorate for a considerable time, usually one week, since the Gnathostomulida are very slow in coming to the surface of the sediment, probably due to their anaerobic metabolism. Superficial sand layers, 250 to 500 ml, are then placed in an Erlenmeyer flask (2,000 ml) and left for 10 minutes with isotonic $MgCl_2$ solution (Section 4.2.1.2). Two washes with $MgCl_2$ and one wash with seawater are filtered through a 62-μ mesh diameter nylon net mounted on a plastic ring (height 2 cm, diameter 9 cm; see Section 4.2.2.2). The filter and deposit are then placed in a dish with seawater, whereupon the animals tend to move through the net into the dish and can be collected free from the sediment.

For quantitative work the same filter is used, but upside down. The deposit is then washed into a dish. The Uhlig Seawater Ice Technique (Section 4.2.1.1) also has been found to be effective. Since only live animals can be identified, whole sediment preservation is unsatisfactory.

In general, examination, fixation, and preservation are the same as for "soft" fauna. For the purpose of determination by a specialist, drawings (and photographs) of the following characters should be made on the living specimen: dimensions and proportions of body and head; number and exact location of sensory bristles; position and dimensions of pharynx, ovary, bursa, testes, and male organs; jaws and basal plate; and sperm. Furthermore, two whole mounts per species should be made, one only slightly squeezed and the other maximally.

No keys are available as yet. For the systematics see Sterrer (in press, *b*), and for reviews see Riedl (1969) and Sterrer (1969, 1971, and in press, *b*).

6.6. Nemertina.—Although an abundant, mainly marine phylum, the Nemertina (about 850 species, ranging in length from 1 mm to 30 mm) are not a major group in the meiobenthos. Comparatively large volumes of sediment are necessary to obtain an adequate number of specimens of the small forms.

From sandy sediments, samples of 5 to 7 liters are taken, and for extraction the deterioration technique (Section 4.2.1.3) is used, allowing the sand to stand 3 to 5 days before the upper sediment layer is removed and treated with the anaesthetization technique (Section 4.2.1.2). Mud samples should comprise 30 to 40 liters of sediment, and for extraction the Swedmark technique (Section 4.2.2.2) is recommended.

The systematics of the Nemertina is based on internal anatomy, since earlier workers studied preserved material for which external characteristics were not recorded. Thus, identification of live Nemertina often is exceedingly difficult. It is essential to combine the anatomical account with a detailed description of the external features of the animal.

The following details should be noted from the living animal: shape, arrangement of eyes, cephalic slits or grooves, main color and color patterns, behavior, size of relaxed animal. Under phase contrast the proboscis armature (present in Hoplonemertina) can be drawn. Probosics eversion can be achieved by adding a few drops of 70 percent alcohol to the seawater containing the animal. The proboscis can be cut off and squeezed under a coverslip. Statocysts and the number of statoliths, if present, should be observed by squeezing preparations of whole animals.

No satisfactory technique is available for the preservation of Nemertina within the sediment samples. Before fixation all Nemertina must be relaxed slowly (urethane, chloral hydrate, or $MgCl_2$ are suitable). This may take from ten minutes to two hours. Animals are best fixed in Bouin's solution (5 hours or more) and preserved in 70 percent alcohol, which should be renewed when it turns yellow. Collecting procedures and treatment prior to preservation of benthic Nemertina in general were outlined by Kirsteuer (1967a).

Microsections (5 to 7 μ) are necessary to study the internal organization. All descriptions of Nemertina should include a graphic reconstruction of at least the anterior body region (see Adam and Czihak, 1964; Kirsteuer, 1967b). Ecological data are very important to new species descriptions and should be included also in redescriptions. Useful keys can be found in Bürger (1895), Coe (1905, 1943, 1954), Corrêa (1961), Friedrich (1936, 1950, 1955, 1961), Hylbom (1957), Iwata (1954), and Müller (1968). Kirsteuer (1971) has reviewed the systematics and ecology of interstitial Nemertina.

6.7. Gastrotricha.—Some 160 species of Gastrotricha from 34 genera are known from marine and brackish water habitats. Densities as high as 400/cm^3 have been recorded, but those of 2/cm^3 to 10/cm^3 are common. The Gastrotricha range from strap-shaped animals possessing lateral and posterior adhesive tubes to bottle-shaped animals with a prominent caudal furca. Species with thin cuticles are easily damaged, and even $MgCl_2$ can cause cuticle blistering in some species.

Sediment samples of up to 10 liters of sand can be easily handled for qualitative studies, although for quantitative work 10 cm^3 has been found to be preferable.

Animals can be extracted quantitatively from the sediment by using the Uhlig Seawater Ice Technique (Section 4.2.1.1) or the anaesthetization technique (Section 4.2.1.2). With the Uhlig technique, the extraction efficiency varies from 60

to 90 percent, depending on the species. Extraction efficiency of 95 percent can be achieved by the anaesthetization technique but observational efficiency is reduced. Thus, both methods probably give equivalent qualitative data. As $MgCl_2$ is detrimental to some species, the Uhlig technique is preferred. Whole-sediment preservation in 5 percent formalin must be preceded by relaxation, using $MgCl_2$ or propylene phenoxytol. Staining the sample with Rose Bengal is the best technique for subsequent extraction.

Whole mounts are best prepared by adding increasing mounts of formalin to relaxed specimens. The animal can then be transferred by means of an Irwin loop (see Appendix, Section 10.1.16) to a solution of one part glycerin and one part formalin (10 percent). The water is then evaporated, leaving pure glycerin. The specimen can then be mounted in glycerin, using a Cobb aluminum frame (see Appendix, Section 10.1.19), allowing examination of both dorsal and ventral surfaces.

For taxonomic purposes the following should be especially noted: size of relaxed animals, caudal appendages, other appendages, adhesive tubules, scale pattern, ciliation pattern, digestive tract, reproductive system, and protonephridia.

Keys published since 1950 include those by Boaden (1963b,c), Brunson (1959), Clausen (1965a,b), d'Hondt (1965, 1966), Kaplan (1958), Pennak (1953), Voigt (1958), and Wieser (1957a). Hummon (1971) has reviewed the status of the systematics of Gastrotricha.

6.8. Kinorhyncha.—The Kinorhyncha are small (less than 1 mm) and, although abundant in certain areas, have been little studied quantitatively. Typically they are mud dwellers and are least abundant in sandy biotopes. Sublittoral deposits yield much fewer specimens than littoral ones. Most Kinorhynca are restricted to the upper 1 to 2 cm of sediment, although *Cateria* Gerlach (a sand beach inhabitat) has been found to a depth of 90 cm.

Qualitatively, Kinorhyncha are best obtained by using an epibenthic sledge (Section 3.3.2) in which 10 to 20 liters of interface sediment are collected. Quantitative samples can be made by using corers or subsampling with cores from a grab (Section 3.3.2).

Whole mud samples can be preserved with 7 to 10 percent formalin or, less satisfactorily, with 70 percent ethyl alcohol. It is best to sieve the sediment through a 62-μ screen and preserve the retained material.

The most efficient extraction method for mud samples is the bubbling technique (Section 4.2.2.3), which can be 90 percent efficient. If less than two liters of sediment are to be extracted, however, the method is less efficient and sieving through a 62-μ sieve may be more satisfactory. Sorting of the fauna is greatly facilitated by the use of an Irwin loop (see Appendix, Section 10.1.16). From preserved sediment, Rose Bengal stained material is easiest to extract by the sieving method, but not all animals stain.

Relaxation, preferably in propylene phenoxytol, is recommended (prior to fixation) in 4 to 7 percent formalin or 70 percent ethyl or isopropyl alcohol (fresh water contact for 1 to 2 minutes aids extrusion of the head). For histological purposes hot Bouin's or Gilson's fixative are satisfactory, but fixation is difficult.

Preservation should be in 70 percent ethyl or isopropyl alcohol to which glycerin has been added. Storage in anhydrous glycerin is excellent. Glycerin is not so useful as a mounting medium because of difficulties in clearing, and Hoyer's medium colored with Lugol's iodine is recommended. A double coverslip mount

using Cobb aluminum frames (see Appendix, Section 10.1.19) is essential. Murrayite (see Appendix, Section 10.1.18) is an excellent ringing compound.

Most specimens should be dorsoventrally oriented. If sufficient specimens are available a few should be laterally oriented. Hoyer's medium eventually clears eggs and sperm beyond recognition, and these should be noted immediately after the animal is mounted as external sexual dimorphic characters are often lacking.

Spines and hairs are critical diagnostic characters for species identification and great care should be taken in noting their presence or absence. Spines may break off at the base; hence, a spine scar may be the only remaining evidence. This is a difficult character and requires the utmost caution. In general the species characters outlined by Higgins (1967, 1968, 1969a,b) should be followed.

No satisfactory keys have been developed. The monograph of Zelinka (1928) and works by Higgins (1967, 1968, 1969a,b, 1971) should be consulted.

6.9. Priapulida.—Priapulida have only recently been found in the meiobenthos. They are known from the Red Sea and from Bermuda and Curaçao, and live in shallow water. Since no quantitative data are yet available, as much surface sediment (mud and muddy shell gravel) as possible should be collected.

The specimens obtained were extracted by sieving on a 62-μ sieve and sorting the debris. The fixation and preservation techniques are identical with those described for the Kinorhyncha (Section 6.8).

It is important that both larval and adult characteristics are noted. Extension of the proboscis is essential, as the critical taxonomic characters are based on the teeth. Only three references are available, those of Coull (1968), Remane (1963), and van der Land (1968).

6.10. Nematoda.—The Nematoda usually are the most abundant metazoans found in marine sediments. They generally occur in greatest numbers in the top 5 cm of sediment, so care must be taken not to disturb the surface layers while sampling. Qualitative sampling of exposed sediments may be accomplished simply by skimming off the upper layers with shovel, trawl, or similar device. The Hope corer (Section 3.2.3.1) works well when collecting by diving in relatively shallow waters, and the various grabs, corers, and dredges may be used there as well as in deeper waters. Direct coring or subsampling grab samples (if the sediment surface has not been disturbed) with a corer are recommended for quantitative samples.

Several extraction techniques have been developed by soil nematologists (see Goodey, 1963), few of which serve well in the extraction of marine forms. The modified Baermann funnel technique, used alone or in combination with screening, is not effective because marine Nematoda will not pass through the tissue paper barrier used in that method.

The Seinhorst elutriator has been demonstrated to be one of the most effective means of extracting Nematoda from soil. It has not been widely used by nematologists, however, because it takes nearly 30 minutes to process one sample if operated as recommended, and the equipment is fragile, impractical to use in the field, and difficult to clean.

A sugar flotation technique for soil Nematoda has been modified for extraction of marine Nematoda and other small invertebrates (Teal, 1960). While this technique is effective in separating Nematoda from small particles having a substantially higher density, it is much less satisfactory for samples containing low-density organic detritus.

Perhaps the technique with fewest objections is the following sieving technique: Sediment (usually 100 ml or less) is thoroughly stirred in a container of

approximately one liter of seawater; after allowing the heavier particles to settle for about 15 seconds, supernatant is poured through a sieve having approximately 50-μ openings; Nematoda and other material are rinsed in a second container by pouring clean seawater through the inverted screen. Final separation of Nematoda from detritus residues may necessitate hand sorting. Staining with Rose Bengal may help in hand sorting, but not all Nematoda will stain and some may be overlooked. Four or five sievings of each sample may yield better than 90 percent extraction.

Many marine Nematoda attach themselves by caudal adhesive glands to solid surfaces they contact, thus reducing the efficiency of extraction. To overcome this problem Hopper (personal communication) has used a mixture of seawater and fresh water while extracting Nematoda, thus temporarily disabling them by osmotic stress. Some of the methods of relaxing might also be used for this purpose.

Nematoda should be relaxed at the time of fixation, as they otherwise bend or coil. This may be accomplished with osmotic stress, or heat (50° C for 10 minutes), but these methods may impair specimen quality and often are impractical in the field. J. B. J. Wells (personal communication) found that in the tropics hot water at a temperature of 50° to 55° C was ineffective in killing and preventing coiling unless the treatment was prolonged up to one hour. He found, instead, that heating rapidly to 60° to 70° C was very effective. Chemical relaxants may be most effective and practical, but these materials and methods for their use need further study.

Five percent formalin in seawater for at least 24 hours is recommended for fixation. Alcohol should never be used unless necessary for histochemical purposes, as this fixative may cause Nematoda to collapse and possibly shrink. Five percent formalin buffered with $CaCO_3$ is recommended for preservation. Anhydrous glycerin also is an excellent preservative, and it is routinely used as a mounting medium for the preparation of permanent microscope slide mounts.

Cobb aluminum frames (see Appendix, Section 10.1.19) should be used when preparing microscope slide mounts, and coverslips should be supported with glass beads (see Appendix, Section 10.1.20) corresponding in size to the diameter of the Nematoda.

Identification of marine Nematoda is based on numerous anatomical features, some of the more important being the cuticle, amphid, cephalic and somatic setae, stoma, esophagus, tail, and reproductive organs (especially the male copulatory apparatus). Most keys are outdated. The most extensive keys are those of de Coninck (1965) and Wieser (1953, 1954, 1956), although they have inadequacies. Hope (1971) has reviewed the current status of the systematics of marine Nematoda.

6.11. Bryozoa.—Only two groups of meiobenthic Bryozoa are known. One, the genus *Monobryozoon* Remane, is considered a typical member of meiobenthos. The other group, the lunulitiform Bryozoa, are questionable components because as adults most are usually larger than the upper size limit of meiobenthos and they live on sand surfaces.

The genus *Monobryozoon* has only two species, one of which lives in mud (*Monobryozoon limicola* Franzen) and one of which lives in "*Amphioxus* sand" (*Monobryozoon ambulans* Remane). *Monobryozoon limicola* can be collected with an Ockelmann sledge, whereas *Monobryozoon ambulans* is sampled with a dredge or grab. The lunulitiform Bryozoa can be sampled with any gear. The meiobenthic Bryozoa are not abundant and so far only qualitative sampling has

been used. *Monobryozoon limicola* has been found in fair quantities in the Gullmar Fjord, Sweden (Franzen, 1960), and at Roscoff, France (F. Monniot, unpublished observation). *Monobryozoon ambulans* is exceedingly rare and is known only from about 20 specimens obtained from Helgoland, Germany (Remane, 1936, 1938), and Robin Hood's Bay, England (Gray, in press, *a*). The lunulitiform Bryozoa have been reported from eastern Africa and the northern Indian Ocean (Cook, 1966), Gulf of Mexico (Lagaaij, 1963b), and Brazil (Marcus and Marcus, 1962).

Animals can be extracted from mud by the Swedmark technique (Section 4.2.2.2), from "*Amphioxus* sand" by the anaesthetization technique (Section 4.2.1.2), and from sand in general by the deterioration technique (Section 4.2.1.3). Extreme care must be taken to thoroughly examine all sample washes as *Monobryozoon* is the same color and of smaller size than the substratum it inhabits. Examination under low magnification is sufficient for the identification process. Animals can be relaxed in $MgCl_2$ or propylene phenoxytol. Preservation in 6 percent formalin or 1.5 percent propylene phenoxytol and fixation in Bouin's fluid or 6 percent formalin are recommended.

As so few species are known, keys are unnecessary. For *Monobryozoon,* see Franzen (1960), Gray (1971b) and Remane (1936, 1938); for lunulitiform Bryozoa, see Cook (1966), Lagaaij (1963a,b), and Marcus and Marcus (1962). Gray (1971b) has reviewed meiobenthic Bryozoa.

6.12. Brachiopoda.—Up to the present time only one species of Brachiopoda, *Gwynia capsula* (Jeffreys), is known to occur in meiobenthos. It is about 1 mm in size and possesses primitive anatomical characters. The species is known from the shallow sublittoral in sediments containing a high proportion of shell debris. The animals live inside fragments of serpulid tubes or in crevices on shell fragments of Gastropoda, barnacles, and other Brachiopoda.

Dredging (Section 3.3.2) is the most satisfactory method of sampling. Samples of 1 liter yield sufficient specimens, and 75 to 80 percent alcohol is a good preservative. Since *Gwynia* Jeffreys is sedentary, no techniques of extraction can be employed. For fixation 75 percent alcohol or neutral formalin can be used. Bouin's fixative is recommended. Whole mounts can be made in Canada balsam for histological studies for which the shell is not to be preserved.

Live specimens should be used for observations on behavior and study of external characters. Animals can be stored for long periods in embryological cups in a small volume of seawater which should be changed twice a week. Under such conditions, the animals probably feed on bacteria or on dissolved organic matter (McCammon, 1969) and it is not necessary to add food. For literature, see Beauchamp (1960) and Swedmark (1967, 1971).

6.13. Archiannelida.—The Archiannelida include a heterogeneous group of five families ranging in size from 250 μ to over 2 cm long. Most Archiannelida live in sand or mud and are interstitial forms. *Troglochaetus* Delachaux is the only freshwater genus. The Archiannelida can be easily damaged by most extraction techniques and, for accurate identification, living animals in good condition are essential. Since the Protodrilidae and Saccocirridae have to be sexually mature before they can be identified as to species, samples have to be taken at different seasons.

Most Archiannelida occur below mean tide level. For qualitative samples it is necessary to bring 1-liter samples of sediment to the laboratory for extraction.

Quantitative samples can be taken with almost any core sampler. Subtidally, dredges, cores, or the Ockelmann sledge can be used effectively.

For live animals, the optimum extraction method is the anaesthetization technique (Section 4.2.1.2). The Uhlig Seawater Ice Technique (Section 4.2.1.1) or a simple seawater wash also can be used. For mud samples, the Boisseau technique (Section 4.2.1.4) has been found to be excellent. Whole sediment samples can be fixed with 10 percent formalin but should be stored in 70 percent alcohol. From such preserved samples, the Boisseau technique (Section 4.2.1.4) has been found to be effective for extraction.

Animals, after relaxation, should be fixed with Bouin's, Helly's or Zenker's fixative. Carm-alum (5 percent) and light green have been found to be useful for the study of internal organs, especially gonads and salivary glands in the Protodrilidae. After clearing in butyl alcohol, mounting should be made in Canada balsam.

The principal characters used in identification are behavior, color, size and number of segments, characteristics of epidermis (ciliation, sensory hairs, glands), reproductive and associated organs, and setae when present.

Mature specimens often are white, and on live examination they can be separated easily. Internally, the salivary glands, number of fertile segments, lateral organs and spermaducts (for Protodrilidae), position and shape of spermducts and sacs and of oviducts and spermatheca (for Saccocirridae), and size of ova and their number per segment should all be noted. The shape of the head of the spermatozoa is a good character even on preserved specimens, especially for the Polygoridae and Nerillidae (Jouin, in press).

Outdated keys appear in works by Fauvel (1927), Remane (1932), and Hempelman (1931). Keys for the Saccocirridae have been published by Alikunhi (1948), Jouin (1971), and Wu and Yang (1962); for the Polygordidae by Marcus (1948); and for the Nerillidae by Jouin (1971). Other important references include Aiyar and Alikunhi (1944), Ax (1967), Boaden (1961), Fraipont (1887), Gray (1969), Jägersten (1944, 1952), Jouin (1966), Jouin and Swedmark (1965), Kirsteuer (1966, 1967c), Pierantoni (1908), Swedmark (1959a,b), Tenerelli (1967), Westheide (1967b), and Wieser (1957b). Jouin (1971) has reviewed the status of the systematics and ecology of Archiannelida.

6.14. Polychaeta.—Approximately 70 families including 10,000 species of Polychaeta are known. The group is heterogeneous, with species varying in size from only a few millimeters to more than 3 meters. Representatives of many families are found in the meiofauna, and the literature on the meiofaunal species is diffuse. The characters needed for identification differ greatly within the families.

Qualitative samples using the technique described in Section 4.1.1 are recommended. For quantitative littoral sampling, subsampling with a core (3 to 5 cm long) into the sides of a pit (Section 3.2.4) is satisfactory. In deeper layers with subsoil water, an iron corer can be inserted vertically into the sediment. For sublittoral sampling, the techniques given in Section 3.3 are satisfactory. Extraction can be effected by using the Uhlig Seawater Ice Technique (Section 4.2.1.1) for sandy sediments or the deterioration technique (Section 4.2.1.3) for mud.

The total sediment sample can best be preserved in 10 percent formalin. There are no special preservation methods needed for the Polychaeta, except that alcohol should not be used for fixation. After fixation, it is well to transfer the specimens to 70 percent alcohol. The general provisions described in Section 5 should be observed.

Histological sections usually are not necessary for species determinations. Differences in specific characters of smaller animals, however, are slight. Some delicate features such as color, shape of body appendages, glands, etc., can be seen only in the living animals. Thus, a combination of living and fixed material is optimal. Anaesthetization with isotonic $MgCl_2$ or $MgSO_4$ is often necessary for observation of living animals as well as for fixation. An addition of a small amount of acetic acid often causes the evagination of the proboscis, which is important for identification in some families such as the Phyllodocidae. Clearing in glycerin or oil of cloves is profitable for recognition of specific internal features in fixed whole animals. For a complete examination of setae it often is necessary to mount in lactophenol (one part lactic acid and one part phenol; Gee, 1964). The slides are warmed gently before applying a coverslip. Lactophenol partially dissolves the body tissues, leaving cleared chitinous setae.

The general characters used in identification include shape of anterior part of body; number and shape of tentacles and cirri; sensory organs; number, arrangement, and exact shape of setae in parapodia; shape of pygidium; shape and position of proboscis; color in living and fixed specimens; and measurements of length. Special characters of each genus have to be added.

General works on the Polychaeta that cover keys to the families, genera, and species include those of Berkeley and Berkeley (1948, 1952), Day (1967), Fauvel (1923, 1927, 1953), Hartman (1968), Pettibone (1963), and Uschakov (1955, 1965). A nearly complete list of meiofaunal Polychaeta exists only for interstitial species of sandy biotopes (Laubier, 1967). Publications dealing with some meiofaunal genera include the following: for *Pisione* Grube (Pisionidae), Aiyar and Alikunhi (1940), Alikunhi (1947, 1951), Hartman (1939), Laubier (1967), Siewing (1955a), Stecher (1968), Tenerelli (1965); for *Eteonides* Hartmann-Schröder, *Hesionura* Hartmann-Schröder, *Mystides* Théel, and *Protomystides* Czerniavsky (Phyllodocidae), Hartmann-Schröder (1963), Laubier (1967); for *Hesionides* Friedrich and *Microphthalmus* Metschnikow (Hesionidae), Ax (1966), Laubier (1968), Westheide (1967a), Westheide and Ax (1965); for *Petitia* Siewing (Syllidae), Siewing (1955b); for Syllidae in general, Cognetti (1957); for *Psammodrilus* Swedmark and *Psammodriloides* Swedmark (Psammodrilidae), Swedmark (1955, 1959a); for *Stygocapitella* Knöllner and *Parergodrilus* Reisinger (Stygocapitellidae), Karling (1958). The "Catalogue of the Polychaetous Annelids of the World," by Hartman (1959, 1965), should be consulted for questions of nomenclature. Westheide (1971) has reviewed the interstitial Polychaeta.

6.15. Oligochaeta.—Although the Oligochaeta are predominantly freshwater and terrestrial, some families are marine. The coarse and medium sand of the supralittoral zone is a highly favorable habitat, but "*Amphiouxus* sand" and muddy sands also are inhabited. Taxonomic characters are not likely to be easily damaged.

Samples are taken with coring devices, the Ockelmann sledge, or grabs. The sample size varies from 100 ml to 0.1 m^3 in poor sediments. Whole sediments samples can be preserved in 5 to 10 percent formalin, but 80 percent ethyl alcohol is best for long storage.

Animals can be extracted qualitatively by simply washing and filtering on a nylon screen. Quantitative samples can be extracted by the Uhlig Seawater Ice Technique (Section 4.2.1.1) or the Boisseau technique (Section 4.2.1.4) for

preserved sediment. With mud samples, the Swedmark technique (Section 4.2.2.2) is preferred.

For living animals, 5 percent alcohol or chloroform vapor is a good anaesthetic. J. B. J. Wells (personal communication) found that tropical Oligochaeta can be relaxed and killed by heating water rapidly to 60° to 70° C, a technique that was almost 100 percent effective. Fixation can be effected in 5 percent formalin, but for storage 70 percent alcohol should be used. Bouin's fixative should be used for histological purposes. For mounting in water, miscible media such as polyvinyl lactophenol is recommended. Permanent mounts should be stained in chlorophydric carmine or acetic haematoxylin. Canada balsam is recommended for permanent mounts.

The haploid chromosome number is a useful aid for species determination. Acetic orcein (orcein, 1 g; glacial acetic acid, 45 ml; distilled water, 55 ml) has been found to be a successful chromosome stain. The acetic orcein is usable for only one month. With live animals, considerable practice is necessary. The examination of immature specimens generally serves no useful purpose in the process of identification.

The principal criteria used in species identification are coloration, size, number of segments, setae, brain morphology, shape of gut and diverticula, septal glands, nephridia, blood vessels, and shape of male reproductive organs. Useful keys to the marine Oligochaeta can be found in Brinkhurst (1963, 1965, 1966a,b), Brinkhurst and Cook (1966), Bunke (1967), Cook (1969), Lasserre (1964, 1966, 1967a,b), Nielsen and Christensen (1959, 1963a,b), and Sperber (1950). Lasserre (1971) has reviewed the taxonomy and ecology of marine Oligochaeta.

6.16. Mollusca.—The Mollusca are represented in the meiofauna by Solengasters, a few Prosobranchia, Scaphopoda, and Protobranchia, and by Opisthobranchia. The latter includes species belonging to the orders Acochlidiacea (12 species in 1969) and Philinoglossacea (2 species in 1969) and the aeolid family Pseudovermidae (8 species in 1969). As yet, the Solenogasters, which are known from the European Mediterranean and Atlantic coasts and the northwest Pacific coast of America (Friday Harbor), are incompletely studied. Scaphopoda (*Siphonodentalium* M. Sars and *Cadulus* Philippi) and some Protobranchia are represented in the meiobenthos in subtidal sediments to depths of 3,000 m.

For qualitative sampling in sand and mud, epibenthic sledges such as the Ockelmann sledge (Section 3.3.2) are most satisfactory. Other sledges and grab samplers have been used with success. For quantitative sampling in mud, corers (Section 3.2.3) or the Petersen or Smith-McIntyre grab (Section 3.3.2) covering an area of 0.1 m^2 are used.

Eighty percent alcohol is a suitable preservative. Extraction techniques include the Uhlig Seawater Ice Technique (Section 4.2.1.1) and the Swedmark technique (Section 4.2.2.2). The specimens should be anaesthetized (Section 4.2.1.2) carefully for live examination and before fixation. Bouin's fixative is recommended as a standard, but any fixative for histological purposes may be used. If shells (Scaphopoda and Protobranchia), spicules (Acochlidiacea), or scales (Solenogasters) must be preserved, acid fixatives must be avoided and buffered formalin used. Whole mounts can be made in Canada balsam.

Spicules, scales, epidermal glands, radula, statocysts, and extension of cilia should be studied on living material. Most of the anatomy can be studied on squeezed specimens. The radula should be analyzed in detail, using the formula n (2.1.2) where n = number of radula series, 2 = one median plate plus one

lateral plate, and 1 = central tooth. Keys for the identification of species have been published for the Acochlidiacea (Odhner, 1952; Marcus, 1953; Swedmark, 1971), for the Pseudovermidae (Salvini Plawen and Sterrer, 1968), and for the Scaphopoda (Muus, 1959). Other useful references include Marcus and Marcus (1954) and Marion and Kowalevsky (1886).

6.17. Mystacocarida.—The Mystacocarida are morphologically and behaviorally distinctive; thus, they are easy to sample. Where present they are abundant, and since they do not cling to the substratum they are easy to extract.

Sediment samples from holes 10 cm in diameter and 40 cm deep are extracted in the field following the technique described in Section 4.1.1. The animals are durable and may easily be brought back to the laboratory alive, or they may be preserved in the field. Four percent formalin is the most convenient field preservative despite its disadvantage of killing the animals in a variety of contorted positions, thereby making uniform measuring techniques impossible. Alcohol tends to distort animals less than formalin, but it is disadvantagous for field use because it forms a precipitate with seawater.

Where subsequent measurement is desired, it is best to bring the live filtrate back to the laboratory and pick out the desired individuals and kill them in lactic acid which has been stained lightly with methyl (not methylene) blue. This kills the animals in a uniform, relaxed position and stains them perfectly for microscopic investigation of external anatomy, which should be carried out in unstained lactic acid. The animals may be left in lactic acid for a maximum of several weeks. Dilute lactic acid (1:1) can be used to kill the entire filtrate in the field, but later, during sorting, it is difficult to see the animals in this medium. Formalin is best for long-term preservation. Whole sediment samples may be preserved in formalin and extracted by an abbreviated decantation technique (Section 4.1.1).

All present evidence indicates that Mystacocarida are extraordinarily conservative; the species are differentiated on the basis of just a few slight details. Therefore, it is important to note even subtle differences between populations. Ten to twenty individuals from each population should be checked for individual variation.

Delamare Deboutteville (1960) and Hessler (1971b) give introductions to the literature on Mystacocarida. The works of Dahl (1952), Delamare Deboutteville (1953), Hessler and Sanders (1966), Noodt (1954), and Hessler (1969) are especially useful in systematic studies.

6.18. Ostracoda.—Benthic marine Ostracoda range from 0.4 to 1.5 mm in size. They occur on sand, mud, and algae at all depths. Some species are truly interstitial while others are epibenthic. Most species occur in the upper few centimeters of sediment but are not generally abundant. Thus, large quantities of sediment must be collected and sorted. One hundred milliliter samples of sediment may give from 10 to 300 animals. Probably a more suitable sample size for littoral work is 0.1 m^2. Coring devices usually do not provide enough living specimens, so dredges and grabs are preferred for sublittoral work. Deep-sea samples of at least 5 liters of surface sediment usually are necessary to obtain enough specimens.

Animals can be extracted from the sediment by sieving. Most species are retained on 140-μ sieves. Care should be taken that highly ornamented shells are not damaged. The Boisseau technique (Section 4.2.1.4) or the bubbling technique (Section 4.2.2.3) can be used but the sediment must be reexamined after using these techniques as separated valves and highly calcified species often remain.

Buffered 5 percent formalin or propylene phenoxytol can be used to preserve the whole sediment. Though little histological work has been done on Ostracoda, Bouin's fixative is recommended. Animals can be preserved in 5 percent buffered formalin or 70 percent alcohol. Buffering is essential for whole sediment samples to prevent decalcification of the valves. Calcium carbonate, hexamine, and borax can be used as buffering agents.

Ostracoda are best dissected in glycerin and the dissected appendages left in glycerin or mounted permanently in glycerin jelly. Glycerin mounts, if sealed, are satisfactory. Polyvinyl lactophenol is a useful semipermanent medium for mounting appendages; however, it cannot be used for valves because it causes decalcification. CMC-S (see Appendix, 10.1.21) is a water-soluble staining and mounting medium, appendages can be arranged in position prior to the coverslip addition.

It is preferable that the valves be mounted on micropaleontological slides. The specimens should be glued to the slides with a very slight amount of gum tragacanth. A few drops of formalin, phenol, or oil of cinnamon can be added to the tragacanth to prevent the development of molds. As the glue is water-soluble, the valves can be removed from the slides.

Particular attention should be given to a description of both right and left valves, appendages, and copulatory organs, and distinction should be made between molts and adults. Moore (1961) and Morkhoven (1962) give detailed techniques for the study of ostracod valves and some information on appendage morphology. Morkhoven (1963) gives generic descriptions and references to studies of appendage morphology of all post-Paleozoic Ostracoda. Hartmann (1964) has integrated the neontological and paleontological classification schemes. Hulings (1971b) has reviewed the status of the taxonomy and ecology of marine benthic Ostracoda.

6.19. Copepoda.—Next to the Nematoda, the Copepoda are the most abundant group in the meiobenthos. Probably less than 50 percent of the existing species have been described. Copepoda inhabit not only sand and mud areas but are associated with algae and other biotopes. Sediments may be sampled fairly easily; however, sampling of other biotopes is more difficult, and this is especially true for the Copepoda, most of which swim and jump actively and are not, therefore, sampled by traditional techniques.

Qualitative sampling is easy for most biotopes, and samples of sand, mud, algae, etc., can be easily obtained. No satisfactory quantitative techniques are available for biotopes other than sediment. Core samplers (5 cm in diameter) have been used effectively for sampling littoral and sublittoral sediments down to SCUBA limits. Subsampling from grabs by cores or the Thiel "Meiostecher" (Section 3.2.3.4) can be used in the sublittoral.

Whole sediment samples are best preserved in 5 percent formalin or 75 to 80 percent alcohol. Preserved sediment should be extracted as soon as possible since some specimens become fragile in preserved conditions.

Copepoda can be extracted from the sediment by the anaesthetization (Section 4.2.1.2) or Boisseau (Section 4.2.1.4) techniques; however, washing and decantation may give greater extraction efficiency. For preserved sediment the Boisseau technique is highly effective, and the addition of Rose Bengal aids sorting, although all the animals may not be stained.

Copepoda may be extracted from algae by shining a light from one side of a dish and collecting animals that migrate to the light. Washing and sieving may be more effective.

Preservation in 70 percent alcohol with 5 percent glycerin has been found successful, and the addition of 1 percent phenol prevents growth of fungus in this preservative. Animals can be dissected easily in lactic acid or glycerin, and stains such as methyl blue (Section 6.15) may be useful. Mounts made in glycerin or Zeiss W15 (see Appendix, Section 10.1.22) mounting medium ringed with Murrayite or Glyceel (see Appendix, Sections 10.1.18, 10.1.23) are preferred. Polyvinyl lactophenol or CMC (Appendix, Section 10.1.21) also can be used for mounting.

Most taxonomic work has been on preserved material but some information on species biology is available. The basic literature includes the monographs of Lang (1948, 1965). Species and references subsequent to Lang's (1948) monograph are summarized by Bodin (1967). Noodt (1971) has reviewed the ecology of the Harpacticoida.

6.20. Palpigradida.—The Palpigradida are very primitive Arachnida. They were originally discovered in the interstitial environment by Condé (1965) and later by Monniot (1966). Two species are known, *Leptokoenenia gerlachi* Condé from beaches of the Red Sea and *Leptokoenenia scurra* Monniot from high-tide level on the beach at Pointe Noire, Republic of Congo. The Palpigradida are very fragile and the techniques of collecting, fixing, and preserving them are the same as those used for the Mystacocarida. For the systematics of this group, see Condé (1965) and Monniot (1966).

6.21. Halacaridae.—The Halacaridae generally are less than 600 μ in size and thus may pass through the screens of many types of gear. While some species are discontinuously distributed, others are continuously distributed. Thus, only qualitative sampling techniques are usually employed. For sand and gravel forms, four to five liters of sediment are sufficient. Animals can be extracted by using the anaesthetization technique (Section 4.2.1.2) but substituting chloroform for $MgCl_2$. The animals can be preserved in 65 percent ethyl alcohol. The alcohol concentration should be increased gradually to this concentration. Algal living species can be extracted in a similar manner.

Whole sediment samples can be preserved in 65 percent ethyl alcohol. Screens of 1 mm and 62 μ can be used. Nearly all mites are retained by a 62-μ sieve. Rose Bengal staining aids sorting.

Halacaridae are best preserved in Hyrax, which has a high refractive index (about 1.70) and is suited for resolving fine details. Because of the high refractive index, it is essential to remove the soft parts as fully as possible, otherwise the specimens are nearly opaque and cannot be studied with the microscope. The most suitable method for removing the soft parts is hydrolysis with pepsin. KOH and NaOH cannot be employed because they affect the cuticle adversely and damage delicate setae. Even lactic acid and chloral hydrate weaken the cuticle perceptibly and produce distortion in the final mounts.

The use of pepsin as a hydrolyzing agent imposes two additional requirements. First, the specimens must be pierced with fine needles to permit the entrance of pepsin molecules; second, the specimens must not at any time be placed in formaldehyde, which makes the soft parts insoluble in pepsin.

Halacaridae should not be placed in formalin, but some considerations make it desirable or even necessary to have determinations made on such material. This is possible by the use of alternative but less-desirable methods.

Material which has been preserved in formalin can be handled by mounting it in Hoyer's medium, but this does not yield permanent mounts, and the specimens are less suitable for descriptive purposes because of the refractive index.

Hoyer's mounted specimens can be made permanent by soaking off the coverslip in water and transferring the specimens to anhydrous glycerin by the evaporation technique. Permanent mounts can then be made by one of several available techniques.

If formalin-fixed material is being sent for determination, it is very important to point out that the specimens have been in formalin at one time; otherwise, much time may be wasted in trying to mount the material in Hyrax.

No completely satisfactory keys are available. The most comprehensive keys are those of Viets (1939, 1940) for the European fauna and of Newell (1947) for the North American fauna. Newell (1971) has reviewed the problems of studying subtidal Halacaridae.

6.22. Tardigrada.—The marine meiobenthic Tardigrada have been found from the littoral to depths of 5,000 m. Only 25 marine species are known, 9 of which are in one genus. Densities of $90/cm^3$ of sand have been recorded. The animals are small (300 μ) and generally are attached to sand grains or algae.

In general, 50-ml samples of sediment are adequate for taxonomic and ecological purposes. Tardigrada can survive long periods in dry sand and are not damaged by such treatment, reviving immediately upon addition of seawater.

Whole sediment samples can be preserved in 10 percent formalin; however, nearly dry sand samples can be used to keep Tardigrada indefinitely.

For extraction of live animals from sediment the anaesthetization technique (Section 4.2.1.2) is preferable to the Uhlig Seawater Ice Technique (Section 4.2.1.1). The latter is only 20 percent efficient for Tardigrada. For quantitative studies the Boisseau technique (Section 4.2.1.4) has been used effectively.

For preserved sediment, a simple washing technique is effective. Tardigrada should be relaxed before being fixed in 70 percent alcohol or 10 percent buffered formalin. Preserved specimens should be mounted in anhydrous glycerin. For histological sections, alcohol Bouin's solution seems to be the best fixative. Preembedding in a drop of agar jelly prior to paraffin embedding is recommended. The agar jelly can be stained with fast green to aid viewing. Sections are stained in haematoxylin eosin.

Particular attention should be given to study of the head and cirri, mouth and stylets, cuticular ornaments, genital openings, legs and toes, and claws and secondary spurs. The definitive work of Ramazzotti (1962) includes a key to the genera and species described. Renaud-Mornant and Pollock (1971) have reviewed the systematics and ecology of marine Tardigrada and have included a key to the genera.

6.23. Echinodermata.—Among the Echinodermata, only a few Holothuroidea belonging to the order Apodida are known as permanent members of the meiofauna. Their body lengths range from 2 to 8 mm; all are more or less vermiform. Along the European coasts, two species, *Rhabdomolgus ruber* (Keferstein) and *Leptosynapta minuta* (Becher), are frequent in "*Amphioxus* sand." A third species, *Labidoplax buski* M'Intosh, is common in subtidal mud. Rao (1968) reported a new interstitial genus and species (*Psammothuria ganapatii*) from beach sands in India.

Qualitative samples of subtidal sand can be taken with any type of dredge or grab, and quantitative samples with a suitable corer. Qualitative samples of subtidal mud meiofauna can be taken with an Ockelmann sledge or other epibenthic sledges (Section 3.3.2), and quantitative samples with a corer (Section 3.2.3).

For preservation, 75 to 80 percent alcohol is satisfactory. The extraction

technique commonly employed is that of Swedmark (Section 4.2.2.2). Anaesthetization (Section 4.2.1.2) using $MgCl_2$ or $MgSO_4$ should be carried out. For fixation, Bouin's fluid is recommended, but it should be replaced by neutralized formalin or 75 percent alcohol if the calcareous spicules are to be preserved. But neutralized formalin, as a long-term preservative, seems to affect calcareous structures adversely. Whole mounts can be made in Canada balsam.

There are no special keys for the identification of species of Echinodermata belonging to the meiofauna. For European species, Mortensen and Lieberkind (1928) can be used. Swedmark (1971) has reviewed meiobenthic Echinodermata.

6.24. Tunicata.—Meiobenthic Tunicata live only in coarse sand at a depth greater than 5 m. As they are rare, sediment samples should be as large as possible (20 liters). Sampling with a dredge or anchor dredge (Section 3.3.2) is the best method. The animals penetrate the sand and have a fine network of rhizoids to hold themselves in place. Samples cannot be held longer than 48 hours if live specimens are wanted. Preservation of whole sediment samples may be pointless, as the animals are so rare.

The only successful extraction method found is that of simply washing the sediment with a strong current of water on a fine screen. No quantitative methods have been employed.

Animals can be preserved for 48 hours in buffered 5 percent formalin or in 75 percent alcohol. For fixation purposes, 10 percent formalin is effective. Specimens should be carefully dissected and the mantle, attached organs, and branchial sac stained in Masson's hemalum for 1 to 3 minutes for the former two and for 5 to 10 minutes for the latter. Following a 10-minute wash in fresh water and dehydration in 70 percent and 95 percent alcohol and 10 minutes in butyl alcohol, the mantle with attached organs and the branchial sac can be mounted in Canada balsam on separate slides.

For taxonomic purposes the following should be noted: size (in three dimensions), appearance and thickness of tunic, form of siphons and atrial tentacles, branchial sac, neural gland, mantle, gut, gonads, kidney, incubatory pouch, larva, and environmental data. No keys are available. Monniot (1965) should be consulted for subtidal species. For deep-sea ascidians see Millar (1959, 1960, 1964), Monniot (in press), and Monniot and Monniot (1968, 1970). Monniot (1971) has reviewed meiobenthic Tunicata.

7. Culture and Experimental Techniques

As the experimental treatment of the meiobenthos is still at an early stage, the aim of this short survey is not to give an exhaustive description of various types of experiments but rather to stress the great need for experimental work in the hope of encouraging further studies.

The great number of continuously varying parameters in the field makes it impossible to ascertain fully the correlation between a morphological character and sediment type (long bristles/coarse sediment) or an environmental parameter and a faunal distribution in a beach. The experimental approach, both in taxonomical and ecological studies, must, therefore, be highly recommended.

The taxonomist may be able to explain morphological modifications as adaptations to different media by means of culture experiments or preference experiments. Life cycle studies, already stressed as most important in taxonomic

descriptions, should be more standardized and controlled in order to deliver maximal ecological information about the animals.

The ecologist has great need of tolerance and preference experiments for explanation of field distributions (for a discussion see Jansson, 1967a–d, 1971) and of behavioral studies to recognize the types of reactions of the organisms (Boaden, 1963a, 1968; Gray, 1966a). In productivity studies there is a great need for more information on community metabolism, including a preferred way of measuring laboratory studies of the most important pathways of the energy flow (Odum and Hoskin, 1958; Pamatmat, 1968).

The physiologist has an important role in studying the physiological background of the tolerance and preference reactions of the organisms and in explaining field distributions (Lasserre, 1969, 1970, in press) when simple ecological experiments are not adequate. The very small size of meiofauna, however, precludes most of the common methods of experimental physiology (e.g., Warburg apparatus, microrespirometers, inorganic estimation in coelomic fluid, etc.) There difficulties merely present a challenge either to adapt the classical methods or develop new ones. Some recent and refined microtechniques and ultramicrotechniques used in cytochemistry (see Glick, 1962) may be very useful in physiological experiments with meiofauna.

7.1. The Experimental Population.—The experimental population should be described as clearly as possible. The following points should be recorded: (a) name of species (with possible morphological aberrations), age, sex, physiological condition (eggs and/or sperm, grade of activity); (b) the habitat where the animals were collected and the microclimatic conditions before and at sampling. Pretreatment in the laboratory under controlled conditions can in many cases be recommended. The environmental conditions of the animals prior to the experiment should be carefully noted.

7.2. Culture Techniques.—Although a vast number of meiobenthic organisms are known to species level, few life history studies have been completed. This emphasizes the great need for culture experiments.

An adequate culture of organisms presupposes a good knowledge of the species and of the environmental conditions in the normal habitat. The usual approach to culture techniques is to imitate the natural habitat; this may be not only the best method but also may be necessary in order to secure normal specimens. General principles and successful culture methods for different phyla are given in Costello, Davidson, Eggers, Fox, and Henley (1957), Needham (1959), and Kinne and Bulnheim (1970).

For the culture of meiobenthic animals a substratum usually is necessary. Glass beads (Appendix, Section 10.1.20), which can be obtained lead-free, have been found useful for culture work. Animals can be seen through the beads, and the chemical nature of the beads is infinitely easier to control than the natural substrata. This method has been used successfully with the archiannelid *Dinophilus gyrociliatus* Schmidt and the harpacticoid *Amphiascus tenuiremis* (Brady), changing the seawater monthly. On the other hand, the interstitial harpacticoids *Paraleptastacus spinicauda* Klie and *Paramesochra constricta* Nicholls have been reared only in the natural substratum, using darkened vessels and changing the seawater weekly. The gastrotrich *Turbanella hyalina* Schultze has been cultured using glass beads but it reproduced only when bacteria were kept at a low level in the culture vessels. Predatory species such as the kalyptorhynchid Turbellaria are

more difficult to culture. This is probably due to lack of knowledge of their food requirements (Gray, unpublished). Other methods may be found in the taxonomic literature of different groups, e.g., Nematoda (von Thun, 1966), Enchytraeida (Christensen, 1956).

7.3. Experimental Techniques.

7.3.1. Field Techniques.—The most common type of field experiment is the colonization experiment (Boaden, 1962; Renaud-Debyser, 1959, 1963). Sterile substratum is enclosed in permeable gauze bags or tubes open at both ends and buried in the sediment. After the experiment the animals are sorted out and counted. In such a way the attractiveness of graded sand (Boaden, 1962) or migration due to tidal movements of the interstitial water (Renaud-Debyser, 1959, 1963) have been studied. As the environmental parameters except for the one studied are allowed to fluctuate in a normal way, these variations must be considered when evaluating the results.

Other experiments such as measuring wave exposure and meiofaunal distribution or the tolerance of introduced meiofaunal populations in polluted areas are possible but have not been attempted.

7.3.2. Laboratory Techniques.—In all laboratory experiments adequate control tests must be set up. Without suitable controls the results of the experiments are open to criticism.

7.3.2.1. Tolerance Experiments.—A simple but much-criticized type of experiment is that for tolerance or resistance. The organisms are kept in graded concentrations of a variable factor (e.g., salinities from 5 to 35 ppt), while the other environmental factors are kept constant, and the survival of the organisms is noted. While most other parameters are rather easy to keep optimal, the food factor may cause some difficulties. If "natural" seawater is used for the experiment a moderate bacterial growth usually develops, enough to keep the animals alive even for months as noted by Jansson (1968) for Turbellaria, Oligochaeta, and Harpacticoida. Sometimes a vigorous bacterial growth may spoil some of the results. It may, therefore, be necessary to feed the animals regularly, intermittently changing the water to new, sterile solutions. The time of exposure to air must be correlated with the normal exposure endured by the organism.

For comparison with other investigations, the results should be expressed as LD-50 values (lethal dose 50), which means the time or dose at which 50 percent of the experimental population died. Corrections should be made for eventual deaths in the control population according to Abbott (for a description of this type of experiment see Andrewartha, 1961). This method has been criticized, and rightly so, for its crudeness. But it gives a rough estimation of the end-points of the physiological ability of the organism and, in a simple way, makes possible comparisons between quite different types of organisms. A careful observation of the organisms during the experiment, noting whether they are sluggish, in coma, etc., will provide information for further studies and give the data a greater biological significance.

A better way of studying the tolerance is to find some way to measure the "efficiency" of the organism. Good examples are growth, respiration, and locomotory rates of species.

It is better to observe the grade of recovery of the experimental population after transfer to optimal conditions than simply to note LD-50 values. Quantification poses difficulties, however.

7.3.2.2. Preference Experiments.—In a preference experiment the organism is allowed to choose the most favorable of two or more alternatives or the most attractive area in a gradient. The divergence from the theoretical random distribution is taken as a measure of the intensity of the preference.

The number of animals should be as large as possible to represent the reactions of the whole population from which the organisms were sampled and, thus, to represent the morphological and physiological variation within the species. This emphasizes the need of working with several populations, isolated in time and geographical distribution. The results should be treated statistically.

It is convenient to divide the preference experiments into alternative experiments and gradient experiments. In alternative experiments, the animals are offered two or more alternative properties of the parameter studied, e.g., types of food, grades of sand, salinities (Gray, 1966a,b, in press,*b*; Jansson, 1962, 1968).

In gradient experiments, the organisms are transferred to a gradient of the factor studied, e.g., temperature (Gray, 1965; Jansson, 1966). Compared with the alternative experiment, not so many runs have to be made in order to establish the preference area. Alternative experiments, however, usually are easier to carry out and to evaluate.

The following important points have to be considered when making preference experiments: (a) parameters other than those studied must be kept constant; (b) the alternatives or gradients must be stable during the experiment, and new equipment must be tested; (c) the behavior of the organisms during experimental conditions should be observed in order to make sure that the prerequisites for a proper choice are fulfilled, i.e., that the animals really migrate in the gradient, that no gregarious activity occurs, etc.; (d) time of experiment must be sufficiently long for the animals to try every alternative or part of the gradient many times (this requires introductory studies of the locomotory ability of the organism).

7.3.3. Physiological Techniques.—Where the ecologist ends his task, the physiologist sometimes can carry it further to explain the metabolic processes which determine the reactions of the animals. When pure ecological techniques fail to explain the distribution of the organisms, physiological proofs may be shown. The following important subjects, closely related to ecology, deserve serious attention.

7.3.3.1. Osmoregulation and Ion Regulation.—Previous studies on osmoregulation and ion regulation are summarized by Kinne (1964), Potts and Parry (1964), and Remane and Schlieper (1958). The diverging composition occurring in sediments and in polluted areas stress the need of further research in connection with field studies.

7.3.3.2. Oxygen Reactions.—The often low oxygen tensions in sediments indicate a great stress for the organisms (for a recent summary see Theede, Ponat, Hiroki, and Schlieper, 1969). Tolerance to oxygen deficiency and hydrogen sulfide should be studied. Wieser and Kanwisher (1961) and Theede, Ponat, Hiroki, and Schlieper (1969) give techniques for studying such tolerances.

Oxygen consumption often has been taken as a measure of the metabolic activity of the organism. Studies of the oxygen consumption in waters of different salinities and ion composition similar to those of Lasserre (1969, 1970, in press) give valuable information for the ecologist in explaining field distributions.

The most accurate method of measuring the oxygen consumption of small organisms is the Cartesian diver technique (Glick, 1962; Holter and Linderstrøm-Lang, 1943). The minute volume capacity of the divers allows measurements on

single planktonic animals (Zeuthen, 1947) and interstitial animals (Lasserre, 1969, 1970, in press). All measurements require statistical analysis (see Snedecor and Cochran, 1967); thus, they should be replicated (Zeuthen, 1953).

7.3.3.3. Metabolism.—The food and feeding processes of meiofauna are incompletely known. The large group of "detritus consumers" certainly consists of animals of great specialization. For example, one species may eat certain bacteria present in detritus, with the rest of the material being returned as fecal pellets to the detritus pool of the sediment and then perhaps consumed by another animal species. Thus, another part of the detritus is utilized and competition is avoided. There is an almost complete lack of information in this area, and much work is needed.

7.3.3.4. Sense Physiology.—The organisms are guided in the field by their sense organs. Little is known about the function and even morphology of the various types of sense organs of the meiofauna. This, again, is an important field for future work.

8. Biotopes other than Sediment

The meiobenthos of nonsedimentary habitats usually is different from that of the sediment habitat, with only a few taxa being common to both. Few of the faunal elements are directly associated with the macrobenthos (e.g., Cnidaria, Bryozoa).

The most important nonsediment biotopes are sessile and floating algae (littoral, sublittoral and Sargassum), sessile macrofauna, vagile macrofauna, rock surfaces, and dead organic materials.

Quantitative sampling of the nonsediment meiobenthos is difficult. Dahl (1948), Hagerman (1966), A.-M. Jansson (1966), Ott (1967), Segerstråle (1944), and Wieser (1952, 1959) have attempted such sampling in a variety of ways. The extraction procedures outlined in Section 4 are generally applicable to the nonsediment meiobenthos.

The following data should be noted, in addition to those mentioned in Section 1: name of macrobiotic organism, if any; special characteristics (e.g., branched algae, bryozoan encrustations, mobility of organism); microclimate associated with macrobiotic organism (e.g., light, currents, salinity changes, oxygen, organic material); and microflora (e.g., diatom film on rock or algal surface).

This section is necessarily brief because of the lack of expertise within the group that prepared this manual. Knowledge of these important biotopes is poor and further detailed studies on lines parallel to the sediment meiobenthos are needed.

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10. Appendix

10.1. Equipment, materials, and manufacturers.

10.1.1. Telethermistors.

10.1.1.1. Rustrak Instrument Company, Municipal Airport, Manchester, New Hampshire 03103, U.S.A.

10.1.1.2. Shandon Scientific Company Ltd., 65 Pound Lane, Willesden, London N.W. 10, England. (Prices from $400.)

10.1.1.3. Spembly Technical Products, Trinity Trading Estate, Sittingbourne, Kent, England. (Price, $380.)

10.1.1.4. Veco Victory Engineering Corporation, Union, New Jersey 08611, U.S.A.

10.1.1.5. West Instruments, The Hyde, Brighton 7, Sussex, England. (Prices from $200.)

10.1.1.6 Yellow Springs Instrument Company, P.O. Box 279, Yellow Springs, Ohio 45387, U.S.A.

10.1.2 Chart Records.

10.1.2.1 Amprobe Recorder Service Division, 630 Merrick Road, Lynbrook, New York 11563, U.S.A.

10.1.2.2 Rustrak Instrument Company, Municipal Airport, Manchester, New Hampshire 03103, U.S.A.

10.1.3. UNESCO Oceanographic Tables.

10.1.3.1. Librairie de l'UNESCO, Place de Fontenoy, 75 Paris 7e, France.

10.1.3.2. M. M. Stationery Office, P.O. Box 569, London S.E. 1, England.

10.1.4. Conductivity Cells and Wheatstone Bridge.

10.1.4.1. Mullard Equipment, Ltd., Aboyne Works, Aboyne Road, Tooting, London S.W. 17, England.

10.1.4.2 Pye Unicam Ltd., York Street, Cambridge, England. (Conductivity cell: Philips PR 9513 microcell, 0.5 ml; price, $35.)

10.1.4.3. Electronic Switchgear Ltd., 58 Wilbury Way, Hitchin, Herts, England. (Conductivity meter Type MC1 Mkv; price, $180. Conductivity cell (3 ml), specify CJ1B; price, $30.)

10.1.5. Salinity-Temperature Bridge.—Electronic Switchgear, Ltd., 58 Wilbury Way, Hitchin, Herts, England. (Price, $500.)

10.1.6. Goldberg Refractometer.—American Optical Corporation, Scientific Instruments Division, Buffalo, New York 14245, U.S.A. (Price, $210.)

10.1.7. pH-Redox Meters.—Analytical Measurements, Ltd., Feltham, Middlesex, England. (Price, $200.)

10.1.8. Oxygen-Availability Meter.—Direct inquiries to: Dr. B. O. Jansson, Askolaböratoriet, Trosa, Sweden. (Price, $170.)

10.1.9. Speedy Moisture Tester.

10.1.9.1. Griffin and George, Ltd., Ealing Road, Alperton, Wembley, Middlesex, England. (Price, $105.)

10.1.9.2. Thomas Ashworth and Company, Ltd., Sycamore Avenue, Burnley, Lancs, England. (Price, $100.)

10.1.9.3. Townson and Mercer, Beddington Lane, Croydon, Surrey, England. (Price, $95.)

10.1.10. Hydrogen Sulfide.—Hach Chemical Company, P.O. Box 907, 713 South Duff, Ames, Iowa 50011, U.S.A.

10.1.11. Crystic 28C.—Scott Bader and Company, Ltd., Wollaston, Wellingborough, Northhamptonshire, England.

10.1.12. Araldite.—C.I.B.A. (A.R.L.), Ltd., Duxford, Cambridge, England.

10.1.13. "Meiostecher."—Hydro-Bios Apparatebau GmbH, Am Jagersberg 7, 23 Kiel, Holtenau, West Germany. (Price, $5.)

10.1.14. Reineck Box Corer.

10.1.14.1. Hermann Siefken, Mozartstrasse 16, Wilhelmshaven, West Germany. (Price, $800, including shipping.)

10.1.14.2. Friedrich Leutert, D–3141, Erbstorf, West Germany. (Price, $2,200, including shipping.)

10.1.14.3. Tetra Tech, 742 Herschel Avenue, La Jolla 92037, California, U.S.A. (Price, $5,570.)

10.1.15. Siliclad.—Clay-Adams, Inc., New York, New York 10012, U.S.A.

10.1.16. Irwin Loops.—Welch Scientific Company, 7300 Linder Avenue, Skokie, Illinois 60076, U.S.A. (Price, $3 per set of three.)

10.1.17. Eukitt.—O. Kindler, Deckglaszuschneiderei, Freiburg/Breisgau, West Germany.

10.1.18. Murrayite.—Edward Gurr, Ltd., London S.W. 14, England.

10.1.19. Cobb Aluminum Frames.

10.1.19.1. Metaalwarenfabrik "Amstel," Gijsbrecht van Aermstelstraat 16–18, Amsterdam O, Netherlands.

10.1.19.2. Sartory Instruments, Ltd., 7 Steele Road, Chiswick, London W 4, England.

10.1.20. Glass Beads.

10.1.20.1. Catathote Corporation, Microbeads Division, Jackson, Mississippi 39205, U.S.A.

10.1.20.2. Ballotini Manufacturing Company, Ltd., Barnsley, Yorkshire, England.

10.1.21. CMC, CMC–5.—General Biological Supply Company, 8200 South Hoyne Avenue, Chicago, Illinois 60620, U.S.A. (Prices $2 for 4 ounces; $7.25 for 1 pound.)

10.1.22. Zeiss W15.—Carl Zeiss, Inc. (Price, $1 per 30 cm^3.)

10.1.23. Glyceel.—Edward Gurr, Ltd., London S.W. 14, England.

10.2. Wheatstone Bridge.—The Wheatstone bridge incorporates a magic-eye detector unit in conjunction with a dip-type conductivity cell. Two of the ratio arms of a conventional Wheatstone bridge are combined in a single potentiometer so arranged that for maximum rotation of the dial the ratio change (i.e., R_3/R_4) is exactly 100:1. The scale is calibrated from 0.1 to 10; hence, when the bridge is balanced, the dial reading when multiplied by the resistance inserted at R_2 allows the resistance of the conductivity cell to be calculated, at balance, as

$$\frac{R_1}{R_2} = \frac{R_3}{R_4} \text{ and } R_1 = R_2\left(\frac{R_3}{R_4}\right)$$

When the bridge is balanced the magic eye indicator shows the minimum fluorescence. Figure 12 shows the circuit diagram for the Wheatstone bridge.

10.3. Oxygen Availability.—The oxygen availability can be expressed by the formula

$$f = \frac{i_t}{(n)(F)(A)},$$

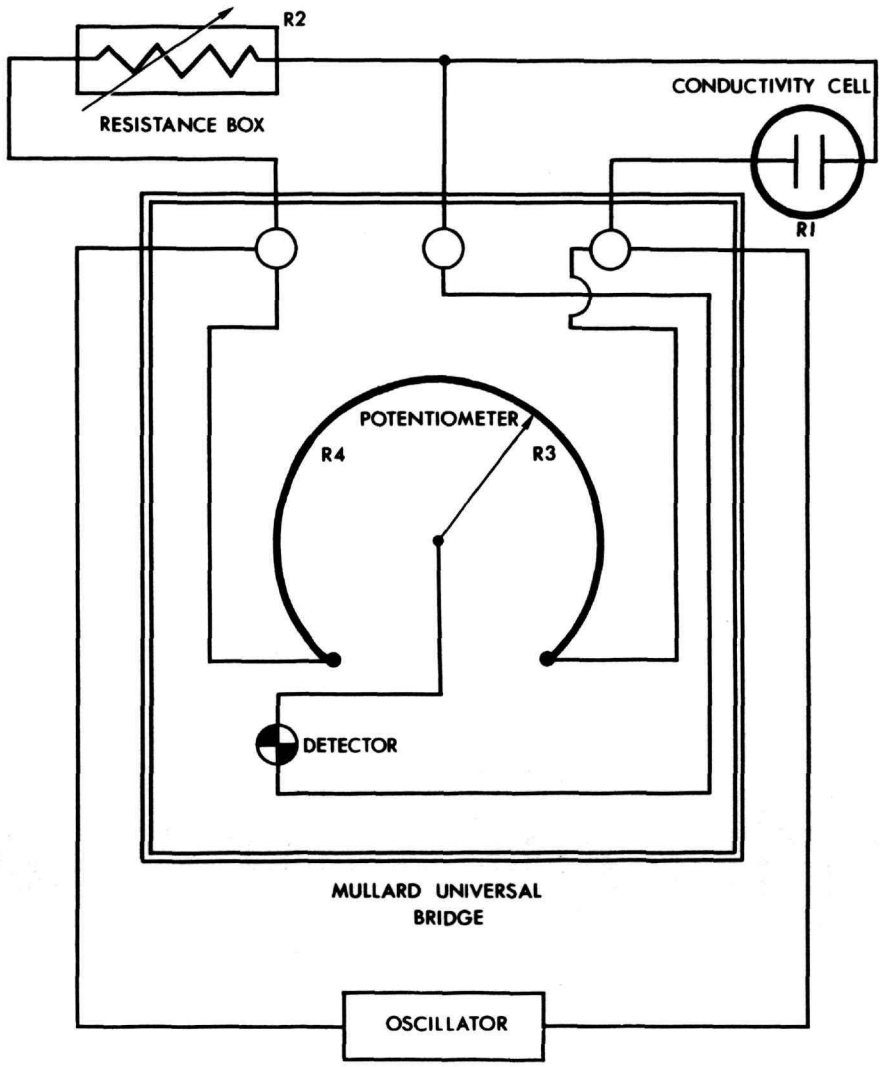

FIGURE 12.—Circuit diagram of the Wheatstone bridge.

where f = flux of oxygen (96,500 coul), i_t = amp, n = number of electrons involved in oxygen reduction at Pt surface (=4), A = electrode area (cm^2). For example, if $A = 0.11$ cm^2, then

$$f = \frac{i_t}{(4)\,(96{,}500)\,(0.11)} \text{moles } cm^{-2}sec^{-1}.$$

To convert moles $cm^{-2}sec^{-1}$ to g 0_2 cm^{-2} min^{-1}, multiply by 32 (mol wt 0_2) and by 60 (sec/min). Then,

$$f = \frac{(32)\,(60)\,(i_t)}{(4)\,(96{,}500)\,(0.11)}\ (g\ 0_2\ cm^{-2}\ min^{-1}) = \frac{i_t}{22.11}$$

Readings of i_t are made in microamps; thus, the formula becomes:

$$f = \frac{A}{22.11}(10^{-6}\ g\ 0_2\ cm^{-2}\ min^{-1}).$$

The reference electrode can be made either by using a commercial pH reference electrode (e.g., Beckman), or a self-manufactured one. A filter crucible of porosity 15 to 40 μ is filled with 0.1 N KCl. A wire is led through a tight-fitting rubber stopper and is attached to a bundle of silver wool (available from any laboratory apparatus supplier). The wire lead then can be attached to the oxygen availability meter. In operation, the reference electrode must make a good contact with wet sand, and care should be taken to avoid trapped air from remaining under the crucible.

The naked platinum electrode is made by first attaching the platinum to a wire lead, preferably of single strand copper. Soldering should be avoided as it interferes with the weak electrode current. The platinum is made to protrude from the end of a glass tube and is sealed in. The surface area of the exposed platinum should be calculated accurately in cm^2 by using a micrometer screw.

For the circuit diagram (Figure 13), the units are as follows: B = 1.35v, Mallory ZM-9; P_1 = 1000 ohm; P_2 = 150 ohm; R_1 = 100 kohm; R_2 = 1000 ohm, 1%; R_3 = 1000 ohm, 1%; R_4 = 2000 ohm, 1%; R_5 = 4000 ohm; R_6 = 400 ohm; R_7 = 3900 ohm; R_8 = 35 kohm.

10.4. Preparation of Silicone-Coated Pipette.—A water-soluble silicone such as Siliclad (see Section 10.1.15) can be used. This concentrated solution is diluted into 100 parts of distilled water. The pipette should be washed thoroughly and

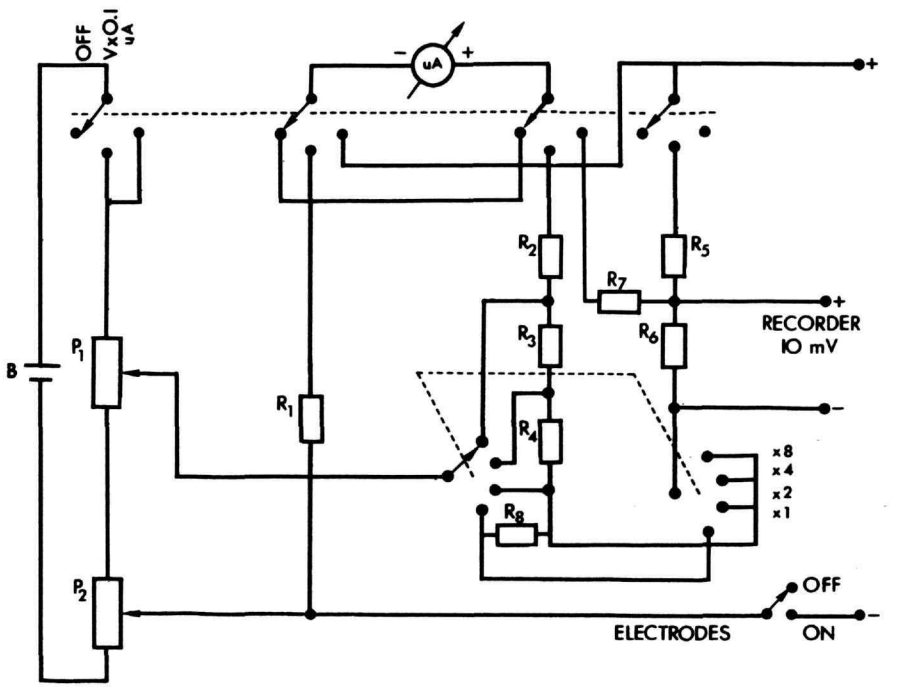

FIGURE 13.—Circuit diagram of the Jansson-Oden oxygen diffusion meter.

rinsed in distilled water. The inside of the pipette is coated by forcing the silicone solution through it. After complete immersion, it is removed immediately and rinsed thoroughly with clear water to remove any excess silicone from the surface. It then is air dried for 24 hours at room temperature. The pipette may be heated at 100° C for 10 minutes to speed the curing process. The Siliclad solution gives a coating that is physiologically inert and under normal conditions is unaffected by heat, moisture, and seawater. Rinsing the pipette is all that is necessary to keep it clean.

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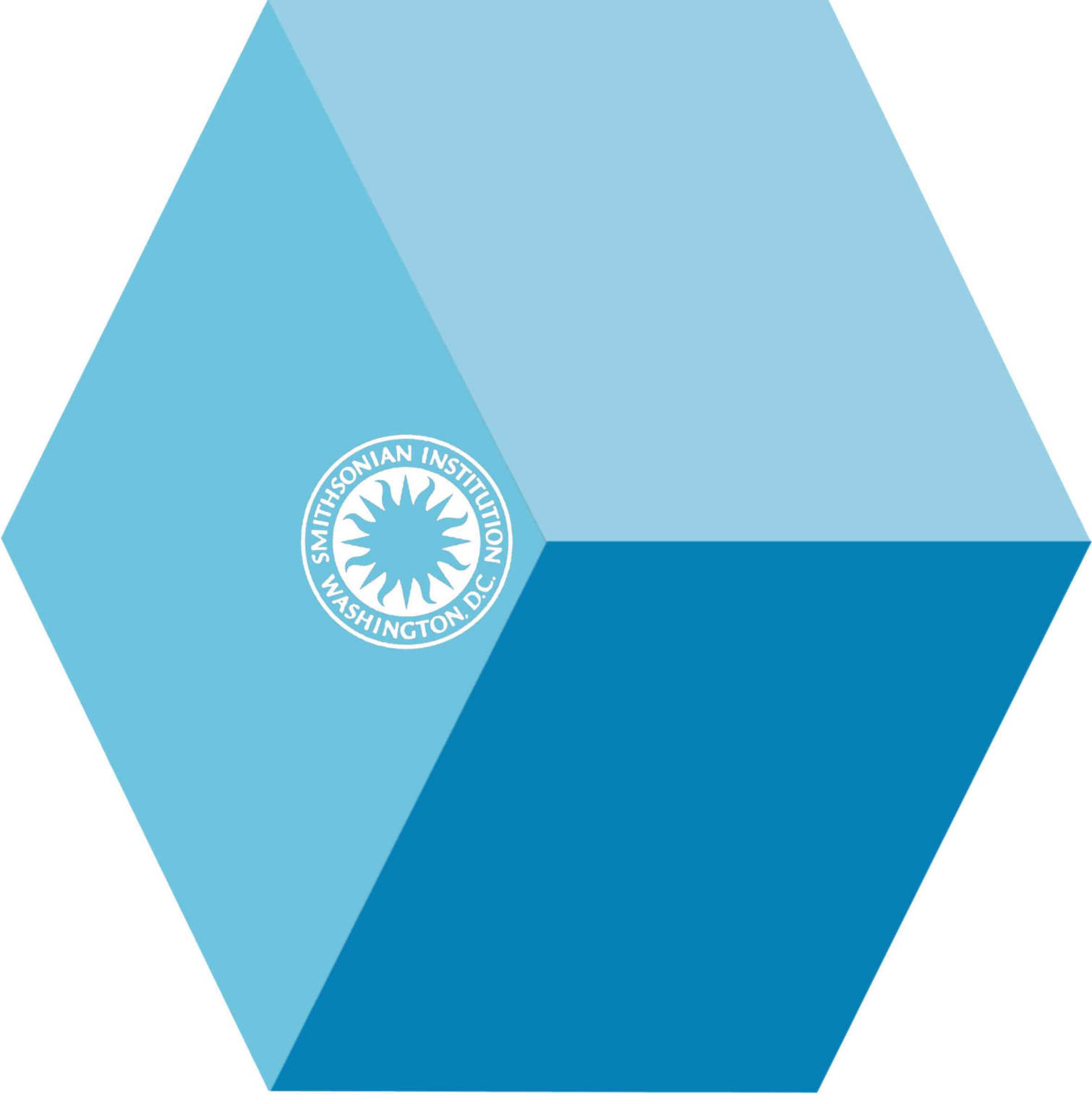
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