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Laboratory methods in the study of marine zooplankton

A summary report on the results of Joint Working Group 23 of the Scientific Committee on Oceanic Research and the United Nations Educational, Scientific and Cultural Organisation 1968–1972[1]

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Introduction

Because of the extreme range of types of life in oceanic zooplankton and the worldwide location of marine laboratories with their different and varying ambient temperatures, problems of fixation and storage of marine zooplankton have occurred at intervals throughout the history of this branch of marine biology. In response to some of the difficulties experienced and voiced by many specialists, an inquiry was made into the methods commonly employed by planktologists relative to curatoreal techniques and associated practices.

The inquiry was instituted by the Scientific Committee on Oceanic Research (SCOR) of the International Council of Scientific Unions, and a questionnaire was distributed during 1967. This was followed by a meeting of the Joint Working Group between SCOR and UNESCO (WG 23), Chairman V. Kr. Hansen, at the Smithsonian Institution Oceanographic Sorting Center, Washington DC, in March 1968, and ended with a symposium at Bath University, England, in July 1972. The members of the Working Group and other participants are listed in Table 1. An experimental programme with the purpose of evaluating and improving fixation and preservation methods was instituted in June 1968 under the direction of H. F. Steedman. This project was supported financially mainly by the Natural Environment Research Council (UK), the Smithsonian Institution (USA), SCOR and UNESCO, and was aided internationally in many ways by institutions and individuals: the Royal Society, London, the University of Bath, England; the Indian Ocean Biological Centre, India; the Regional Marine Biological Centre, Singapore; the Higher Education and Science Bureau, Tokyo, Japan; the Ministry of Fisheries, Copenhagen; the Danish Institute for Fisheries and Marine Research, Charlottenlund, Copenhagen and N. V. Organon, Holland, contributed generously in a variety of ways towards the furtherance of the research. Results of the experimental programme, as well as other aspects of plankton handling in the laboratory, were considered at the Bath symposium.

Specific topics in the monograph included plankton handling techniques on board ship and in the laboratory, A. Flemminger and B. Griffiths; biomass, J. R. Beers; aldehydes, D. Jones and H. F. Steedman; narcotising agents, calcium, oil, aqueous examination fluids, milliosmole pressures, H. F. Steedman; freeze-drying, R. H. Harris; storage

Table 1. Members of SCOR Working Group 23

V. Kr. Hansen	Denmark (chairman)
J. Beers	United States
H. Flugel	Federal Republic of Germany
E. Paasche	Norway (consultant)
H. F. Steedman	United kingdom (UNESCO)
B. Kimor	Israel
T. Tokioka	Japan
M. Vinogradov	Russia

[1] Complete results of the WG23 project will be published in the UNESCO series: "Monographs on Oceanographic Methodology", September, 1974. It will be between 120,000 and 150,000 words in length.

container types and usage, H. A. Fehlmann; automated plankton counting and analysis by electronic devices, J. K. Fawell; and applied specialist techniques to cover all the main taxa by a number of international research workers eminent in their own fields:
Microplankton (B. Kimor; A. W. H. Be; J. R. Beers; K. Gold; F. J. R. Taylor.)
Coelenterata and Ctenophora (K. Peterson).
Chaetognatha (L. Furnestin, and T. Tokioka).
Mollusca (R. D. Turner.)
Annelida (P. E. Gibbs.)
Crustacea (A. Flemminger, V. Kr. Hansen, A. O. Omori).
Appendicularia (R. Fenaux).
Salps and doliolids (T. Tokioka).
Fish eggs and larvae (E. H. Ahlstrom).
Outline of results in India (T. Balachandran).
Outline of results in Japan (R. Marumo, S. Motoda and T. Tokioka).

General plankton handling techniques (A. Fleminger and B. Griffiths)

Details are given of plankton handling methods on board ship and in the laboratory. A step-by-step summary of appropriate techniques is given starting from when the net is pulled out of the sea and ending in the laboratory where the samples are sorted. The care of nets, choice of containers, label materials, papers and inks, formulae for fixatives and preservatives, data recording; transport, storage and maintenance of samples are dealt with in detail. Some of the recommendations are as follows:

1 Make duplicate hauls if possible rather than splitting one haul for a variety of purposes.
2 The minimum information necessary for a field log is as follows: Station number including ship and cruise. Date, time, geographical ordinates; type of net, mouth size and mesh size. Type of haul. Sampling depths. Metres of wire out and wire angle. Flow meter serial number. Flow meter readings at the start and finish of the haul. Length of tow (time). Estimated volume of water filtered (time × speed × mouth area). Other measurements and collections taken at the station. Comments. Collector's name.
3 For shipboard storage of fixative solutions, use unbreakable material such as polypropylene carboys.
4 Use internal and external labels with storage jars.
5 Paper labels are recommended. The paper should be good quality 100% rag paper, free from starch and mineral matter. It should have a high wet strength and should take India ink on both sides without feathering.
6 Use waterproof India ink on all paper labels.
7 Use glass preserving jars in preference to those of plastic.
8 Keep a regular check on the condition of stored material with particular reference to pH, evaporation of fluid, appearance of haziness, moulds, precipitates, etc.
9 An addendum by B. Kimor and F. J. R. Taylor, deals with procedures suitable for micro-and nanoplankton.

Biomass (J. R. Beers)

Biomass measurements provide quantitative information which is useful for characterizing plankton taxa and populations. Several basic properties of zooplankton, including size, weight, volume, chemical composition and calorific content are commonly used as indices of biomass. However, the major taxa comprising plankton populations show wide differences in these properties. Even among individuals of the same species significant variations can be found within and between such properties due to differences in the stage of development, season of the year, and geographical location. This natural heterogeneity restricts the meaning and hence the usefulness of these properties as indices of abundance.

No single measure of biomass can be recommended as the best for all purposes. For each study, the method chosen for expressing biomass should be pertinent to subsequent use of the data obtained. This requires careful consideration of practical aspects such as the availability of sample material and analytical facilities. For example, a common reason for measuring zooplankton biomass is to gain insight into the nutritional state of the organisms and their potential value as food. Determination of calorific content or chemical composition (e.g. organic carbon) reported on the basis of the ash-free dry weight provide the most relevant measure of biomass for this purpose but requires specialized equipment and skills. Other methods of analysis, such as volume and wet weight, include measures of the highly variable water and ash contents of the organisms which are of little nutritional value. These are, however, generally less demanding of time and skill and, with several such methods the sample remains intact for taxonomic and other study.

The following guidelines and recommendations are made in an effort to standardize the use of biomass measures, and to provide the most meaningful, precise, and generally most accurate determinations.

General

1. A complete description of the procedure followed in making a biomass determination should always be provided. Several procedures which differ markedly in precision and accuracy are in common use for determining some properties of plankton (e.g. the displacement and settling methods of volume determination).
2. The natural variability within plankton of the various properties used as indices of biomass makes any generalized equivalents at best gross approximations. Their use in producing conversion factors between various measures of biomass cannot be recommended.

Collection of samples

1. Whenever possible two samples should be collected, one for the purpose of biomass determination only. The use of a single sample for several purposes is not desirable. Paired net systems (e.g. Bongo samplers) can provide two simultaneous samples.
2. If only one sample can be obtained the subsampling should cause a minimum of damage to the plankters, and the time of handling should be minimised.

Sample preparation

1. Alteration of many properties used to measure biomass may occur in zooplankton preserved in solutions (e.g. formaldehyde). Samples preserved in liquids should not be used in biomass measures if possible.
2. Freeze-drying, i.e. lyophilization, of fresh material immediately upon collection provides the most unequivocal means of preparing samples for methods in which the natural water content of the organism is removed. If freeze-drying apparatus is not available, oven-drying at a temperature not exceeding 60°C may be used to halt enzyme activity rapidly and to prevent any microbial decomposition.
3. In measures of biomass which include the natural fluid content of the organisms (e.g. size, volume, wet weight) it is suggested that in order to be more precise, determinations should not be made until approximately two weeks to one month after fixation. Rapid and significant but variable changes in biomass depending upon the taxonomic composition of the samples have been observed commencing immediately upon fixation. Measuring volume or wet weight of fresh material prior to fixation may result in damage to the organisms jeopardizing their use for future taxonomic study as well as possibly causing significant loss of material.
4. The complete removal of interstitial fluids is a common requisite of several biomass measures, but this is difficult to achieve especially in mixed plankton samples. Direct determination of the volume of interstitial fluids, for example by calorimetric methods, should be considered as a desirable alternative to its removal for procedures in which both interstitital water and salts have to be excluded. For methods in which the water is removed by drying and where it is necessary to eliminate only the interstitial salts, brief rinses with distilled water or easily volatilized substances such as ammonium formate at concentrations iso-osmotic to the animal's normal seawater environment can be used.
5. The biomass of large gelatinous forms (e.g. coelenterates, tunicates, etc.) of relatively high water content should be measured separately from other plankters.

Gravimetric methods

1. Ash-free dry weight is the most desirable basis for reporting the chemical or calorific content of zooplankton, and is not in error if removal of interstitial salts is incomplete.
2. The oven-drying of plankton material should be done at temperatures not exceeding 60°C in order to minimize possible losses of volatile organic substances. Drying should be carried out until a constant weight is achieved, but should not be prolonged beyond this point.

Volumetric methods

1. Displacement volume generally provides a more precise and accurate measure than settling. Settling volume alone cannot be recommended for biomass determination.
2. Several techniques for the determination of plankton volume call for the use of suction or air pressure to remove interstitial water. These must be used with care in order to avoid physical damage to the plankton.

Chemical/calorific content

1. Measurements of chemical components or of the calorific content of organisms provide good biomass indices, but in view of the effort involved in their determination they are generally practical to make only when applied to relatively restricted and well-defined taxonomic groups.
2. Because many plankters have a relatively high content of calcium salts which may be partly decomposed at the high temperatures used in calori-

metric investigations, the residue remaining in the bomb following combustion should not be used for a measurement of ash weight.

Freeze-drying (R. H. Harris)

Methods of freeze-drying adapted for use with marine zooplankton show such promise that wider use of these techniques is envisaged, particularly in the preparation of material for biochemical assays.

Fluid fixation and preservation (H. F. Steedman)

Most of the research of Working Group 23 was concerned with fluid fixation and preservation of marine zooplankton, with the naturally imposed requirements of faithful morphological and anatomical clarity of detail visible for fifty to one hundred years or longer. It was also essential that the fluids should be suitable for open-dish sorting.

In addition, methods for scanning electron microscopy, particularly for marine protozoa, were considered in some detail (F. J. R. Taylor).

Preliminary work showed that of the standard reagents and fixatives already in use, formaldehyde was the reagent most easily modified for different taxonomic needs, and many thousands of experiments were devoted to its use.

The criteria used in evaluation of the fluids produced and tested were those which had a direct bearing on taxonomic requirements, open-dish sorting, toxicity, evaporation rate; effects on jelly plankters, crustacea, chaetognaths, molluscs, protozoa, fish eggs and larvae; osmotic pressure, pH, refractive index and price. Storage room conditions regarding light and temperature were given special attention. Biochemical effects of formaldehyde solutions on proteins and on lipids are dealt with in detail by Dennis Jones.

The following principles and data, some of which are based on long usage, are regarded as desirable for the satisfactory fixation and preservation of marine zooplankton for taxonomic purposes:

1. Fixation of plankton should take place within five minutes of its arrival on deck.
2. The ratio of specimens to fixative should be as one is to nine (1:9) total ten, by volume.
3. With formaldehyde as the fixative, specimens in excess of the 1:9 ratio will lead to increased acidity of the fixing and preserving fluids. Calcareous plankters may dissolve completely.
4. Good preservation of plankton will occur at any pH between 6·5 and 7·5.
5. Calcareous plankters require a pH of 8·2 to 8·4 at fixation and during preservation, otherwise the calcium carbonate shell will dissolve.
6. Though a high pH such as 8·4 may be necessary for the successful preservation of calcium carbonate materials in formaldehyde solutions, it may lead to softening and gelatinisation of protein in warm storage conditions and it will also accelerate colour loss.
7. Specimens with skeletons based upon strontium sulphate will keep longer in fixative/preservative fluids containing strontium salts.
8. Strong formaldehyde as purchased (37% to 41%) may have a pH between 3·3 and 5·5.
9. This pH may be raised by using borax or sodium glycerophosphate, 5 g to 95 ml of 40% formaldehyde.
10. Borax in the amount given is in excess and will raise the pH to 8·2 to 8·4. Sodium glycerophosphate will raise it to 7·2 to 7·4.
11. When diluted with sea water or with distilled water strong formaldehyde buffered as above will maintain roughly the same pH as the strong solution.
12. Where purity of formaldehyde is essential use paraformaldehyde. Dissolve it in sea water or in distilled water with the aid of heat. Four grams of paraformaldehyde will dissolve in sea water in 20–30 minutes at 70°C (100 ml of sea water) to make 4% formaldehyde.
13. Formaldehyde, commonly used at 4% in sea water, may be used at 2% provided that the ratio of 1:9 specimens to fixative is not exceeded.
14. When formaldehyde is diluted to make a 2% solution it should be made up as follows:

2% formaldehyde	
40% formaldehyde	5·0 ml
sea water or distilled	95·0 ml

This solution should be referred to as 2% formaldehyde, not as 5% formalin.

15. Though there is a good margin of excess formaldehyde in the 2% solution adequately to fix and preserve zooplankton if the recommended proportion of specimens to fixative are not exceeded, the action of the fluid is improved by the addition of propylene phenoxetol and propylene glycol. These two reagents bring improved penetration of the fixative, anti-oxidant, humectant, bactericidal and fungicidal properties to the fluid.

General purpose zooplankton fixative/preservative

Propylene phenoxetol[1]	0·5 ml
propylene glycol	4·3 ml
40% formaldehyde	5·0 ml
sea water or distilled	90·0 ml

shake vigorously

The ratio of active ingredients is as 0·5:4·5:5. A stock solution may therefore be made up with these proportions and diluted as required. Variation in the osmotic pressure may be achieved by altering the amount of propylene glycol thus 1:10:5, as shown in Table 2. Formaldehyde is essential in the fixative fluids but is not required in the preserving fluids nor in sorting fluids.

Two stock solutions may be prepared and diluted as shown in Table 2. The diluted fluids are suitable as fixatives and preservatives. Fix for a minimum of one week. The fluids will keep plankters in good, flexible condition indefinitely. The osmotic pressure readings in Table 2 are derived from a Fiske osmometer at Keele University, courtesy of Professor A. R. Gemmell, readings and calculations by Nicholas Cartlidge.

The osmotic pressures given in Table 2 – though taken with great care – are given as approximate because their repetition would depend on the use of identical samples of commercial formaldehyde and sea water. These two fluids may vary. The figures given are therefore advanced as guides rather than exact criteria.

The formaldehyde content of fluids 3, 4, 7 and 8 is 1%. In fluids 1, 2, 5 and 6 it is 2%. Fluids 7 and 8 are of low concentration and are therefore useful for small plankters only. Whatever the fixative, the ratio of specimens to fixative should not exceed one to nine (1:9) by volume if the best results are to be obtained.

All solutions are made up more readily if distilled water is used instead of sea water. They also keep specimen colour longer. Propylene glycol is an aid to propylene phenoxetol dissolution.

Once plankters have been thoroughly fixed – whatever the fixative – they may be stored in formaldehyde-free fluids. Gently drain off the fixative or old storage fluid and replace with the following fluid:

propylene phenoxetol	0·5 ml
propylene glycol	4·5 ml
sea water or distilled	95·0 ml

This mixture should be shaken vigorously to get the phenoxetol and the glycol into solution.

The use of glycols as preservatives for biological specimens is the subject of British Patent 1263565.

Sorting fluid

The storage fluid 0·5:4·5:95 given above is suitable for sorting plankton, but it has the disadvantage that in a very hot, dry laboratory the water content of the mixture may be reduced so rapidly by evaporation that propylene phenoxetol is soon forced out of

[1] Propylene phenoxetol (3-phenoxy propanol) $C_9H_{12}O_2$ mol. wt. 152 obtainable from Nipa Laboratories Ltd., Treforest Industrial Estate, Pontypridd, Glamorgan, South Wales, Gt. Britain.

Table 2. Stock solutions and dilutions suitable for producing working solutions for fixing and preserving plankton

Stock solution	Propylene phenoxetol	Propylene glycol	40% formaldehyde	Total ml
1	100 ml	1 000 ml	500 ml	1 600
2	100 ml	500 ml	500 ml	1 100

No.	Stock solution 1	Stock solution 2	Sea water	Dist. water	Milliosmoles (approximate)	Suitable for
1	10	–	84	84	4 050 calculated	mixed plankton
2	16	–	–	84	3 035 calculated	mixed plankton
3	8	–	92	–	2 350 measured	mixed plankton
4	8	–	–	92	1 327 measured	mixed plankton
5	–	11	89	–	2 735 measured	mixed plankton
6	–	11	–	89	1 680 measured	mixed plankton
7	–	5	95	–	1 775 measured	small plankton
8	–	5	–	95	580 measured	small plankton
9			100		1 023 measured	
10	4% formaldehyde in sea water				2 500 measured	

solution and will appear in the form of small globules rather like oil drops. To avoid this trouble add distilled water to the 0·5:4·5:95 mixture in the petri dish at the beginning of sorting. One part of distilled water to one part of storage fluid should enable many days of sorting to take place without propylene phenoxetol being precipitated. Should precipitation take place a few drops of alcohol added to the sorting fluid will make it clear again.

Alternatively a special supply of sorting fluid may be prepared as follows:

propylene phenoxetol	0·3 ml
propylene glycol	5·0 ml
distilled water	94·7 ml

This fluid will show no sign of constituent separation even after many days of sorting in shallow dishes. All sorting dishes should be covered when not in use.

For general fixation and pH preservation of marine zooplankton, as distinct from the specialist requirements of histology, cytology and electron microscopy, solutions of formaldehyde may be raised to around pH 8 by adding 2 g of borax ($Na_2B_4O_7 . 10H_2O$) to each 98 ml of 40% formaldehyde. This will be in excess, and for making up fixatives the supernatant fluid should be decanted. Additional borax added to such fixatives may be precipitated as white, sticky or crystalline patches on or inside plankters.

These fluids may be used both as fixatives and as preservatives. The choice of sea water or of distilled water is dependent on the osmotic pressure required.

Fixation of planktonic foraminifera for cytological studies (A. W. H. Bé and O. R. Anderson)

The following procedure gives excellent results:

1. Prepare two solutions of phosphate buffer:

   Stock solution A 0·4 M monobasic sodium phosphate solution

$NaH_2PO_4 \cdot H_2O$	27·8 g
distilled water	500 ml

   Stock solution B 0·4 M dibasic sodium phosphate solution

$Na_2HPO_4 \cdot 7H_2O$	53·65 g
distilled water	500 ml

   For use:

Solution A	17·0 ml
Solution B	183·0 ml
pH 7·8 with a pH meter	

2. Saline adjustment of phosphate buffer tonicity. Sodium chloride is added to the buffer solution

Sodium chloride	72 g
buffer solution	1000 ml

   This is twice the concentration of the final fixative solution and allows for the addition of an equal volume of glutaraldehyde fixative.
3. Remove buffer from the refrigerator when needed and allow to warm to room temperature (25°C). Stir vigorously to ensure that all buffer crystals are dissolved.
4. Mix equal volumes of 8% glutaraldehyde at 3°C (pH 7·0) and phosphate buffer to produce a 4% glutaraldehyde solution in 0·2 M phosphate buffer. This should be prepared immediately before use. The glutaraldehyde should be of the highest purity and with a pH 7·0. Otherwise delicate cell organelles will not be obtained.
5. Suspend freshly obtained plankton samples and allow them to settle in sea water. Then drain off excess sea water leaving only a thin layer of water covering the sample. Add the buffered fixative with gentle swirling. The volume of the added fixative should be 6 to 10 times the volume of sea water covering the sample. After adding the fixative the samples should be placed in a refrigerator at approximately 3°C. Samples may be stored in the fixative solution at 3°C for as long as three weeks if necessary without noticeable deterioration in the quality of fine structure preservation. It is better, however, to complete steps 6 and 7 as soon as possible after glutaraldehyde fixation.
6. After fixation in the above mixture for at least 4 h the sample may be post-fixed for 24 h at 3°C in 2% osmium tetroxide solution prepared in 0·2 M phosphate buffer. Before addition of the osmium tetroxide the sample must be thoroughly washed in 0·2 M phosphate buffer for one hour to remove residual glutaraldehyde. If the sample is insufficiently washed, electron dense particles may become precipitated on the cell membrane during osmium tetroxide fixation and staining.
7. Wash the fixed cells in phosphate buffer after the osmium tetroxide bath. Dehydrate in graded series of aqueous ethanol solutions. Clear in propylene oxide. Embed in Epon 812.

   The calcareous test may cause sectioning difficulties. It may be removed after the osmium tetroxide has been washed out in the phosphate buffer by washing the cells in distilled water and suspending them in 0·05 M acetate buffer, pH 6·5, until all traces of the test disappear. An alterna-

tive method is to replace the test with Epon after embedding (Gorycki, 1971).[1]

8. Ultrathin sections may be stained on the grid to enhance contrast by using an alkaline lead hydroxide stain.

Strontium sulphate plankters (J. R. Beers)

The celestite (strontium sulphate) skeletons of some acantharians show rapid dissolution in formaldehyde solutions whether at a low pH such as 3·3 or at levels in the region of pH 8·0 to 9·0. A tenfold increase of the strontium level of normal sea water added to the fixative/preservative retards or even prevents dissolution of the skeleton framework of these forms. A suggested formula for a fixative and preservative for acantharians is shown below:

40% formaldehyde	5·0 ml
strontium chloride ($SrCl_2 \cdot 6H_2O$)	0·026 g
sea water	95·0 ml

The amount of strontium chloride which can be added without precipitation will vary depending on the seawater media and the conditions of fixation/preservation. The extent of the problem of skeletal dissolution in all taxa of acantharians has not been determined.

Oily plankters (H. F. Steedman)

Because oil cannot be fixed and made relatively insoluble, as can protein, the chief interest in its preservation is concerned with prevention of its breakdown. As a preservative alcohol dissolves oil readily and is therefore of little use. Formaldehyde also breaks down lipids or reacts with them in such a way that after storage in formaldehyde solutions of two years and longer there is a greatly reduced amount present in tissues. The biochemical reasons for this are discussed by Dennis Jones in the UNESCO manual.

In practice many planktologists have observed a severe loss of oil in specimens stored in formaldehyde solutions. The reaction with formaldehyde is not very rapid, however, and fixation of oily plankters in 2% formaldehyde, or in the formaldehyde phenoxetol glycol mixtures, may be safely undertaken for two or three weeks. After this, carefully pour off the fixative and rinse the plankton in distilled water or in sea water and store in:

Propylene phenoxetol	0·5 ml
propylene glycol	4·5 ml
sea water or distilled	95·0 ml

Propylene phenoxetol acts as an anti-oxidant as well as a bactericide.

Staining oil globules in Calanus

The following new method enables oil droplets to be stained a clear blue or green depending on the original colour of the oil.

1. Fix fresh material in

propylene phenoxetol	0·5 ml
propylene glycol	4·5 ml
40% formaldehyde	5·0 ml
sea water	90·0 ml

Shake the mixture vigorously

Fix for ten days or for an indefinite period above that time. The fixative/preservative may be used on all types of plankter. If specimens have been in 2% or in 4% formaldehyde solutions, transfer them to

propylene phenoxetol	0·5 ml
propylene glycol	4·5 ml
distilled water	95·0 ml

Shake vigorously

Keep the plankters a week in this fluid before staining.

2. Prepare the blue stain

Terasil blue GR liquid (Ciba-Geigy)	2 drops
the 0·5:4·5:95 mixture above	100 ml

Stir well to disperse the dye.

3. Drain the preserving fluid gently from the plankters and replace it with the stain solution. Stain for a minimum time of one week. Plankters may be left in the stain solution for six months or longer without damage. Cover the jars during staining.
4. Before examining the specimens drain off the coloured fluid and wash them with the 0·5:4·5:95 fluid given above. Keep the coloured fluid. It may be used again. Examine the plankters in the 0·5:4·5:95 mixture, or if prolonged examination lasting several days is envisaged use the 0·3:5:94·7 mixture given for sorting purposes.

Note that Terasil blue GR liquid is a dispersion of dye particles. It is not a solution. The dye was designed for the staining of polyester fabrics. It ap-

[1] A compound bedding technique for the microscopical study of living and fossil foraminifera. Micropaleont., 17 (4): 492-500.

pears to be specific in its staining of such mono-esters as fatty acid esters because even long staining such as one month fails to produce any take up by protein. The long time taken for fat globules to accept the stain is due partly to the resistance of the *Calanus* exoskeleton and partly to the particulate nature of the dye.

Resinous mounting media
(H. F. Steedman)

Replacement of Canada balsam as a mounting medium for tissue sections and for stained or unstained whole mounts has met with only partial success during the last thirty years largely because the plastic materials brought forward as substitutes were of such high molecular weight that their viscosity in solution prevented a high proportion of resin to solvent. In practice this means that a high proportion of solvent is used, and when that has evaporated only some 30% or 40% of the original volume remains. With whole mounts this leads to air-bubble intake and consequent damage to the mount.

Many materials were investigated during the WG 23 project to reduce these weaknesses and the best of these was sucrose benzoate. Its molecular weight is 1100 as compared with 3000 to 400 000 for polystyrene. It is colourless, amorphous, has good adhesion to glass and a refractive index of 1·577. The mounting medium may be made up as follows:

Sucrose benzoate (Velsicol)[1]	65·0 g
polyethylene glycol 600 benzoate	1·0 g
benzyl benzoate	2·0 ml
xylene	16·0 ml
ethyl acetate	16·0 ml

Dissolve the sucrose benzoate in the xylene with the aid of heat on an electric hot plate. Stir. When dissolved allow to cool to room temperature. Add, with stirring, the ethyl acetate and the other two constituents. The result will be a transparent, colourless, easy-flowing fluid which may be used for whole mounts and stained tissue sections. The refractive index is 1·52. For very large whole mounts use 75% sucrose benzoate in the formula instead of 65%. Mount specimens or sections from xylene. Mounts may be dried at room temperature or on a warm plate. There is a rapid thickening and partial drying as the more volatile solvent – ethyl acetate – quickly leaves the mixture. This is followed by a slower thickening and hardening as the xylene leaves.

[1] Sucrose benzoate and polyethylene glycol 600 benzoate. Obtainable from Velsicol Chemical Corporation, 66 Tilehurst Road, Reading, Berkshire, England.

Electronic measuring devices in the sorting of marine zooplankton
(J. K. Fawell)

Sorting plankton is one of the major difficulties in current studies because the manual techniques used are difficult, slow and tedious. Three major possibilites for automation exist at present: particle sizing instruments, television image analysis and computer pattern recognition.

Particle sizing by the Coulter counter and specially designed systems have been successfully used in sorting plankton into size classes in which the percentages of individual species are estimated by a visual scan. This is of particular value in food chain investigations and low diversity samples.

Television image analysers are able to distinguish discrete particles within preset gray level and can separate these on non-orientation dependent criteria such as area and perimeter or according to the formula $A \times B/Cn$ where n is 1, 2 or 3 and A, B and C are any of the following: area, perimeter, horizontal or vertical projection, horizontal or vertical Feret and volume. Some commercial systems also have facility for interfacing into the more sophisticated programmable calculators. These systems have been used to sort low diversity samples on a species basis.

Computer pattern recognition requires an optical interface into a large computer which is programmed to recognise shapes presented to it. The obvious problem associated with this is the generation of software to deal with new problems as they arise. This reduces the versatility of the system in routine sorting.

All these systems provide a basis from which future developments in instrumentation may be made while having immediate application to current research.