Instrumentation for measuring radiant energy.

Analytical techniques for determing primary productivity using C^{14}: (in situ and simulated in situ).

Descriptions of equipment for C^{14} work.

Relationship between radiant energy and photosynthesis.

Review of WG-15 reports.

Notation for Optical Oceanography.

Diurnal variation of radiant energy.

Estimates of radiant energy in the sea based on simple measurements at specified wavelengths.

Description and use of quanta meter.

It was suggested that the Chairman (WG-15) act as editor and that suitable sections be assigned to the various members of the Working Group. It was also suggested that at an appropriate time the Working Group should meet to work over the manuscript. Mr. Jitts stated that he would be pleased to act as host to the group at CSIRO.

Recommendation: It is recommended that Working Group-15 undertake the preparation of a monograph as outlined and that arrangements be made to have the manuscript professionally published in book form (perhaps by UNESCO).

It is recommended that Working Group-15 plan to meet to work on the manuscript at an appropriate time and place. The time and place will become evident with progress on the monograph and will be communicated to SCOR by the Chairman WG-15.

J. Tyler

ANNEX V

REPORT OF WORKING GROUP 23 ON ZOOPLANKTON LABORATORY METHODS

Meeting in Washington, 25 - 30 March 1968

The first meeting of WG 23 was held on 25 - 30 March 1968 in Washington, D.C. at the invitation of the Office of Oceanography and Limnology of the Smithsonian Institution. The following persons participated:

MEMBERS: Chairman, V. Kr. Hansen (Denmark); H.J. Flügel (FRG); B. Kimor (Israel); H.F. Steedman (UK); T. Tokioka (Japan); M.E. Vinogradov (USSR).

OBSERVERS: D.M. Damkaer (Smithsonian Oceanographic Sorting Center); D.J. Faber (Canadian Oceanographic Sorting Center); H.A. Fehlmann(SOSC); A. Fleminger (Scripps Institution of Oceanography); N.C. Hulings (Mediterranean Marine Sorting Center); P.A. McLaughlin (SOSC); E.J. Ferguson Wood (Institute of Marine Science, Miami).

A wide range of subjects pertinent to plankton fixation and preservation was discussed

in detail, and agreement was reached on a set of interim recommendations. Brief summaries of several topics, not considered in the interim recommendations, are included in Attachment I. In addition to the discussions, a comparative examination of fixation and preservation methods was carried out through the actual observation of plankton samples from several sources. Prior to this, WG 23 members had been asked to examine critically the state of preserved samples available to them and to make these samples available to the group. Samples were also furnished by the Canadian Oceanographic Identification Centre, Ottawa, Canada; the Oceanographic Laboratory, Edinburgh, Scotland; Indian Ocean Biological Centre, Ernakulam, India; and Smithsonian Oceanographic Sorting Center, Washington, D.C.

A questionnaire on standard methods employed for fixation and preservation of zooplankton samples for taxonomic studies and on biomass determinations had been prepared by the members of WG 23 through correspondence prior to the meeting; this was distributed to about 300 institutions, individuals with current sampling programs, and museums. More than 30% were returned. A preliminary analysis of the replies was made during the meeting.

INTERIM RECOMMENDATIONS ON METHODS OF FIXATION, PRESERVATION AND BIOMASS DETERMINATION

As a result of the group's activities during the meeting a set of interim recommendations on fixation procedures to be used on shipboard (items 1 - 9) on preservation and storage (items 10 - 18), and on biomass determination (items 19 - 24) were adopted. Many of these recommendations will be considered further in future discussions and experimental studies proposed by the Working Group.

Fixation of Plankton

1. Fixation should take place immediately after the catch is taken aboard.

2. Plankton samples to be used for taxonomic studies should be separated into calcareous and non-calcareous specimens prior to, or immediately after, fixation. The separation should be made by techniques, such as the gravimetric methods, resulting in minimal damage to the specimens. Great care should be taken when separating the specimens. The fixation and preservation of non-calcareous plankters should be in a 4% formaldehyde solution made up with the addition of sea water on the site (40%formaldehyde diluted in the ratio of 1:9 with sea water). Calcareous plankters should be preserved by freeze drying, in 70% ethanol or in 4% formaldehyde (as above). 40% isopropanol may be used although it causes heavy shrinkage of the tissue. If the formaldehyde is to be buffered, this should be done carefully and the pH should be checked at very frequent intervals, at least during the first three months and then later at half yearly intervals.

3. The use of buffered formaldehyde is subject to some criticism. Its use is a matter of personal choice and presently can be neither accepted nor rejected. When a buffer is needed or wanted we recommend sodium bicarbonate or calcium carbonate, until future experiments yield a better buffering agent. Sodium borate in excess may have an unfavorable effect.

4. The quality of formaldehyde should be U.S.P. or reagent grade (solutio formaldehydi concentrata) or, eventually, deionized formaldehyde.

5. The fixative should be stored, prior to use, at a temperature of 20° C or less, but still above a few degrees C° in order to prevent polymerization.

6. The sample container should always be filled completely, leaving the minimum volume of air. This reduces the movement of the specimens in the container.

7. Air proof closures should be used.

8. Immediately after adding the fixative and closing the container, the sample should be transferred and kept at a temperature somewhat cooler than sea temperature. This prevents formation of gas bubbles during the period of fixation. The sample should be kept in the dark.

9. Although plastic containers are most practical for field work, the plankton samples should be transferred into glass containers for permanent storage in laboratory or other depository.

Preservation and Storage of Plankton

10. Storage should be in the dark.

11. Ambient temperature variations, both daily and seasonal, should be kept at a minimum.

12. Glass containers with air-proof closures should always be used for long term storage.

13. Ethylene glycol (5-10%) may be added to prevent complete dessication during storage. As a fungus inhibitor, it is preferred to glycerine.

14. For preservation, formaldehyde of the quality described under (4) should be diluted with deionized or distilled water with or without addition of 3.3% NaCl.

15. Volumetric measurements should be made only when essential to the program.

16. Subsampling should be kept to a minimum.

17. Technicians should be thoroughly trained in careful handling techniques.

18. Processing (sorting) centres should be encouraged not to use stains, at least not long lasting ones, to facilitate sorting.

Plankton Biomass Determination

19. For biomass determination, whenever possible,replicate hauls should be taken, one for the purpose of biomass determination only. The use of a single sample for several purposes is not desirable.

20. 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 kept at a minimum.

21. Measurements should be made as soon as possible after obtaining the sample. If fixation is necessary, the time between bringing the catch aboard and fixation should be kept to a minimum.

22. Sedimentation volume by itself cannot be recommended for biomass determination.

23. When measuring displacement volume, coelenterates and tunicates should be measured separately from the other plankters.

24. For exact chemical analysis freeze-drying appears to be the best preservation method, giving the least alterations in the chemical constituents of the sample.

PROPOSALS FOR FUTURE ACTIVITIES

Zooplankton Fixation and Preservation

As the result of our critical examination of plankton samples from throughout the world we concluded that now is the time to reconsider standard fixation and preservation techniques. This should be done by making detailed tests of the present methods on a range of defined plankton components (see Attachment I). Criteria for quality of fixation and preservation must be developed and defined. Long term effects of preservation should be measured by chemical, histochemical, histological and morphological tests. Electron microscopy would be considered suitable and may be essential.

New reagents which may fulfill the theoretical requirements of a stable preservative, but which so far have not been used for this purpose should be selected and tested. These experiments should preferably be conducted on a world-wide basis so that variation of climatic and other effects may be observed. The WG proposed that such an experimental program be initiated. Dr. Steedman, in his capacity as UK observer on WG 23, referred to a meeting of the British National Committee on Oceanic Research where interest and intention to assist in such a program was clearly expressed. Dr. Fehlmann, representing the Office of Oceanography and Limnology, Smithsonian Institution, expressed the strong interest of his institution in such a project; he was asked to ascertain whether his institution would be willing to accept responsibility for some of the proposed tests and experiments.

For planning and implementation of an experimental program it was recommended that a coordination body within the framework of WG 23 should be established and that Dr. Steedman be invited to be the chairman with Drs. Tokioka and Beers as members.

Zooplankton Biomass Determination

Our interim recommendations for biomass determinations are given above. The suggestion that WG 23 should consider methods for preserving zooplankton material for subsequent biochemical analysis was noted (SCOR Proc., Vol. 3, No. 2, p. 70). The wealth of recent data on changes that occur in plankton material, depending on type of preservation, timing of analysis, etc., was discussed. It was agreed that Drs. Hansen and Beers should discuss these problems and the replies to the biomass questionnaire by correspondence and determine what future action should be recommended.

Micro-zooplankton Fixation and Preservation

The growing need for proper techniques to determine the significance of micro-zooplankton, from both a taxonomic and biomass standpoint, was acknowledged. The small zooplankters such as flagellates, ciliates (including tintinnids), foraminifera, and radiolarians need to be considered separately for research and preservation.

We found that in the absence of Dr. Beers only Dr. Kimor had relevant experience in this field and concluded that no decision should be made at present as to recommendations on detailed procedures nor on the desirability of establishing a SCOR/UNESCO Working Group on micro-zooplankton. Drs. Kimor and Beers were asked to study further the problems relating to micro-zooplankton through correspondence.

Manual on Zooplankton Fixation, Preservation and Storage

We recommend that a manual describing the standards of fixation and preservation should be compiled through the effort of WG 23 and that it should be published in a loose leaf system depending on the successful outcome of the planned experiments. Existing sorting centers should be encouraged to prepare a section on procedures and techniques used at such specialized laboratories. The manual should include a bibliography.

Workshop on Zooplankton Fixation and Preservation

It was concluded that the methods of fixation and preservation used by zooplankton researchers have remained stagnant; even when considering the new efforts made and planned, we are of the opinion that the interest of these technical subjects could be intensified and new ideas brought forward by bringing zooplankton researchers together with scientists dealing with related technical problems from other fields. It was felt that the usual type of symposium was an unsuitable medium for presenting new facts, and that a workshop would be more appropriate. We therefore recommend that a workshop be organized at a suitable time. Specialists in disciplines with problems of fixation and preservation similar to those of the planktologists should be invited to contribute their experience. They should be from such fields as medicine, terrestrial biology, histology, histochemistry, pathology, food technology, etc. The proceedings would enable planktologists to evaluate and eventually to utilize the facts presented. The workshop should not be convened until sufficient information is available; it should be organized at least one year in advance.

Acknowledgements

Sincere appreciation is extended to Drs. I.E. Wallen and H.A. Fehlmann of the Smithsonian Institution, Office of Oceanography and Limnology and their staff for the hospitality and valuable assistance accorded us during the meeting. We are grateful to Dr. A. Fleminger for participating and contributing significantly to the group's activities. We also wish to acknowledge the valued contributions of the observers to our discussions and to thank the various institutions which loaned plankton samples for the comparative studies.

ANNEX V
ATTACHMENT I

OTHER TOPICS DISCUSSED BY WORKING GROUP 23

Criteria for Measuring the Quality of Fixed and Preserved Plankton

It was concluded that well defined criteria for measuring the adequacy of fixation and preservation of plankters are needed. Reports were presented by Drs. Fleminger and Flügel on their preliminary studies of morphological structures as measures for describing the state of plankton preservation. They were encouraged to continue and, if possible, to enlarge the fields of their respective investigations.

It was felt that criteria for recognizing progressive deterioration during long term storage can be found by closer histological and cytological examinations. With the development of such methods it may be possible in short periods to check whether a preservative or method can be used safely for long term storage without waiting decades for a result.

Specific groups of plankters should be accepted as monitors. We recommend six monitors: 1) calanoid copepods, 2) pteropods and Atlanta (heteropod), 3) Foraminifera, 4) chaetognaths, 5) salps and doliolids, and 6) fish larvae. Comparative tests should be made on four types of plankton: 1) fatty crustaceans, 2) non-fatty crustaceans, 3) non-crustaceans, and 4) calcareous plankton. It was decided to refer further details of these problems to our proposed implementation of comparative studies of fixation and preservation methods.

Comparison of State of Preserved Plankton Samples and Recommendations for Continued Studies

The members of the WG 23 carried out comparisons of the state of preservation of plankton in samples collected by R/V "Anton Bruun" during IIOE and stored separately at IOBC and SOSC. In addition, a variety of other samples brought by us were exhibited and studied. Although moderately different views were sometimes expressed regarding the condition of different taxonomic groups in the collection, it was agreed that the comparisons were highly informative since they provided an opportunity to compare material treated in a similar fashion but stored under a variety of climatic and ambient shelf conditions. The comparative studies indicated that it is possible to attribute the causes of improper state of preservation to factors such as 1) fixation made too long after death, 2) too weak formaldehyde concentration used for fixation and/or preservation, 3) duration of sorting too long and combined with a too weak preservative. Extension of these comparisons to the IIOE collections held by other participating institutions may clarify significant aspects of the problem of preservation.

The International Collection of IOBC provides an excellent opportunity to broaden these observations since parallel collections treated in a similar manner on board ship have been maintained partly by the donor institutions and partly by the IOBC. We recommend that these comparisons be made by specialists who have worked on the national IIOE collections of the donor institutions. Approximately ten IOBC samples in generally poor condition from each of the national collections listed below should be compared with available samples collected at similar stations and fixed in a similar manner but stored in the country of the donor institution. Each IOBC sample chosen should be represented by a small aliquot of the archive fraction, a small aliquot of the residue fraction, and by one or more sorted categories, the choice being according to the specialities of the senior investigators who have been examining their IIOE national collection. The specialist should compare the preservation of both external features and prominent internal organs among the taxonomic groups they will consider. Comparisons should be made with the national collections of at least the following national IIOE collections: Institute of Oceanology, Moscow, USSR, R/V "Vityaz"; Institute of Marine Research, University of Kiel, FRG, R/V "Meteor"; National Institute of Oceanography, Wormley, England, R/V "Discovery"; Scripps Institution of Oceanography, La Jolla, USA, R/V "Argo"; Tokyo Fisheries University, Tokyo, Japan, R/V "Umitaka Maru"; "Koyo-Maru", "Oshoro-Maru", and "Kagoshima Maru"; Smithsonian Oceanographic Sorting Center, Washington, D.C., USA, R/V "Anton Bruun".

MISCELLANEOUS NOTES AND REPORTS ON TOPICS UNDER THE TERMS OF REFERENCE OF WG 23

1) Oxidation of Formaldehyde. A proposal by Mr. R.I. Currie to prevent oxidation of formaldehyde in a container by placing a layer of liquid paraffin on the top of the preserving fluid was considered. It was noted that there often are two types of collections, "archives" and "daily use". The former type might be sealed as proposed, whereas this is not practical in the latter type.

2) Narcotization. The necessity of narcotizing specific plankton groups such as siphonophores and ctenophores was discussed. We agreed to refer at present to methods described in publications such as:

G. Tregouboff and M. Rose: Manuel de Planctonologie Mediterraneenne. Tome I, Paris, 1957.

R. Wagstaffe and J. Havelock Fidler: The Preservation of Natural History Specimens 1. Invertebrates, London, 1955.

J. Marr: Some Notes on the Preservation of Marine Animals. J. Cons. Explor. Mer, 28 (1): 121-125, 1963.

3) The International Collections, Indian Ocean Biological Center, Ernakulaum, India. WG 23 noted the draft report of the 6th meeting of the Consultative Committee of the Indian Ocean Biological Center and the resolution on preservation of the IOBC International Collection. It was agreed to draw their attention to the interim recommendations given above on methods of fixation, preservation and biomass determination.

WG 23 will review and report to the UNESCO curator of the IOBC their proposals with regard to Mr. T. Balachandran's report on Experiments of Fixation and Preservation of Zooplankton. (Mr. Balachandran has been allotted the responsibilities of these studies at the IOBC.)

4) Miscellaneous Fixatives and Preservatives. A report was presented on Dr. Beers' tests on plankton fixation in glutaraldehyde and subsequent preservation in 70% ethanol. A preliminary check showed a seemingly wide degree of variability in the state of preservation of the samples. After further study Dr. Beers will report his conclusions.

Dr. Flugel suggested fixation of micro-organisms in glutaraldehyde and post-fixation in a Dowacide solution.

Internal reports from various institutes on the use of Phenoxetol solutions as a preservative and as a medium during sorting and display of specimens were discussed. It was noted that bulk use of Phenoxetol should be considered with some caution.

As for the fixatives, it was concluded that it may be difficult or impossible to find a fixative more satisfactory than formaldehyde but esters may prove better preservatives than aldehydes and alcohols

5) Displacement Volume. Dr. Vinogradov reported on a standard Russian procedure for measuring the displacement volume. Before the measurement is made all specimens untypically large in size for the type of net used, together with post-larvae and older fishes, are removed. Dr. Vinogradov was asked to report to the WG on the improved instrument for measuring the displacement volume and also other relevant information.

6) Microplankton. Dr. Ferguson Wood happened to be at the Smithsonian Institution and he was invited to join this session. He informed us that a U.S. working group on microplankton consisting of Drs. Holmes, Snyder, Norris and himself is studying the problems of microplankton fixation and preservation and will report at a later date. It was also noted that a booklet on phytoplankton methods is planned by ICES.

7) British National Committee of Oceanic Research. Dr. Steedman, member of and observer for the Working Group on Zooplankton Laboratory Methods of the BNCOR, gave a report on its meeting held in February of 1968. WG 23 welcomes the invitation of the British Working Group as to how they can implement or assist the work of WG 23. Dr. Steedman was asked to inform his Working Group of our proposals and to examine future cooperation.

8) International Association of Biological Oceanography. The Symposium on Design and Analysis in Plankton Sampling, of the International Association of Biological Oceanography, IUBS, was noted. We regret that the timing of the Symposium and of our meeting was such that they could not have been held in succession.