REPORTS OF WORKING GROUP 13 ON ZOOPLANKTON SAMPLING METHODS

Report of the Chairman

Introduction

WG 13 can be said to have originated in January 1964 following discussions by various international and national bodies which had expressed keen desire to consider the possibilities of standardization of zooplankton methods where it was reasonably practical to do so.

A joint working group of SCOR, UNESCO and ICES was therefore set up to deal with this problem and at their first meeting in Paris 1964 a general plan was prepared.

Present were the convener, J. H. Fraser (ICES), Dr. G. Humphrey (SCOR), Dr. T. Parsons (UNESCO) and Prof. J. Krey (chairman of the Plankton Committee ICES). As plankton covers such a great range of organisms, in size, shape, consistency, behaviour and taxonomic grouping no single standardized method is possible and arbitrary divisions become necessary, each with its disadvantages as well as advantages. Of these size was chosen as the most practicable and innocuous, and the following terms of reference were agreed:

Terms of reference

To set up small working parties from experts in their particular fields of work, who will examine and consider the methods used at sea and in the laboratory in sampling zooplankton of various categories, and make recommendations concerning the methods they consider the most satisfactory for general adoption. Where they consider present methods inadequate, new methods should be recommended, based if necessary on new hydrodynamic or other research. Where it is possible to do so the working parties should compile a series of intercalibration factors between the methods especially where much data have been published.

There should be four such working parties in zooplankton (including fish eggs and larvae) to deal with:

1) The microzooplankton, at present samples by water bottle, very fine meshed nets and pump filters.

2) The zooplankton now sampled by a great range of techniques, but largely dependent on filtration through a No. 3 mesh (about 60 meshes per inch).

3) The larger zooplankton, often sampled by stramin or other coarse meshed nets.

4) The faster moving macroplankton, such as the larger euphausids and small fish.

We agree that the working parties should be of a practical size and there should be five members to parties 1, 3 and 4 but seven for w.p. 2 which has more complex problems, chosen from suitable experienced experts and representing as far as reasonably practical a world wide coverage of interests.

It is important to emphasize that while standardization of plankton methods would assist greatly in the comparisons of one area with another, and would help in the selection of gear by those needing such help, standardization should in no way be interpreted as a bar to progress towards further improvements of methods, nor as a discouragement in the use of other more specialised gear for purposes where this is considered desirable

It was agreed that an effort should be made to complete the work of WG 13 in two years time.

Personnel of the working parties

It was regarded as essential that the members of each working party should not only be experienced in the appropriate field but should be selected on as wide a geographic basis as possible. With a view to this all the countries represented in SCOR were asked to nominate suitable personnel for consideration by the convening committee and these lists were very helpful in selecting the w.p. members. Not all those first selected were able to give their services and those finally selected were:

Working Party 1

R. Currie	(U.K.) Convener
J. Krey	(Germany)
K. Banse	(U.S.A.)
V. Hansen	(Denmark)
I. McLaren	(Canada)

Working Party 2

A. W. H. Bé	(U.S.A.) Convener
N. Della Croce	(Italy)
A. Boudillon	(France)
A. de Decker	(South Africa)
B. Kimor	(Israel)
E. Hagmeier	(Germany)
B. Bogorov	(U.S.S.R.)

Working Party 3

D. Tranter	(Australia) Convener
M. Vannucci	(Brazil)
J. Gehringer	(U.S.A.)
M. Vinogradov	(U.S.S.R.)
M. Anraku	(Japan)

Working Party 4

P. Foxton	(U. K.) Convener
W. Aron	(U.S.A.)
M. Legand	(New Caledonia)
T. Nemoto	(Japan)

Although these members are given with their nationality, their representation on the working parties is entirely international. The chairman of WG 13 would endeavour to attend any working party meeting if this was desired by the convener and would also act in an advisory capacity by correspondence with all four working parties.

Financial

To help keep expenses within reasonable bounds we expected the working parties to do much of their work by correspondence, but some meetings would certainly be necessary. The costs of the working parties would be borne on an equal basis between SCOR, UNESCO and ICES. Although an assessment of costs before hand could not be expected to be accurate, a figure of 12,000 U. S. dollars was estimated, i.e. 4,000 dollars for each of these three organizations over a two year period. Each organization agreed to meet costs up to this estimated figure.

When arranging from which source the various expenditures would come, it has not been possible, in practice, to divide these very evenly.

In part this is due to the way different people naturally fall heir to patronage by one organization rather than another. Certain expected expenditures failed to materialize; for example, Soviet members were unable to attend meetings at Villefranche and Sydney, and international expenditure for the Sydney meeting earmarked for ICES was generously born by CSIRO. Also, working parties 1 and 4 have done their work by correspondence, thus saving the expense of meeting.

As things stand, UNESCO payments have slightly exceeded the quota ($4,302). In addition, UNESCO helped very considerably in meeting expenses of the Sydney symposium. SCOR also supported the symposium generously, and has spent most of its quota. ICES still has about $2000 in hand which will be held for future expenses of WG 13 in producing their reports.

Symposium on the Hydrodynamics of Plankton Samplers

The suggestion to hold this symposium was first proposed by David Tranter as a valuable way to provide the background knowledge necessary to the proper functioning of w.p. 3. It was logical to recommend that it be held at Sydney, where the right facilities were readily and freely available at the University of Sydney through the courtesy and co-operation of the Department of Aeronautical and Mechanical Engineering, and where all the prior organization could be done on the spot without additional expenditure.

The idea was strongly supported by w.p. 2 and by ICES, and thanks to help from SCOR and UNESCO it was possible to hold the symposium at Sydney in February 1966. There were 27 participants plus 7 observers from Australia, and 38 contributions were presented.

The Symposium started with two days practical testing at the University of Sydney. Here experimental work was carried out:

1) Using model nets in wind tunnels with visible smoke trails. Models of various meshes and length of cone were tested without and with clear perspex cases to represent encased nets such as the Gulf III; nets were held at different angles, and various degrees of artificial clogging were used.

2) Testing the w.p. 2 net in a larger wind tunnel. A hot wire anemometer was used to give a full picture of the flow and turbulence over the whole diameter of the net at various distances in front of it, and including the effects of the bridles and to ascertain the best position for the flowmeter.

3) Testing the Clarke-Bumpus net for flow and filtration coefficient using various meshes and lengths of cone and at various speeds in the water test tank.

The w.p. 2 net was also tested from a boat in Sydney Harbour to ascertain wire-angles and behaviour at sea.

Much of great value was learned at the Symposium and it is a pleasure to record appreciation of the very helpful co-operation of the staff of the University of Sydney, of the excellent organization by the convener David Tranter of CSIRO through the courtesy of Dr. Humphrey.

It was suggested that UNESCO publish in their monograph series a review based on the papers contributed to the Symposium and prepared in seven chapters (see w.p. 3 report). Some of the papers presented at the Symposium were being prepared for publication elsewhere, others were concerned with work not yet completed, and it was considered best to publish in this review form rather than publish the individual papers (as suggested in SCOR Proceedings, 1 (2): 50).

Implementation of Recommendations

Working Group 13 has essentially completed its studies on the standardization of zooplankton sampling. Reports of the individual working parties are attached to the present document. The final integrated report is now to be written.

No progress has been made towards the production of intercalibration factors between various methods as this is considered impracticable or would involve an undue amount of effort that could be better spent on more fruitful pursuits.

We have made recommendations for the adoption of a standardized net for biomass sampling, which we hope will be internationally adopted, and for the design of a net for sampling the larger zooplankton until a more satisfactory encased high speed sampler has been designed. This, too, we hope will be internationally adopted. ICES Plankton Committee at their 1965 meeting in Rome recommended "that as soon as the recommendations from the working parties on the Standardization of Zooplankton Methods organized by ICES/UNESCO/SCOR are available, the member countries of ICES should co-operate in comparative tests with the recommended gear." It is hoped that other countries and institutions throughout the world will also adopt these recommended gear, where necessary as an addition to their own specialized equipment.

We have also made recommendations for future work, some of which we consider can be left in their present form to stimulate relevant research by interested laboratories, but SCOR should help by publicising them. Other recommendations should be given special impetus. The most important of these is the recommendation to ask appropriate institutions which have the necessary facilities, to design the best possible encased high speed sampler to meet as far as practicable the points laid down by w.p. 3. Such an impetus could come either from the SCOR executive or from the convener of WG 13 with the backing of SCOR, UNESCO, and ICES.

SCOR could also, we believe, help by organizing the preparation of the list of facilities available for testing plankton nets (w.p. 3 recommendation 3). The recommendation of w.p. 3 (Rec. 4) concerning telemetry is closely linked with w.p. 2 (Rec. 2) and in this connection it is useful to state that at the October 1966 meeting of ICES a joint session of the Plankton and Hydrographic Committees will have as its main theme "The present use of recording and telemetering apparatus in plankton research and hydrography."

Working party 2 has recommended that "a small permanent international committee be set up to keep standardization of plankton sampling under review and to make such recommendations as may be advisable." I envisage this not as an unlimited prolongation of WG 13 but that SCOR should periodically set up a working group to look into this question. The choice of period for review would be decided by SCOR Executive, but I would expect something in the order of five years or more would be adequate, and the personnel would be chosen afresh each time. I believe this to be a sound recommendation as further research will undoubtedly lead to improvements in methods that will gradually make the present recommendations of WG 13 out of date.

WG 18 has suggested (SCOR Proceedings 1 (2): 88) that the terms of reference of WG 13 "be extended to include a guide for the presentation of biomass data and qualifying information to World Data Centres (and specialised centres, where such exist, for international expeditions)." They also recommend (page 89) that WG 13 (with other WGs) consider whether data on presence and abundance of the various taxa "can be submitted to W.D.Cs. in a breakdown and with the supplementary information as far as possible compatible with the data forms given in the 'Manual' of NODC, Washington."

These suggestions of WG 18 need special consideration in the question of the future of WG 13 as they came too late to be included in the topics discussed by the WG 13 working parties. The members of the four w.ps. were selected for their abilities to discuss the original terms of reference and it may well be that they should complete only their original duties. Rather than prolong the existence of WG 13, SCOR should consider the advisability of selecting new personnel for the new topic and to set up a new WG.

As Chairman of WG 13, I wish to express appreciation of the help given by SCOR and by UNESCO in the duplication and dispatch of letters and reports, and of the work and time given by the working party conveners and members with such generous enthusiasm.

James H. Fraser
Chairman WG 13

Report of Working Party 1 (Microzooplankton)

The activities of working party 1 have been conducted by correspondence. The progress made has thus been subject to the customary limitation of this approach, but the convener has had personal meetings with all but two of the members and believes this report is a fair statement of opinion.

1. Size range of organisms considered

Members of the w.p. have assumed that they are concerned with the animals ranging in size downwards from those with which w.p. 2 are concerned. Since no joint discussion has as yet taken place with w.p. 2 we have assumed arbitrarily for the present an upper size limit of about 200 μ. The size group dealt with thus includes all the smaller animal constituents of the plankton community variously described as nannoplankton, microplankton, etc., and includes everything from the smallest protozoa to the eggs and larvae of a wide range of organisms and the adults of many smaller forms such as the copepods.

2. Objective of investigations

These can be classified as follows:

2.1 Qualitative

2.1.1. Systematic
2.1.2. Distributional
2.1.3. Life histories
2.1.4. Community structure

2.2 Quantitative

2.2.1 Production
2.2.2 Biomass
2.2.3 Population dynamics
2.2.4 Physiological studies

3. Requirements for a standard method

There is a very pressing need to find out more about the composition of the microzooplankton and to find out how important they are in the general ecology of the sea. They are undoubtedly a very important link between the bacteria and phytoplankton and the zooplankton organisms and it is possible that a substantial part of the energy transfer is conducted by the microzooplankton. Qualitatively we need to acquire information about their systematics, their distribution in time and space and their community structure and trophic relationships. Quantitatively we need measurements of their biomass, information on their physiology, particularly of feeding, respiration and excretion, and information about their behaviour.

The main fields in which some unification of methods might help would seem to be in distributional and quantitative studies.

4. Existing methods

Field methods

Water bottle samples varying in size from 100 cc to 100 litres
Pump samples
In situ filtration with
- Membranes
- Micro-mesh nets
- Fine nets ($>$ 40 x 40 μ aperture)

Laboratory methods

Fixation and preservation
- Formalin
- Lugol
- Osmic acid, etc.

Concentration
- None
- Sedimentation
- Centrifugation
- Filtration (membrane, paper or mesh)

Subsampling

Counting
- Staining
- Haemacytometers
- Larger squared slides
- Inverted microscope

Biomass determination
- Dry weight
- Protein, Carbohydrate, etc.,
- Total carbon, phosphorus, nitrogen, etc.
- Electronic volume measurement
- Computation from microscopic size measurements

5. Recommended methods

Clearly there has been no time to conduct any experimental work and make comparisons of different methods and the following comments are thus merely the majority opinions of the working party.

Field sampling - The majority perference is for the use of water samples and it is generally felt that a 10 liter water bottle should give a significant sample of forms up to about 150 µ in size. The main disadvantage of water bottles, however, is that many samples must be taken to cover different depth ranges and for certain purposes water samples integrated over a depth range may be perferable (as used by Lohmann, Strickland and Parsons and others).

Some members of the w.p. expressed a preference for nets for organisms at the larger end of the size range (75-200 µ) and for the more sparsely distributed organisms but it is doubtful whether we should be concerned with the latter at the present state of work in this field. In particular nets have the advantage of eliminating the effects of marked vertical stratification but at the same time existing fine mesh nets are difficult to operate in bad weather, they clog easily and inevitably lose the smaller organisms. Probably the question of the use of nets would best be discussed further when the recommendations of w.p. 2 are available.

Laboratory methods - Little comment has been made on methods of fixation and preservation. Formalin perferably buffered with Hexamine is the most satisfactory general fixative but is of questionable value for use with calcareous forms. Rhode's iodine fixative is preferable for use with the naked forms but is still a poor substitute of the examination of living material. Clearly there is a great need for further research in this field and some liaison with phytoplankton workers dealing with the same problem would seem desirable.

The occasions on which no concentration of material for counting is required are the exception rather than the rule, and generally some technique is required to present the material in a suitable form for counting. Sedimentation appears to be the method most widely favoured. The reason for this is largely the uncertainty of other methods. Both evidence for and against the reliability of centrifugation has been produced and filtration, while adequate for larger forms, can not really be considered satisfactory as a quantitative technique for many of the smaller organisms as they are either disrupted or disappear optically.

Subsampling and counting can, for convenience, be considered together. Generally it is felt that to obtain an acceptable numerical estimate, one should count a sample of such size that some 30-40 organisms of the particular species of interest can be counted. It is thus inevitable that the sample size chosen must be varied inaccordance with the object of the count. The working group is in favour of using a mechanical form of subsampling (as, for example, by means of a Stempel pipette) to make this possible. There seems to be sufficient evidence to cast doubt on the reliability of making counts on only part of the area of a squared slide and counting only part of the area of a filter can be extremely misleading owing to the non-random distribution of material.

The actual technique of counting must inevitably depend on the size of organism being dealt with. The Sedgwick-Rafter cell is satisfactory for the larger organisms and Haemocytometers are adequate for the smallest but care must be taken to avoid counting say nannoplankton on a Sedgwick-Rafter cell about 1 mm. deep as many specimens can be overlooked. A useful halfway measure is a squared slide with a cell of coverslip thickness and having a capacity of about 0.5 ml. This has the advantage that moderately high power objectives can be used for examination. Inverted microscopes overcome some of these difficulties but have the disadvantages of making difficult detailed examination of an object from different aspects. Beyond aiming to make counts as quantitative as possible and avoiding these more obvious difficulties little else can be said with regard to counting.

The estimation of biomass is perhaps the field most calling for standardisation but at the present time no one method seems to offer the potential for widespread adoption. The basic difficulties of separating dead from living material, phytoplankton from zooplankton as yet seem to forestall any completely satisfactory approach and any method adopted would at best be a compromise. Similarly some uniformity of approach to the problem of production must be sought but again little progress has been made towards agreement and at best we can only recommend active investigation in this field.

Report of Working Party 2 (Small Zooplankton)

(NOTE: Not all members of w.p. 2 have had a chance to comment on this draft, which thus must be regarded as an interim report).

INTRODUCTION

A standard sampler of simple, practicable design is proposed as one of a set of four instruments for quantitative, comparative biomass studies of marine plankton in the upper 200 m. of water. The design of the net and other pertinent recommendations were agreed upon during a conference held between September 28-30, 1965 at the Station Zoologique at Villefranche, France, and it will be referred to as the "original version" of the WP 2 Net (for working party two) in this report. An improved version of this net will be described below and recommended for standardization. Because the net material has a mesh aperture width of 202 μ, the WP2 Net can be considered to sample the "microplankton" or the planktonic organisms in the size spectrum from 10 mm downward to a width of at least 200 μ. We realize that the retention of motile organisms by a mesh screen depends largely on their largest cross-section or width dimension. Thus, a copepod 600 or 700 μ in length, having a cross-section of 150μ, has a better chance of escaping than a spherical radiolarian of 210 μ diameter.

The WP2 Net (original version) has been tested in a wind tunnel using a hot wire anemometer and in a towing channel at the aeronautical and hydrodynamic laboratories of the University of Sydney in February, 1966 under the direction of Mr. David Tranter of CSIRO, Cronulla. Profiles of velocity and turbulence in front of the mouth opening were obtained with and without bridles. These measurements yielded data on flow patterns, filtration efficiencies at various towing velocities, and the optimum site for flowmeter placement.

In addition, the WP2 Net was field tested on several cruises in waters of widely variable plankton standing crops. The tests were conducted by Dr. Paul Smith of the U. S. Bureau of Commercial Fisheries in the eastern North Pacific during the spring of 1966. Using two telemetering flowmeters, one mounted outside and the other inside the mouth opening of the net, he was able to monitor the water flow through the net at various velocities. Ten-second readings were taken simultaneously for both meters every 30 seconds on an electronic event counter.

The field tests clearly demonstrate that the filtration efficiency of the WP 2 Net changes during towing due to clogging. From a limited munber of observations in oceanic waters of California, it was noted that the filtration efficiency of the WP 2 Net decreased from 94% efficiency at the start to 85% (level at which clogging is considered to begin) after a period of 4 minutes towing at 2 knots in very rich, neritic California Current waters. In relatively clear waters outside the California Current enrichment, 200 miles off Point Conception, the WP 2 Net began to clog (filtration efficiency dropping to 85%) after 16 minutes of towing at 1 1/2 knots.

In light of the field results, we can now suggest three changes in our preliminary recommendations. First, an increase of filtration ratio from 5:1 to 6:1 should add a margin of safety against clogging. Second, the shape of the net should have a cylindrical front section and a conical end section, each having a filtration ratio of 3:1, giving a filtration ratio for the total net of 6:1. The cylindrical portion will act as a self-cleaning section and has a superior sustained filtration efficiency (Smith, Counts and Clutter, 1965), while it simultaneously helps to shorten the length of the net without altering the filtration area. Third, the net should be towed in a vertical (rather than oblique) manner in the upper 200 m. of water, so as to reduce the towing period, and, hence, avoid the possibility of clogging. Since plankton-rich waters often occur over continental shelves where water depths are less than 200 m., the vertical tows are consequently limited to correspondingly shorter water columns in these regions.

SPECIFICATIONS OF WP 2 NET

Shape = cylindrical - conical. Length of cylindrical section is 95 cm; side length of conical section is 166 cm.

Mouth opening = 57 cm internal diameter, circular, maintained by a brass or galvanized iron ring made of metal rod 1.5 cm in diameter thickness. To give an area of 0.25 m^2, the diameter should be 56.4 cm. However, the 57 cm internal diameter is intended to make up for the thickness of canvass.

Mouth area = 0.25 m^2

Net material = Nylon Nytal 7P or similar net material*, basket weave, with mesh aperture width of 200 microns. Porosity (= ratio of mesh aperture area to total mesh area = mesh transparency) = 55%.

*A list of alternative materials and their manufacturers is being compiled by w.p.2.

Canvas attachment to ring = 10 cm width.

Canvas band for throttling line = 10 cm width (57 cm below upper canvas band).

Bridle (3) = 57 cm long each, attached to swivel.

Filtration ratio (ratio of mesh aperture area to mouth area) = 6:1

Filtration efficiency = 0.94 (94%)

Lead weight = 25 kg (40 kg or heavier when wire angle tends to exceed 25°).

Flowmeter = TSK or equivalent, to be attached from three points on mouth ring and centered at 14.25 cm from rim.

Cod-end = a) bucket with window of same mesh as net (7.5 cm diameter; volume of 150-200 cc) for biomass or taxonomic purposes. PVC (polyvinylchloride) or light brass.

b) bucket with window of same mesh as net (7.5 cm diameter; volume of 500 cc) for living plankton catches.

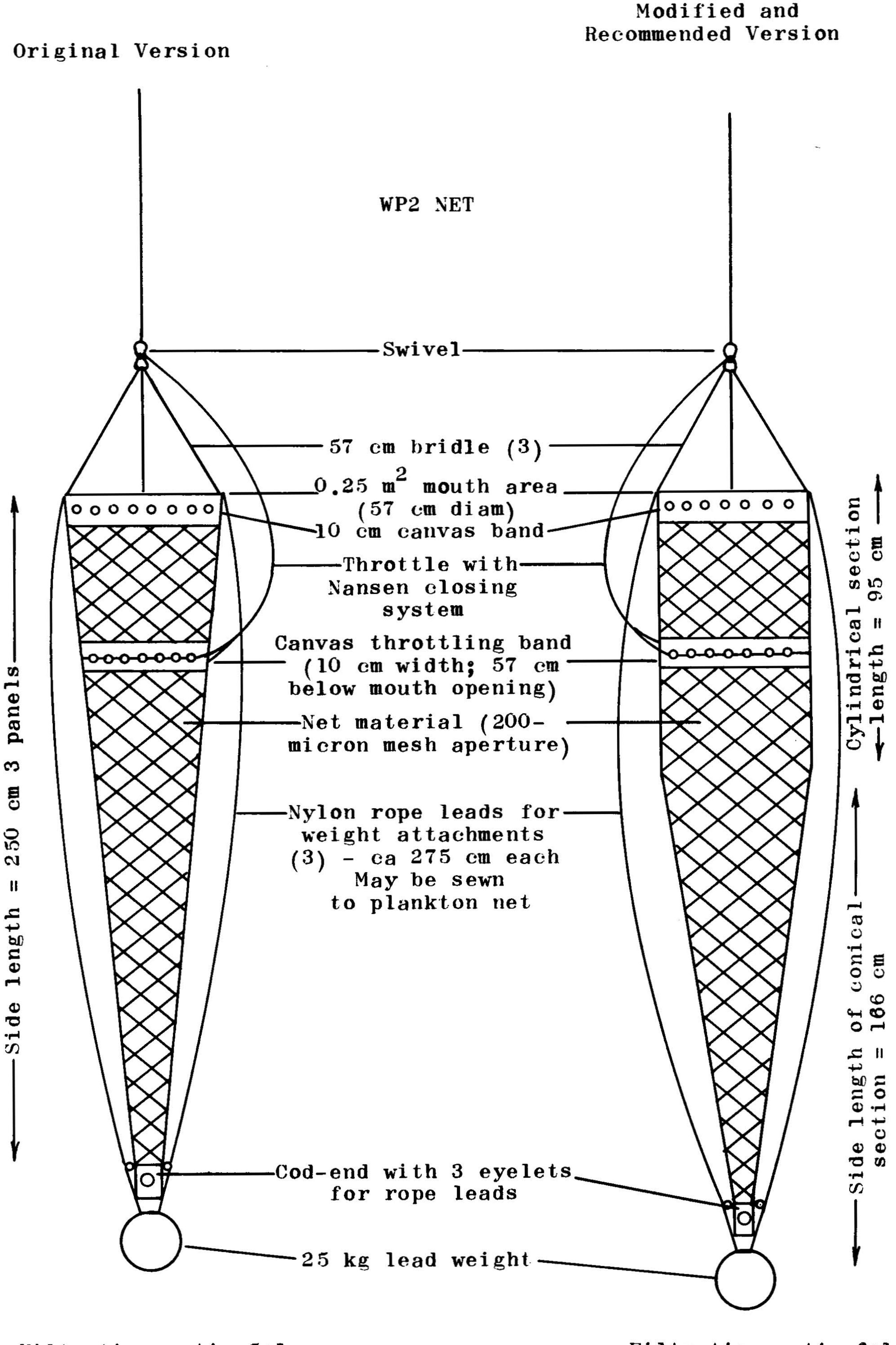
c) bag of same mesh as net (for tows in very rich waters).

Iron ring = 57 cm internal diameter of ring; 1.5 cm diameter thickness; with three eyelets, 120° apart, for bridles and rope lead attachments.

Cod-end arrangements

The hydrodynamics of a conical net are such that precise details of the cod-end arrangement are not of great importance and laboratories can use the method they find most suitable for the conditions in which they operate. If there are no special perferences we recommend:

a) A bucket, with one or more windows of a metal gauze of the same mesh as the net. Where the catch is to be used for research with living material, a reasonably large volume of water - 500 cc or more - below the windows is desirable; where it is to be preserved for biomass or taxonomic purposes, a smaller bucket is preferable, and we recommend a diameter of 7.5 cm and a volume of 150-200 cc. Buckets should be made of PVC (polyvinylchloride) or brass of light construction.


Original Version
Modified and
Recommended Version
WP2 NET
Swivel
57 cm bridle (3)
0.25 m² mouth area
(57 cm diam)
10 cm canvas band
Throttle with
Nansen closing
system
Canvas throttling band
(10 cm width; 57 cm
below mouth opening)
Net material (200-
micron mesh aperture)
Nylon rope leads for
weight attachments
(3) - ca 275 cm each
May be sewn
to plankton net
Cod-end with 3 eyelets
for rope leads
25 kg lead weight
Side length = 250 cm 3 panels
Cylindrical section
length = 95 cm
Side length of conical
section = 166 cm
Filtration ratio 5:1
Filtration ratio 6:1

b) Where plankton is rich, and is not required living, a detachable cod-end bag of the same mesh as the net is more convenient to handle than a bucket.

The end of the net should fit flush into the cod-end so that there is no pocket in which plankton can collect. The bucket, or bag, can be attached by a simple band, screw, or bayonet fitting. The ring supporting the cod-end should be fitted with eyelets to which cords can be attached so that the weight of the cod-end is not taken directly by the filtering material of the net.

Flowmeter

The flowmeter should be of the Tsurumi-Keiki Kosakusho Co. (Japan) type or of any other equally effective or robust manufacture. It should have a stop to prevent reversing and another stop to prevent turning in air, although they are not so necessary with a depth-flowmeter. Recent hydrodynamic tests in the U.S.A. have indicated that the optimum placement of the flowmeter for the most representative measurement of the flow of water into a net should be half-way between the center and the rim of the sampler's mouth. Accordingly, we recommend that the flowmeter be centered at 14.25 cm from the rim. Whenever possible a second flowmeter should be placed an equivalent distance outside the rim. The ratio of the inner to outer flowmeter readings will yield the integrated filtration efficiency for each tow. A filtration efficiency less than 85% would indicate that clogging has occurred and the tow should not be regarded as quantitatively accurate.

Flowmeters should be calibrated at least every three months in a swimming pool; these calibrations may be checked at sea under very calm conditions by hoisting the meter in a vertical manner at velocities of about 90 m/min.

Net material and mesh size

Nylon has several advantages over silk as netting material. The former is more durable and does not shrink when wet as silk, although it is subject to considerable deformation in shape under stresses. We recommend nylon monofilament, basket-weave netting material (e.g. nylon Nytal 7 P and the polyester Estal Mono P.E.) because of their good resistance against friction and moderate deformation. The mesh should be entirely basket-weave rather than alternate basket and twist weave, which are deformed more easily. The mesh opening should have an aperture of 200 microns by 200 microns when the net is wet and used.

SAMPLING METHODS

Depth of towing, towing speed, duration and time of towing

In view of the field tests results which have shown that clogging of the Villefranche net can occur rapidly in rich, neritic waters, we recommend that this net be towed in a vertical manner in the upper 200 m. of water. In shallower waters, the vertical tows should be taken from just above the sea bottom to the surface.

In order that the tows be taken in as vertical a manner as possible, a lead weight of 25 kg is recommended when sea conditions are relatively calm. When the ship drifts rapidly, a lead weight of 40 kg or more may be required to keep the wire angle below our suggested maximum of 25°.

The net may be lowered at 60 m/min. and raised at 45 m/min. Since the Villefranche net will be lowered with weight and cod-end first and the mouth opening is not presented to the direction of water flow, it is assumed that no plankton is collected in the net during lowering. The plankton is collected during hoisting, which should give a towing duration of about 4 1/2 minutes for the 200 m. water column at a raising speed of 45 m/min.

In order to minimize the influence of diurnal migration on biomass studies, it is recommended that sampling be done as far as possible during the 3-hour period after sunrise and after sunset. This does not preclude sampling at other times, but these may not be so strictly comparable in biomass studies.

OPENING-AND-CLOSING SAMPLES

For vertical distributional studies, sampling should preferably be carried out in accordance to the water structure.

When hydrographic information is not available, the following water columns should be sampled by vertical tows while the boat is drifting:

0 - 200 m
200 - 500 m
500 - 1000 m
1000 - 1500 m
1500 - 2000 m
2000 - 3000 m

Two alternative ways of obtaining such samples are by:

a) Modified Nansen method. A throttling line can be attached to the lower canvas band of our standard sampler and linked to the release mechanism (Discovery Rept., vol. 1, pp. 151-222, 1929), and a weight shou . be used below the cod-end for vertical series.

b) Multiple opening-and-closing plankton sampler (Bé, Deep-Sea Research, vol. 9, 1962) for obtaining larger quantities of plankton by oblique towing. Three quantitative samples from three predetermined depth ranges can be collected during a single lowering.

PRESERVATION OF PLANKTON SAMPLES

We recommend the use of formaldehyde as a fixative and preservative. The saturated solution known as "Concentrated formalin" contains 38-40% formaldehyde. One part of concentrated formalin should be added to nine parts of sea water including the plankton sample, and this should be done as soon as possible after collection. As a precaution against the dissolution of calcareous plankton in highly concentrated samples, the strength of buffered formalin should be doubled in such samples.

It is essential that the formalin be neutralized before use by addition of either borax (sodium tetraborate), marble chips or another suitable buffering agent. Marble chips have the advantage of dissolving gradually in the same measure as acid is prod ced in the formaldehyde solution. Checking on the presence of undissolved marble chips or checking with pH indicator paper are two simple ways of ascertaining whether the solution is still neutral. Hexamine, apart from being expensive, has the disadvantage of easily crystallizing around organisms when the sample is subject to even a slight amount of evaporation, e.g. while being examined under the microscope in an open dish.

Commercial formalin is often contaminated with iron componds which, on neutralization, produce a brown precipitate of iron hydroxide. This precipitate spoils the sample by sticking to the surface of the organisms and obliterating their finer structures. It is, therefore, recommended that analytical grade formalin be used and stored in glass or plastic containers.

GENERAL CARE AND MAINTENANCE

Nets should be hosed before being brought aboard to wash plankton into the cod-end.

Nets that show signs of clogging can be washed by towing the net without a cod-end or when clogging is more severe the nets should be washed in detergent. Nets should be washed in fresh water after each cruise before storage.

Nets should not be left in the sun for prolonged periods nor left where there is risk of unnecessary damage by friction wear and tear.

Flowmeters should be washed with fresh water after use.

DETERMINATION OF BIOMASS

If possible, two identical plankton samples should be taken simultaneously -- one for biomass study, the other for taxonomic use. If not, a single sample is taken and split in half by a method still to be recommended.

The first sample (or sub-sample, in case of splitting) is preserved in formalin for taxonomic and counting purposes.

The second sample (or sub-sample) is deep-frozen, then dried according to the technique recommended by Lovegrove (In: H. Barnes, edit., "Some Contemporary Studies in Marine Science", pp. 429-267, 1966) and finally weighed for dry-weight measurement. The organic matter will be determined on this sample by loss upon ingition. The results (dry weight and organic matter) are expressed in mg/m^3.

Tests should be made to determine the effect of formalin fixation upon dry weight and organic matter content. If formalin preservation should be found to have no undesirable effect, it could be used as a routine technique and samples preserved in formalin could be split in the laboratory ashore, one-half to be used for dry-weight and organic matter measurements.

RECOMMENDATIONS FOR FUTURE WORK

1. Hydrodynamic and field testing of our standard samplers and to acquire basic knowledge of net design and high-speed samplers in relation to water flow, plankton behavior, concentration, and patchiness. Research on materials (e.g. net material).

2. Development of a telemetering depth-flowmeter, electrically opening-closing samplers, a shipboard flowmeter calibration tank, and a flowmeter with digital dial operated magnetically.

3. Research toward elimination of bridle and other obstructions in front of net -- as related to plankton avoidance of nets.

4. To study effect of ship's shock wave and noise on plankton sampling.

5. Compile and keep up-to-date a bibliography on sampling gear, sampling methods and processing plankton samples.

MEASURING TOTAL PLANKTON BIOMASS

Assuming that agreement can be reached on standardization of a set of plankton samplers of different dimensions and mesh apertures, we are then faced with the problem of integrating our various catches to obtain "total biomass".

The plankton catches from these various samplers will overlap with one another with respect to kind and size. A net can theoretically select the lower size limit of organisms, but can not discriminate for the upper size limit which depends largely on the kind of plankton present, the towing speed, and the area of the mouth of the net.

A scheme is proposed for eliminating the overlapping upper size fractions from each haul that are duplicated in the samples from the coarser-meshed nets by means of a series of graded sieves whose meshes are equivalent to those of the standard samplers used. Each plankton catch is filtered through a separatory column and is divided into as many classes as there are standard samplers. For a series of four standard samplers the theoretical total would be 16 fractions as shown in the following arbitrary model:

Separatory column with graded sieves	water bottle (o μ)	202 μ Net	1000 μ Net	5000 μ Net
	A^3	B^2	C^1	**D**
5000 μ	A^2	B^1	**C**	D^{-1}
1000 μ	A^1	**B**	C^{-2}	D^{-2}
200 μ	**A**	B^{-1}	C^{-3}	D^{-3}
0 μ				

The fractions A, B, C and D theoretically contain the plankton organisms that are most ideally collected by each of the standard samplers and their sum (after correcting for the volumes of water filtered by each sampler) should give us the best estimate of "total plankton biomass".

This scheme was proposed by A. Bé at the "Symposium on Hydrodynamics of Plankton Samplers" CSIRO, Cronulla, Australia -- February 14 - 16, 1966 and its validity as well as "sampling suitability" of each of the recommended plankton samplers need to be investigated more thoroughly.

Report of Meeting of Working Party 3 (Large Zooplankton)

February 1966, Cronulla, Australia

A. SYMPOSIUM

A symposium on plankton samplers preceded the meeting of the working party. The theme was 'Hydrodynamics of Plankton Samplers' but the papers and discussion covered a wider range of relevant topics. There were 38 contributions presented or read. The Symposium included demonstrations and tests in the aeronautical and hydrodynamic laboratories of the University of Sydney. The tests were made on a net of mesh aperture 200 μ, recommended by working party 2. There were 27 participants and 8 (Australian) observers. Eight papers were read in absentia.

B. REVIEW OF PLANKTON SAMPLING METHODOLOGY

It was agreed that a review of plankton sampling methodology should be prepared to be published by UNESCO, the work to include the bibliography already prepared and an appendix on terminology. The publication of approximately 100 pages will be prepared by the following contributors:

1. History of sampling - Fraser
2. Gauzes - Heron
3. Flow patterns - Tranter and Smith
4. Loss through the meshes - Hempel and Vannucci
5. Avoidance - Anraku and Clutter
6. Field techniques - Aron and Gehringer
7. Design of sampling - Cassie

The contributions are to be in the hands of the Editor (CSIRO Cronulla) by September 30.

C. RECOMMENDATIONS

Upon the assumption that the goal in quantitative plankton sampling is to take a representative sample of a particular size group in the plankton spectrum, the following recommendations were made:

1. That an encased sampler with a net of mesh aperture 1 mm be designed to sample the larger zooplankton, its specifications to be dictated by the following considerations:

(i) That the sampler should filter at a rate of not less than 20 m^3/ minute.

In addition it would be useful to measure also the speed of the sampler in situ.

(ii) That the flow through the sampler should be metered.

(iii) That the mesh velocity (exit velocity from the meshes) should not exceed 10 cm/sec.

(iv) That there should be no obstructions ahead of the sampler mouth.

(v) That the sampler should tow in a stable manner and the drag be as low as possible.

(vi) That the sampler be fitted with a depressor capable of taking it to a depth of at least 200 m at a speed of at least 6 kt.

(vii) That the sampler should be fitted with an acoustically operated opening closing action and a depth sensor telemetering to the surface, and that space should be left available for further modules if required.

(viii) That the sampler be robust and non-corrodable.

(ix) That the net and the catch be easy to remove and the flowmeter easy to read.

(x) That the sampler be as small as possible consistent with the above requirements.

2. That a simple unencased net with the following specifications would serve as the best interim sampler for the larger zooplankton.

(i) Mouth of 1 m^2 consisting of a 3/4" (approx. 2 cm), outside diameter, ring of galvanized tubing.

(ii) Net with a cylindrical forward part, 57 cm long, and a conical after part, 200 cm long, strengthened with 6 longitudinal tapes not more than 2 cm wide.

(iii) Gauze of monofilament nylon of mesh aperture 1 mm.

(iv) Throat of dacron sailcloth not more than 12 cm wide to be wrapped around the ring. Notches at 3 equidistant points to take the bridle lugs.

(v) Bridle of 3 legs equal in length to the mouth diameter.

(vi) Flowmeter to be placed 25 cm inside the ring. It would be useful also to have a second flowmeter outside the net to measure the speed of the net through the water.

(vii) Bucket to be light in weight.

(viii) Towing speed to be 2 - 3 kt.

(ix) Sinker to be either a dead weight of approximately 40 kg or an equally efficient depressor.

(x) It is considered that there is no satisfactory way of closing this net.

3. That a list be made of the facilities available throughout the world, suitable for testing plankton nets, e.g. test tanks, circulating water channels, wind tunnels etc.

4. That further research be undertaken to find the best practical means of telemetering information from the sampler to the surface and telemetering control signals from the surface to the sampler.

D. J. Tranter
Convener

NOTE: The points made by w.p. 3 for consideration in the design of a sampler are suggested as ideals. In practice these ideals cannot be achieved without making a sampler larger than can be conveniently handled so that a sacrifice of ideals will be necessary. WG 13 will discuss this by correspondence so that some indication of where and how far the sacrifices may be made can be given to the designers.

J.H.F.

Report of Working Party 4 (Micronekton)

INTRODUCTION

The development of sampling methods for capturing the larger plankton and smaller nekton in a quantitative manner is in its infancy and although some progress has been made little gear has been designed specifically for this purpose. A plea for standardisation might thus appear inopportune at this stage. However, it is apparent that one promising design is in widespread use and already a large body of catch data is available. Our recommendations are therefore directed towards introducing some degree of acceptable uniformity in the construction, dimensions, and operation of this sampling device, so that valid comparisons can be made with data from different sources. It is also hoped that these recommendations will serve as a guide to those initiating new sampling programmes.

1. CATEGORY OF ANIMALS TO BE CONSIDERED

We have considered methods by which a representative sample can be taken of pelagic organisms lying within the arbitrary size range 2.0 to 10.0 cms. No single term as yet defined (macrozooplankton, micronekton, forage organisms) is strictly applicable under this definition but if a term is to be used the "micronekton" is recommended since it implies less of an overlap with the planktonic animals considered by w.p. 3.

Within the size range 2.0 to 10.0 cms large decapods (Sergestiids, Penaeids, Oplophorids), fish larvae, small adult fish, small cephalopods and large euphausiids will predominate. Gelatinous organisms and animals lying outside the size range will occur in the catches and these must be considered separately from the main sample.

2. TYPE OF SAMPLER

Of the methods available it is apparent that one type of sampler - the Isaacs-Kidd midwater trawl (IKMT) - is in widespread use. Its advantages include a large mouth opening relatively free of bridles and other obstructions, it is self-depressing, it is versatile and easier to fish than conventional conical nets of comparable mouth area, and it can be towed at great depth and at high speed. Of its disadvantages perhaps the most important is that we have no measure of the volume of water filtered so that it is only semi-quantitative. Nevertheless with the realisation that it falls short of the requirements of an ideal sampler we have no hesitation, at this state in the development of sampling devices, in recommending the IKMT as a basis for standardisation.

2.1 Size of Sampler

There are advantages in having as large a net as possible but shipboard facilities vary to such an extent that absolute size could be a limiting factor. For this reason we recommend two sizes: 6 foot and 10 foot, -- this dimension referring to the spread of the depressor.

2.2 Overall physical dimensions and method of rigging

Specifications of net, depressor, spreader bar, etc. and method of rigging can be found in the following reports:

6': Aron, W., 1962. Some aspects of sampling the macroplankton. Rapp. Cons. Expl. Mer, 153: 29-38.

10': Isaacs, J. D. and L. W. Kidd, 1953. Isaacs-Kidd midwater trawl. Scripps Institution of Oceanography S.I.O. Ref. 53-3: 1-18.

2.3 Mesh-size

0.5" stretched mesh nylon, preferably knotless. It is usual to support this finer mesh within a 2 1/2" knot to knot outer net. We strongly deprecate the use of nets having mixed or graded meshes.

2.4 Cod-end

There is evidence that the catch is maintained in better condition if the trawl terminates in a metal or plastic container preceded by a short fine mesh section, of 3 mm mesh aperture. It must be emphasized that thes finer mesh will superimpose on the main catch a fraction of animals smaller than 2.0 cm. which must be excluded in the final treatment of the sample.

3. TYPE OF HAUL

Whether an oblique or horizontal tow is used depends largely upon the nature of the problem under investigation since they meet different requirements. Oblique tows are best suited to small or large scale regional surveys aimed at delimiting distributional patterns, of species or biomass, within the top 200 m. Horizontal tows are applicable to studies of variation in vertical distribution, sampling with respect to thermoclines and scattering layers, and sampling more sparsely distributed organisms.

In the circumstances we can only recommend procedures that should be followed in making these two quite different types of haul. The importance of adopting and maintaining rigorously a standard sampling procedure cannot be overemphasized since only in this way can valid comparisons be made between a series of hauls.

3.1 Oblique haul

3.1.1 Pay out at 40 meters/min.

3.1.2 Haul in at 40 meters/min.

3.1.3 Ship's speed throughout tow: 3 knots (except for shooting and recovering the trawl).

3.1.4 Depth of haul: 200 - 0 m.

3.2 Horizontal tow

3.2.1 Pay out at 40 meters/min.

3.2.2 Haul in at 60 meters/min.

3.2.3 Ship's speed 3 knots (see 3.1.3)

3.2.4 Duration of tow 2 hours at fishing depth

3.2.5 Depth of tow at 50 m intervals down to 1000 m.
at 250 m intervals below 1000 m.

4. SHIP'S SPEED

Recent work has shown that the IKMT is extremely sensitive to variations in ship's speed. It is therefore important to maintain a constant speed throughout the tow if the depth of fishing is to remain uniform.

5. DEPTH OF FISHING

The use of a depth-time recorder or a depth telemeter is a prerequisite of midwater sampling since an accurate knowledge of the depth of fishing is essential to a proper interpretation of the data.

6. DISTANCE OF TOW

Methods of metering the flow of water through the IKMT have yet to be evolved. However as a first approximation of volume filtered, estimates can be made based on mouth area X distance towed through the water (which equals the length of the sampled column). A flowmeter mounted in the cod-end can be used to give a relative measure of the length of water column sampled.

7. TREATMENT OF CATCH

Animals falling outside the size range 2 - 10 cm should be treated separately from the main catch. Consider catch by taxa: measure displacement volume or net weight and enumerate. If possible pool taxa and consider by tropic level.

CONCLUDING REMARKS

In concluding this report it must be emphasized that while our recommendations refer to existing gear we do not wish to imply that the methods available are adequate. On the contrary, it is clear that they leave much to be desired and progress in the quantitative study of these more active pelagic organisms will largely depend upon technical improvements in the IKMT and in the evolution of new devices and approaches. This work must be encouraged, it is long overdue, and we strongly urge that support be given to the following:

(a) Field and laboratory studies of the hydrodynamics and catching efficiency of the IKMT. An objective appraisal of this sampling device can only be made as a result of such work.

(b) Studies to evaluate the effectiveness of existing opening-closing systems and if necessary to design and produce new ones.

(c) The design and production of reliable, accurate depth-telemeters, operative to 5000 m.

(d) The design of devices that can be incorporated in the sampler to telemeter or record environmental parameters (light, temperature, etc.).