6. Recommended Procedure for Measuring Amount of Zooplankton Biomass


It is recommended that amount of zooplankton be reported as dry organic substances per unit volume of water sampled. Determinations of amount of nitrogen and carbon are also recommended.

Dry organic substances: The following recommendations may be carried out either on a separate plankton sample taken specifically for this purpose, or on a fraction of a multipurpose zooplankton collection when only one haul is made at a station. In either case the sample is drained into a filtering sock and the displacement volume determined.

When only one sample is taken at a station, a representative fraction of the sample is obtained by appropriate techniques (fractionating preferred, see McEwen et al., 1954). It need not exceed 5 ml. in volume. Both the volume of the fraction and the remnant are again determined with as much precision as is possible on shipboard. The remnant is then immediately preserved. (It is important that the above work be done as expeditiously as possible in order to prevent deterioration of material before preservation.) The fraction (or the separate sample) is placed in a fine-meshed (bolting silk or nylon) bag, dipped several times in distilled water in order to remove most of the salt present in the interstitial sea water surrounding the plankton, placed in a plastic envelope and frozen for processing ashore.

In the laboratory the sample is thawed, sufficient measured volume of distilled water added to bring the liquid and sample above the blades of an homogenizer: the sample is homogenized, and aliquots taken for drying for dry weight determination, carbon and nitrogen analyses (or nitrogen analysis may be done on an aliquot directly, before drying). Drying of the aliquots to uniform weights should be done at a temperature no higher than 60°C. (see in this regard, Lovegrove, 1962).

One portion is used for determining the ash content. The organic material is removed by oven heating at 500°C. Care must be taken to insure that the temperature does not exceed 550°C., and that no black flakes (organic material) remains. The amount of organic substances in the aliquot is the difference in weight of the sample before and after ashing.

Organic substance is reported in micrograms of dry 0.5 to unit volume of water sampled (preferably 1.0 m³). The amount of carbon and nitrogen are also reported in micrograms per unit volume of water sampled.

In the discussion entitled, "Zooplankton: Determination of Amount (Biomass)", other tentative recommendations are made, especially with regard to depth sampling.
ZOOPLAGKTON: DETERMINATION OF AMOUNT (BIOMASS)

Prepared by the NAS/NRC Committee on Oceanography Working Group on Standardization and Intercalibration of Biological Measurements and Sampling Methods - March 28, 1963

Zooplankton is a term of convenience applied to a diverse assemblage of pelagic animals. The animals range in size from nannoplankters that are only a few micra in length to macroplankters of the size of the large colonial siphonophores, which may be meters in length. A quality zooplankton animals have in common is a relative passivity as compared to nekton. Most zooplankters possess some means of movement, and some groups such as copepods and euphausiids perform extensive vertical movements. Most animals that make up the nekton (fish, crustacea, squid) have younger stages that are planktonic. Hence, within the zooplankton community there is a continuous spectrum as regards motility, from almost complete passivity to fairly rapid swimmers.

In sharp contrast to phytoplanktons, which are limited essentially to the upper euphotic zone, zooplankton organisms occupy an extensive depth range. Another characteristic of zooplankters is a tendency to aggregate—to occur in clusters or swarms—and thus not be randomly distributed either within their depth ranges or their distributional ranges.

Zooplankters are sampled either by hauling fine-meshed nets through the water or by pumping water for filtering on shipboard. Because of their diversity in size, in kind, in distribution and in motility, there is no collecting device that will sample the complete spectrum of zooplankton. All of the collecting devices presently in use (or even planned) are selective. If designed to collect the smaller-sized plankters quantitatively, the gear is not effective in collecting the larger, less numerous, more agile plankters. If designed to collect the latter, the gear must be capable of being hauled rapidly and straining considerable quantities of water—characteristics that require relatively course-meshed netting. Most plankton gear now in use take a swath from the middle of the size spectrum, allowing the escape of smaller organisms through the mesh apertures on the one hand, and the avoidance of the gear by the more agile, larger plankters on the other.

In order to sample the complete spectrum of plankton animals quantitatively, it will be necessary to employ several kinds of collecting devices. How many kinds, we do not know. Planktologists have shied away from the problem, because of its complexity. However, intelligent planning of sampling programs requires the knowledge, hence the problem cannot be avoided indefinitely.

For the present we will have to be satisfied with a partial measure of zooplankton biomass. It is hoped that comparable "relative" measures of biomass can be obtained from the world oceans by standardizing both gear and techniques. Admittedly, there are diverse opinions about what kind of collecting device should be accepted as the standard. Judgements in this area tend to be subjective, rather than based on objective, experimental criteria. This results from a very sufficient reason: such information is largely lacking. One
purpose of this report is to point out some of these problem areas and, in the concluding section, to outline research needed in order to understand principles on which to develop gear with desired characteristics. The ultimate objective is to obtain the minimum "set" of sampling gear with which to completely sample the planktonic biomass.

Characteristics of a "Standard" net

The primary reason at present for having a standard net, as we conceive it, is to obtain comparable measures of zooplankton biomass. As emphasized above, only a portion of the spectrum of zooplankton organisms in the water column being sampled can be collected quantitatively by a single plankton net. The standard net, consequently, should sample quantitatively as wide a part of this spectrum as is possible by a single net. In order to accomplish this, the net should be constructed of as fine mesh as is compatible with good straining characteristics. Clogging is a primary consideration: clogging slows the filtering rate and, if severe, may even prevent straining during a portion of a haul. A clogged net is not a quantitative one. Hence, the mesh-aperture size should not be so small as to clog under usual sampling conditions (even course-meshed nets will clog under some conditions).

On the other hand the net should be able to catch many of the more active plankters. This requires a large mouth opening and ready acceptance of water by the net. The latter is controlled in part, by the ratio of effective straining surface to the area at the mouth of the net, in part by mesh-aperture size, and in part by the speed of hauling. A faster hauled net is more effective in capturing agile plankters than a net towed slowly.

In order to determine the characteristics of an "ideal" standard net (i.e. one that would quantitatively sample as wide a part of the planktonic biomass as is possible with a single collecting device) considerable research must be done. When developed, the standard net should be "calibrated" in relation to the total planktonic biomass in order to determine what fraction is being retained and how variable this fraction is from place to place and season to season.

Until such information is available, any "standard" net accepted must be recognized as an expedient. Decisions as to net size and shape, mouth area, kind and size of netting material, etc., will be based, in part, on arbitrary criteria. An outline of "studies to improve sampling gear" is given later in this report.

Mesh-aperture size: An aperture size of 0.33 mm has been agreed upon for the Indian Ocean and tropical Atlantic programs. No member of our panel has had experience with nets constructed of this particular mesh-aperture size. We can anticipate some problem with clogging, but concur in its use until more basic information is available.

Mouth opening: It is known that nets with rather large mouth openings are more effective in capturing the larger, agile plankters than are smaller nets. The mouth opening chosen for the standard Indian Ocean net (113 cm in diameter, 1.0 m² in area) is within the desirable size range, even though slightly larger
than most nets used by American investigators in the past. The commonest size of mouth opening of nets used extensively has been 100 cms in diameter (0.785 m² in area). Admittedly, there is a convenience in having a net with a mouth area of exactly 1.0 m².

Net size: The question was raised as to the optimal ratio of straining surface (aperture area) to mouth area for nets designed to be hauled vertically, or obliquely, at slow vessel speeds (2-0 knots or less). If experimentally determined information on this point is now available, the panel does not know of its existence. The information is needed to insure good straining characteristics, as well as economical construction.

Measurement of the quantity of water strained: All nets employed should be metered. A reliable and inexpensive flow meter is the Japanese TSK (or equivalent). Precautions should be taken to insure accurate reading of the meter dials. The meter should always be read by two persons, for example, as the dial type meter is somewhat difficult to read accurately. Perhaps a Veeder Type counter could be adapted to this meter, the latter gives a direct reading. Flow meters should be calibrated frequently—before and after cruises of less than a month in duration and during longer cruises as well. When vertical hauls are being taken, the flow meter should automatically lock so that it does not turn in reverse during the lowering.

Depth recording: It is recommended that a depth trace be obtained for each haul. This can be accomplished by using a depth-flow meter, or alternatively by using two separate units, one a depth recorder (preferably not a maximum depth recorder), the other a flow meter. There are a number of depth recorders being developed or already available.

The ultimate aim as regards depth-flow instrumentation is the development of a telemetering unit that would record the performance of the flow meter and the depth of the net continuously on shipboard during a haul. Such a unit would make it possible to determine when clogging impeded flow into the net. It also would be invaluable when conducting tests of the performance characteristics of nets of different mesh sizes.

Depth strata to be sampled: It is advisable for the sake of comparability of results in the investigations of zooplankton stratification that the depth zones be standardized. We wish to recommend that at least the following depth strata be sampled:

a) For all stations: 0-200 m with an open or non-closing net

b) For selected stations: 0-200 m, 200-500 m, and 500-1000 m tows with opening and closing nets. Individual investigators may wish to further subdivide these strata.
a) 0-200 m tows at all stations:

The 0-200 m sample may be obtained either by vertical or oblique tows. If sampling is done vertically, an open one-meter net should be used, which is lowered cod-end first and retrieved at a uniform rate of 1 m/sec (2 knots). The wire angle should not depart significantly from the vertical during a tow. If sampling is to be done obliquely, the open net should be lowered at a rate of approximately 50 m/min (if lowered too rapidly the wire may become entangled), and retrieved at a uniform rate of 10 or 20 m/min depending upon size of sample desired. The vessel speed during an oblique haul should be adjusted so as to maintain a uniform angle of the towing cable. An actual depth of 200 m can be reached by paying out approximately 283 m of wire at a wire angle of 45°.

An essential requirement of quantitative sampling of the 0-200 m zone should be uniform towing, in terms of time duration, through the integrated depth levels. In other words, there should be no sampling bias of any given strata depth level.

Assuming 75% acceptance of the water by the net, the vertical 0-200 m haul would strain approximately 150 m³.Depending on the vessel's speed, the oblique one-meter net haul over the same depth range would filter roughly 600-800 m³ of water if the retrieval rate were 20 m/min and double this amount if the retrieval rate were decreased to 10 m/min.

b) Opening-and-closing tows of 0-200 m, 200-500 m, and 500-1000 m for selected stations

Studies of vertical distribution of zooplankton for determining standing crop or species composition require the use of plankton samplers with opening-and-closing release mechanisms. Such instruments need to have large mouth openings, flow meters, depth-time recorders, and reliable and positive closures. Three basic types of net-closing design now in use or in the experimental stage are: a) closure by throttling the anterior or midsection of the net—e.g. the modified Nansen net (Currie and Foxton, 1957) and the Leavitt net; b) closure at the mouth opening of the net(s)—Clarke-Bumpus sampler and multiple Bé sampler; and c) closure at the tail section of the net by multiple cod-ends—e.g. modified Scripps-Narragansett Gulf III sampler.

In the first category, both the modified Nansen and Leavitt nets have been extensively used. The former is operated in a vertical manner and, therefore, only one net, attached at the end of the wire, can be used during a single haul.

The Leavitt net can be towed obliquely. It is lowered in closed position, opened at the desired depth level by a messenger, towed to the upper depth level and closed again by throttling the mid-section of the net. This requires the use of a second messenger. If several depth zones are to be sampled simultaneously, a series of Leavitt nets need to be attached to the same wire cable. Closing gear using systems of throttling ropes when used in multiples has not proven entirely reliable and failure of the lower sets of nets has often necessitated the repetition of hauls in these deeper strata.
In the second category, closure is effected at the mouth, giving a more positive closing and a minimum risk of loss of catch or contamination. The Clarke-Bumpus sampler is widely used today. Opening and closing of the mouth by a hinged lid require the use of messengers. The original design had a mouth diameter of 5 inches. Paquette and Frolander (1961) and Yentsch, Grice and Hart (1962) have subsequently enlarged the diameters to 10 and 12 inches, respectively. It is felt, however, that large diameters of about one meter or more are necessary for adequate sampling of bathypelagic faunas.

The complete depth range of 1000 m could be sampled by a multiple Beams sampler, in which three 70 cm x 70 cm nets, incorporated in a single frame, successively open and close at the predetermined depth levels of 1000 m, 500 m, and 200 m. The device enables continuous sampling of three successive depth intervals during a single lowering, while the pressure-actuated release mechanism that triggers the opening-and-closing action of each net at preselected depths eliminates the need for messengers.

A third system is that of closure in front of the collecting bucket. Two or more compartments may be incorporated in the bucket to enable multiple samplings through successive depth layers during a single lowering (e.g. Motoda's vertical successive sampler (1953); and the Scripps-Narragansett modification of the Gulf III high-speed sampler). Although these systems have not been tested thoroughly, a basic problem that should be taken into consideration is the likelihood of contamination if flushing of the common net is incomplete.

Prime considerations in choosing or designing closing net gear are reliability of operation to insure a high percentage of successful hauls and positive closure of the nets to eliminate any possibility of contamination of zooplankton from the above-lying water layers.

Measurements of quantity

The preferred measure of zooplankton biomass would be the total amount of zooplankton under a unit area of sea surface, such as 1.0 m² or 10.0 m², expressed in terms of organic content (i.e. weight of dry organic substances). For various reasons, plankton sampling presently is inadequate to obtain a meaningful measure of zooplankton biomass as above defined. Consequently, plankton samples are usually reported as the amount of zooplankton in a unit volume of water (such as 1000 m³). Various measures of amount are being used including displacement volume, wet weight, dry weight, and weight of dry organic substance.

The measures most commonly used have been "wet" displacement volume and "wet" weight. The displacement volume is determined on a drained plankton sample. Unfortunately it is not a measure of plankton animals only, but includes the interstitial liquid held between and by, the bodies of plankton organisms. Interstitial liquid is far from negligible in amount, commonly accounting for 30% to 40% of a volume determination.
Various techniques have been used to partially or completely remove the interstitial liquid, such as vacuum filtration and the blotter drying of plankton organisms. Both techniques can result in "over drying", i.e. the removal of water from within the bodies of plankton organisms. The amount of interstitial liquid in a drained plankton sample can be reliably determined by spectrophotometric techniques.

Weight determinations are made on "wet" plankton, usually after blotting. Most of the interstitial liquid will have been removed before weighing, but "overdrying" of the plankton has to be guarded against.

Studies have shown that there is a decrease in the volume of a plankton sample at preservation and a gradual decrease in the volume of many samples thereafter, depending upon constituent composition. The decrease in volume is least in collections in which crustacean plankters (especially copepods) dominate, most in samples containing a large percentage of salps and doliolids. A decrease in volume of 87% was noted in a test sample containing mostly salps. Most preserved samples come to an equilibrium or stable volume within a year after preservation. Most of the decrease in volume of preserved samples results from loss of water and inorganic salts from the bodies of plankton organisms. One result of this water adjustment is that the organic materials in watery plankters, such as salps and medusae, become much more concentrated in preserved material than in the bodies of the living animals.

Volume adjustments in preserved plankton samples often make it difficult to duplicate earlier measurements. Consistent measurements can only be made on preserved samples that have reached their equilibrium volumes. In order to obtain measurements that correspond to the living volumes of plankton organisms, the volumetric determinations must be made before preservation of zooplankton samples. Even were there no such problem in obtaining comparable measures of wet volume or weight as those discussed above, neither is an adequate measure of biomass.

Because zooplankters differ markedly in the amount of organic constituents in their bodies per unit volume (e.g. as with watery plankters such as salps compared with crustacean plankters such as copepods), the only consistent measure of biomass appears to be the amount of dry organic substances per unit volume of water sampled. Procedures for determining dry organic substances have been outlined in a separate section of this report.

Studies to improve sampling gear

In a preceding section, certain interim recommendations have been made concerning the structure and use of nets for studying one aspect of research into zooplankton, namely the biomass. In order to make more informed recommendations, a better understanding of the physical characteristics of plankton samplers and their catching ability is required. The working group believes that a wide range of problems should be studied.
Design of plankton nets at present is more an art than a science. Designs are not based on hydrodynamic considerations nor on the behavior responses of planktonic animals. The requirements of quantitative sampling have been given insufficient attention. These factors need remediying.

We are working toward an elusive objective—the development of "ideal" sampling devices, in order to obtain the minimum "set" of gear with which to completely sample the zooplanktonic biomass. As well we require the most effective, single piece of gear (standard net) for use on cruises on which the operation of more than one net is impractical. Research on sampling gear may be grouped into two general categories.

First, there are the relatively straightforward problems for which solutions depend on comparisons and intercalibrations of existing collecting gears; perfection of devices, such as opening and closing mechanisms for which prototypes exist; comparisons of the performance and reliability of depth-flow meters; and the like. Some developmental projects may be included in this category—for example the development of a telemetering flow meter. Facilities are available on several research vessels which would enable the necessary testing to be done for evaluating and comparing the gear in this category. There is need to assemble the gear and interest scientists to conduct the tests.

The second category is the more important, and the more demanding of resources. The researches envisaged are basic to the clarification of physical characteristics and the understanding of the principles underlying net design and performance. These include particularly those structural features of a plankton sampler which affect the flow of water through and around it. The "ideal" sampler would be one in which there is uniform and free acceptance of all water in the path of the net. Factors which act adversely to attaining the "ideal" are those affecting the flow patterns—which in turn are a principal cause of the selectivity of a plankton sampler. Again, changes in flow patterns lead to problems of adequately measuring the quantity of water entering the sampler and this in turn is reflected in difficulties of making quantitative estimates of the sample obtained.

The flow patterns in the mouth of a sampler appear to depend on the degree of resistance to the free flow of water through the filter. The filter itself has an inherent resistance which increases with the fineness of the mesh, with clogging by organisms, and with speed of towing. Flow characteristics may also be dependent in part on the ratio of the area of the mouth of the sampler to the effective aperture area of the filter. This ratio can be varied by increasing the length of a net, relative to the sampler's diameter, or by altering the shape of the sampler by incorporating a "reducing cone" at the front end, or by other means.

Factors adversely affecting flow into a net, and its recording by a flow meter situated in front of the filtering surface, can be illustrated in the diagram on the following page.
Resistance to free and uniform acceptance of water by net

Blocking of meshes by organisms

Decreasing speed

Increasing speed

Decrease in mesh-aperture size

Actual clogging

Increasing resistance -- resulting in "relative clogging"

Decreased acceptance of water by sampler

Probable change of flow pattern in and around sampler

Probable change of calibration of flow meter

Deviations in quantitative estimates made on collections
Few of the factors in the outline above are well understood, or can be assessed quantitatively. The consensus of the working group is that all of these should be investigated if the "ideal" in net design is to be approached. Hydrodynamic studies need to be made in a flume or test basin. Initially the tests can be carried out on some arbitrary "standard" sampler, using a range of mesh-aperture sizes and a variety of netting materials (silk, monofilament nylon, and metal meshes), and a series of ratios of mouth area to effective aperture area. Subsequently these tests should be supplemented by experiments with other styles, shapes and sizes of nets (or samplers). Clogging of various degrees and its effect on flow patterns must be investigated, both that ensuing on higher towing speeds and that caused by organisms. Nets incorporating special adaptations, e.g. areas which are opaque to water flow (such as canvas sleeves), could also be tested, as well as the effect of reducing mouth diameter (i.e. with reducing cones) in relation to net diameter.

The relative retention of non-motile bodies by the several nets, with and without clogging, should be tested. Neutrally buoyant beads of smaller, similar and larger sizes than the mesh apertures could be used.

Following on the study of data from these tests it should be possible to construct samplers based on scientific principles. These should be thoroughly tested for physical characteristics and performance. With satisfactory, controlled testing completed, the sampler(s) must be rigidly tested at sea, especially as regards effects of clogging and selectivity on the collections made. Nets that have been widely used, such as the Indian Ocean Net, CalCOFI net, Clarke-Bumpus net, etc., should be intercalibrated with the new samplers.

Concurrently with these series of tests is the need to understand the characteristics of nets encased in a rigid, outer covering. These are the so-called high-speed samplers. Because the meter is behind the filtering surface, in the tail of the sampler, and therefore measures only the water which is actually filtered, it is believed that quantitative estimates of collections relative to volume of water strained can be more readily obtained than when flow meters are mounted in the mouth of uncased nets. Flow characteristics of the encased net may well be very different from the uncased nets; it appears, in fact, that flow may be turned to advantage and assist the overall filtering capacity of the sampler. These and other problems require developing and investigating.