81-21

LABORATORY REFERENCE DOCUMENT NUMBER 81-21

The Use of Particle Counters in Zooplankton Research

Rosalind E. Cohen

☑ Approved for Distribution

☐ Distribution to F/NWC, F/SWC, F/SEC, F/NWR, & F/[illegible] (Technical Writer-Editor)

Signature [illegible]

Date June 29, 1981

National Marine Fisheries Service
Northeast Fisheries Center
Woods Hole, MA 02543

June 1981

The Use of Particle Counters in Zooplankton Research

by

Rosalind E. Cohen

ABSTRACT

Particle counters have only recently become widespread in marine plankton studies. The Coulter Counter is one of the most common sensing-zone counters and has been used to count and size cultures of phytoplankton, measure growth rates, and calculate zooplankton grazing rates. Adaptations of this design have been used in field studies of fine-scale spatial and temporal patterns of distributions in relation to physical structure.

Another newer type of particle counter, the HIAC, working on a different principle, has been tested in several shipboard studies. It has a number of advantages over the Coulter-type of design.

Both devices count bubbles and detritus as well as organisms, record an ambiguous size measurement, are subject to errors produced by organism clumping and breaking, and provide no taxonomic information. They do, however, generate data which are easily logged and analyzed electronically and save time and tedium involved in sorting. They produce semicontinuous records of several biological and physical parameters in real time and might be towed by ships of opportunity in larger scale studies.

INTRODUCTION

Particle counters have been used in other fields for many years, but they have only recently become widespread in marine plankton research. The most common sensing-zone counter is the Coulter Counter which was initially developed for counting blood cells. In addition to its traditional uses in the laboratory for counting and sizing algal cultures, measuring phytoplankton growth rates, and calculating zooplankton grazing rates, the Coulter Counter has been modified and adapted in various ways for use in field studies of fine scale spatial and temporal distributions of plankton in relation to the physical structure of water column.

Another sensing-zone counter operating on a different principle from the Coulter Counter has been produced by HIAC. It has been used in the oil and pharmaceutical industries and there are few published studies evaluating its marine applications.

The operating principles and applications of the Coulter Counter will be discussed first followed by a parallel discussion of HIAC, then a summary of the problems and advantages of both types of counters, and suggestions for future work will be presented.

REVIEW OF PAST STUDIES

Sheldon and Parsons (1967) published an extensive instruction manual for the Coulter Counter, and Maloney (1962) and El-Sayed and Lee (1963) have also written descriptions of its operation. An electric field is maintained across an aperture in an electrolytic medium. A small pump is used to draw mercury into a manometer tube when a tap is opened; when the tap is closed the mercury returns to its former level drawing a set volume of sample solution (0.5 mℓ) with it through the aperture across the electric field. The mercury trips two switches as it runs back into place which cause the counter to start and stop counting (Figure 1). If the particles are non-conductors (plankton) they cause a drop in resistance between the two electrodes which is proportional to the particle volume, and this change is recorded and counted electronically. There are upper and lower threshold controls which define the size range of particles counted. Particles must be within 2-40% of the aperture diameter (0.5-1000 μ). An oscilloscope displays the particle size frequency distributions.

Sheldon and Parsons (1967) have also outlined the calibration procedures using mono-sized particles (plastic spheres, pollen, crab eggs, etc.) and sea water. A table of maximum particle concentrations is presented in order to avoid coincidence of particles within the sensing zone. They say that calibration remains constant within a temperature range of 5-25°C at 30°/oo salinity, and a salinity range of 8-40°/oo at 20°C.

Several authors have tried to compare the Coulter Counter results with standard microscope methods of counting and sizing particles. Maloney et al. (1962) compared haemocytometer counts of a spherical unicellular alga with

Coulter Counter results and found good agreement. El-Sayed and Lee (1963) also compared microscope data with electronic data for several pure cultures of algae and got good correspondence between methods, but had problems from cell clumping and dust particles. Both studies suggested that electronic results were reliable only if used on pure, unialgal cultures of a spherical species.

Another application of the Coulter Counter is to determine growth rates of laboratory and natural assemblages of phytoplankton. Many problems arise in these studies, especially when conducted with coastal samples which may contain up to 76.6% (by number) detritus (Van Volkenberg et al., 1978). Sheldon (1979) discussed the effect of detritus on the calculations of phytoplankton growth rates and made several suggestions for conducting these types of experiments.

The zooplankton grazing studies conducted using particle counters are too numerous to mention and their problems will be referred to later. In general, two parallel phytoplankton cultures are established and copepods are added to one for a specified length of time. Cell counts are taken before and after in both cultures and the results compared.

Sheldon, Prakash and Sutcliffe (1972) took a Coulter Counter to sea and used it to analyze bucket and water bottle samples from the surface and at various depths in the Atlantic, Pacific, and Southern Oceans. They analyzed the water samples for particles in the range of 0.63-100 μm E.S.D. (equivalent spherical diameter), and concluded that there were definite geographic variations in particle size frequency distributions of suspended matter which can be used to characterize specific regions. Relatively few stations were sampled in this study. They say their results suggest that "roughly equal concentrations of material occur in all particle size classes within the range of $1\text{-}10^6$ μm, i.e.

from bacteria to whales," and that since large predators feed on small prey in the oceans, "standing stock can only be maintained if the rate of particle production varies inversely with particle size."

The Coulter Counter has been transformed and modified by several investigators for use on board ship both in situ and in a batch mode using pumped-in sea water samples. One of the first towed devices was constructed by Maddux and Kanwisher (1965). Their detector could be substituted for the cod end of a conical plankton net in order to have more concentrating power in areas where plankton is sparse, and a retaining net could be used to collect samples for standard microscope counts. Large particles were pre-screened to prevent clogging.

Boyd and Johnson (1969) and Boyd (1973) continued development of this type of sampler in order to study small-scale patterns of distribution of marine zooplankton on a finer scale than is possible by conventional means. Their sampler was equipped to measure depth, large and smaller scale temperature variations, flow rate, and zooplankton number and volume (0.078-8.69 mm^3 or 0.531-2.55 mm E.S.D.). Particles separated by about 15 msec or 2 cm could be discriminated. Data were logged on magnetic tape for computer analysis on shore. In his 1973 paper, Boyd presented results taken along a transect between Bermuda and Halifax (July 10-15, 1972) (see Figure 2 for an example). The sampler was actually lowered obliquely to 250 m and retrieved while the ship covered a distance of 11.1 km in 90 minutes. Plots of the data showed larger concentrations of zooplankton in areas of high temperature fluctuations. Boyd suggested that organisms may aggregate around density interfaces.

Mackas and Boyd (1979) used an updated version of this sampler on board ship to record simultaneous records of zooplankton size and number, chlorophyll fluorescence, and temperature using sea water pumped from a fixed depth. Because there were no clogging problems, fairly long transects were possible. Spectral analysis of some North Sea data revealed intense near-surface patchiness with abundances of 10^5 particles/mm^3 and abundance contrasts of 4 to 5 fold with background levels. Small-scale spatial patchiness was greater for zooplankton than for phytoplankton suggesting that zooplankton form more persistent small-scale aggregations; there frequently were negative correlations between zoo- and phytoplankton abundances.

Mackas et al. (1980) again used a similar shipboard system to do near-surface transects in the Georgia Strait off the southwest coast of Vancouver Island in order to map mesoscale (approximately 10 km) patterns of plankton concentrations and to select locations for point sampling by standard methods. There were a variety of physical factors acting to produce the spatial patterns observed (wind-induced upwelling, tidal mixing, fresh water input, estuarine entrainment).

Another Coulter Counter-type sensor on a towed vehicle has been developed jointly by investigators at Bedford Institute and the National Research Council of Canada. The "Batfish" was designed to do vertical profiles, and transports a digital CTD, a Variosens fluorometer and a zooplankton counter-sizer (Figure 3). Herman (1977) and Dauphinée (1977) described the laboratory calibration of the fluorometer and zooplankton counter, and Herman and Dauphinée (1980) reviewed the sampler design and the results of some trial runs. There is a self-cleaning concentrating net (R = 3.5, square pyramid) attached to the front of the sensor

by a tube leading into another straight tube (either 3- or 5-mm diameter) made of insulating material and containing four platinum electrodes; three electrodes are for potential and one for current. A stainless steel tube in front of the device acts as a ground. Because of the extra electrode, information on length as well as volume can be recorded. The size range of organisms detected is 0.08-0.25 mm^3 volume or 0.5-2.5 mm E.S.D. with the 3-mm aperture tube, and 3.0-100 mm^3 volume or 0.8-4.0 mm E.S.D. with the 5-mm tube. A trapping net can be attached to the rear of the sampler to collect organisms for laboratory analysis. They claimed that the sensor's response to changes in temperature and salinity (except for small salt fingers in the size range of zooplankton) can be distinguished from its response to plankton particles. Also, dead animals are not counted because after they are permeated by sea water they become conductors.

During their trial runs they did comparisons between Batfish and Bioness results and got fairly good agreement, but in one series in Nova Scotian coastal waters replicate tows took place on different nights. Several successive depth profiles off the coast of Peru were also compared. The profiles were really oblique, not vertical, ranged from 2-80 meters, and were 0.5 km apart. They estimated the replicability at $\pm$ 25 counts (Figure 4).

Additional studies involved the generation of the calibration algorithm. They used cylindrical beads and live zooplankton and passed each bead through the sensor 200 times recording length and volume each time. Flow through the sensor is laminar and assymmetrical particles line up with their long axes parallel to the direction of flow. Average standard deviations for the beads were 10% for length and 15% for volume, and for live zooplankton compared

to microscope measurements, were 15% for diameter, 19% for length and 25% for volume (Figure 5).

Batfish was used in the cycling mode in the coastal waters off Peru and towed repeatedly along a transect. The dominant species and stages were separable from their records. Additional tows which took place on the Scotian Shelf in the frontal region separating coastal and slope water revealed concentrations in the front an order of magnitude higher than the shelf or slope water. Concentrations beneath the front in slope water were low both by day and night suggesting a block to vertical migration.

Herman and Mitchell (1980) continued work relating electronic counts and sizes to dominant copepod species, and concluded that dominant species and stages could be identified electronically provided microscope counts are taken for verification.

The other sensing-zone particle counter to be discussed is much newer to the marine field, and there are not many published studies to cite. The HIAC has chiefly been used as a field sampling device, but can be used in the laboratory for the same applications as the Coulter Counter. HIAC works on the principle of light blockage. A fluid suspension is passed through an aperture past a window of known area. A collimated light beam is shone through the liquid perpendicular to the direction of flow hitting a photodiode which sends its output to a preamplifier in the sensor, and then to the main particle counter (Figure 6). When no particles are present the preamplifier output is maintained at a constant known level regardless of the optical density of the medium. Flow through the aperture is laminar and particles line up with their long dimension parallel to the direction of flow. A particle passing through blocks part of

the light beam and produces a pulse of reduced voltage. Pulse amplitude is proportional to the maximum side view area of the particle and pulse width depends upon its velocity. There are twelve voltage comparators in the main particle counter which sort the incoming pulses based on threshold settings determined from a sensor calibration curve. If a threshold is crossed the comparator maintains a constant output. Several sensors are available, capable of counting and sizing particles in a range of 1-9000 μm E.S.D.

In one of the published studies using a HIAC, Pugh (1977) focused on phytoplankton and microzooplankton sized particles (1-600 μm E.S.D.). A 9-channel HIAC was used on board ship to sample the upwelling region off northwest Africa. They used an on-line system incorporating 2 sensors, and water from the ship's sea water supply was used for horizontal profiling and the outflow from a submersible pump was used for vertical profiling. Large particles were prefiltered and part of the flow was directed to a fluorometer. Temperature, salinity, and nutrient concentrations were also recorded along with flow rate. A correction for coincidence was applied to the results, and channel settings were set at a $\log_2$ scale or subdivisions.

In a trial run, a transect was taken between 20°50'N, 17°40'W and 22°30'N, 17°40'W recording particles in 6 logarithmically equal size ranges. Counts were taken for 60 seconds out of every 5 minutes and cross spectral analysis was applied to the data. Along this and other transects the different particle sizes contributing to overall fluorescence pattern could be discerned. Vertical profiles were completed in the northwest African upwelling region and the North Sea. The inlet hose of the pump was attached to a CTD unit and particles were counted for 50 seconds out of a minute. Since the CTD was lowered at a rate of 0.1 m/sec, the integrated particle count was for a depth range of

5 meters. These profiles again showed fluorescence peaks composed of several different sizes of particles, possibly different species of phytoplankton (Figure 7). Pugh concluded that although size composition of the phytoplankton may be more important to herbivores than species, taxonomic studies are still necessary whenever possible.

At the Fisheries Laboratory in Lowestoft, England, Tungate and Reynolds (1980) have developed an on-line sampling system using HIAC, and covering a particle size range of 1-2500 μm, using 3 sensors. Each sensor could be programmed to count for from 1-99 seconds at intervals of 1-99 minutes; initially they sampled for 1 minute and had 1 minute intervals. A pulse height analyzer (PHA) was added to this system in order to expand the number of channels for counting and sizing from 12 up to 512 or 1023; a visual display also was added. Data could be logged directly on a computer, magnetic tape, or chart recorder.

This system has been tested on many horizontal transects in the North Sea and Atlantic Ocean pumping in water from a constant depth of 5 meters. The patterns of particle size frequency distributions were related to chlorophyll levels and transparency as expected (Figure 8). A large number of particles were detected in the <10 μm range possibly representing microflagellates which are difficult to sample by other means. They too emphasize the need to collect corroborating samples by standard sampling techniques simultaneously with the particle counter.

PROBLEMS, ADVANTAGES, AND FUTURE WORK

In this section, the major mechanical and biological problems which occur when using sensing-zone particle counters will be outlined. The various mechanical problems of the Coulter Counter were studied independently by several different laboratories (Karuhn et al., 1975; Davies et al., 1975; Grover et al., 1969a and 1969b; Kachel, 1976). Some of these problems have been corrected electronically by the manufacturer.

The height and shape of the voltage pulse of the Coulter Counter are affected by:

1. Applied constant current.
2. Electrical conductivity of the suspending medium; small-scale changes in temperature and salinity.
3. Electrical conductivity of the particle.
4. Geometrical dimensions of the orifice.
5. Path of the particle through the orifice.
6. Shape of the particle.

(Kachel, 1976)

Both the Coulter Counter and the HIAC have the following problems in common;

1. Ambiguous nature of the measurement - E.S.D. represents particle volume or area, but the same E.S.D. may be output for several different shapes.

   (Harbison and McAlister, 1980)
2. Coincidence of particles in the sensing zone, or of electrical impulses.
3. Bubbles, dust, and detritus are counted and sized.
4. Cell or organism clumping and aggregations, and chains.

5. Cell or organism breakage.

(Deason, 1980)

6. No taxonomic information.

There may be additional problems associated with the HIAC which have not yet been discovered because it is so new to this field. The problem of coincidence can be minimized by carefully controlling flow rate and concentration; many of the other problems can be reduced by examining the experimental solutions before and after counting, if possible. The HIAC does appear to have fewer electronic problems than the Coulter Counter and can be used over a much wider range of sizes with a larger number of channels. Future work should involve careful calibration of HIAC using known species of phyto- and zooplankton, tests of the effects of particles and media of different optical densities on the sizing and counting results, and the possibilities of using biological stains and fluorescence to distinguish between dead and live cells and phyto- and zooplankton, respectively.

The major advantages of particle counters over standard samplers are:

1. Semicontinuous records are produced - advantages for statistical analysis.
2. Simultaneous records of particle size and number, temperature, salinity, and other parameters are possible.
3. Real-time information is generated.
4. Data are easily logged and analyzed automatically.
5. Vehicles transporting the sensors can be towed by ships of opportunity.
6. Saves time and tedium of sorting samples.
7. May detect colorless flagellates not detected by other means.

In spite of the problems encountered, particle counters in conjunction with standard samplers, may be able to increase our knowledge of fine-scale spatial and temporal distribution of plankton in relation to physical structure. They are also useful in locating areas of high biological activity, and may be of use in species identifications within patches or areas where only a few species are dominant.

REFERENCES

Boyd, C.M. 1973. Small scale spatial patterns of marine zooplankton examined by an electronic in situ zooplankton detecting device. Neth. J. Sea Res., 7: 103-111.

Boyd, C.M. and G.W. Johnson. 1969. Studying zooplankton populations with an electronic zooplankton counting device and the Linc-8 computer. Trans. Applications of Sea Going Computers Symposium; Mar. Tech. Soc., 83-90.

Dauphinée, T.M. 1977. Zooplankton measurements using a conductance cell. MTS-IEEE Ocean '77, Oct. 1977, Los Angeles, Calif. Session 39B: 1-5.

Davies, R., R. Karuhn, and I. Graf. 1975. Studies on the Coulter Counter. Part II. Investigations into the effect of flow direction and angle of entry of a particle on both particle volume and pulse shape. Powder Technol. 12: 157-166.

Deason, E.E. 1980. Potential effect of phytoplankton colony breakage on the calculation of zooplankton filtering rates. Marine Biology 57: 279-286.

El-Sayed, S.Z. and B.D. Lee. 1963. Evaluation of an automatic technique for counting unicellular organisms. J. Mar. Res. 21: 59-73.

Grover, N.B., J. Naaman, S. Ben-Sasson, and F. Doljanski. 1969a. Electrical sizing of particles in suspensions. I. Theory. Biophys. J. 9: 1398-1414.

Grover, M.B., J. Naaman, S. Ben-Sasson, F. Doljanski, and E. Nadav. 1969b. Electrical sizing of particles. II. Experiments with rigid spheres. Biophys. J. 9: 1415-1425.

Harbison, G.R. and V.L. McAlister. 1980. Fact and artifact in copepod feeding experiments. Limnol. Oceanogr. 25: 971-981.

Herman, A.W. 1977. In situ chlorophyll and plankton measurements with the Batfish vehicle. MTS-IEEE Oceans '77. Oct. 1977, Los Angeles, Calif. 39D: 1-5.

Herman, A.W. and T.M. Dauphinée. 1980. Continuous and rapid profiling of zooplankton with an electronic counter mounted on a "Batfish" vehicle. Deep-Sea Res. 27A: 79-96.

Herman, A.W. and M.R. Mitchell. 1980. Counting and identifying copepod species with an in situ electronic zooplankton counter (In press) 31 pp.

HIAC Instruments Div. Pacific Scientific. 1979. HIAC sensors for contamination monitoring. Pacific Scientific Information Bulletin No. 7901. Montclair, Ca. 4 pp.

Kachel, V. 1976. Basic principles of electrical sizing of cells and particles and their realization in the new instrument "Metricell." J. Histochem. Cytochem. 24: 211-230.

Haruhn, R., R. Davies, B.H. Kaye, and M.J. Clinch. 1975. Studies on the Coulter Counter. Part I. Investigations into the effect of orifice geometry and flow direction on the measurements of particle volume. Powder Technol. 11: 157-171.

Mackas, D.L. and C.M. Boyd. 1979. Spectral analysis of zooplankton spatial heterogeneity. Science 204: 62-64.

Mackas, D.L., G.C. Loutlit, and M.J. Austin. 1980. Spatial distribution of zooplankton and phytoplankton in British Columbian coastal waters. Can. J. Aquat. Sci. 37: 1476-1487.

Maddux, W.S. and J.W. Kanivisher. 1965. An in situ particle counter. Limnol. Oceanogr. 10: 162-168.

Maloney, T.E., E.J. Donovan, Jr., and E.L. Robinson. 1962. Determination of numbers and sizes of algal cells with an electronic particle counter. Phycologia 2: 1-8.

Pugh, P.R. 1977. The application of particle counting to an understanding of the small-scale distribution of plankton. In Spatial pattern in plankton communities. J.H. Steele (ed.), 111-129.

Sheldon, R.W. 1979. Measurement of phytoplankton growth by particle counting. Limnol. Oceanogr. 24: 760-767.

Sheldon, R.W. and T.R. Parsons. 1967. A practical manual on the use of the Coulter Counter in marine research. Fish. Res. Bd. Canada. Pac. Oceanogr. Group. Nanaimo, B.C. 66 pp.

Sheldon, R.W., A. Prakash, and W.H. Sutcliffe, Jr. 1972. The size distribution of particles in the ocean. Limnol. Oceanogr. 17: 327-340.

Tungate, D.S. and E. Reynolds. 1980. The MAFF on-line particle counting system. Fish. Res. Tech. Rep. 58. 11 pp.

Van Volkenberg, S.D., J.K. Jones, and D.R. Heinle. 1978. A comparison by size class and volume of detritus versus phytoplankton in Chesapeake Bay. Est. and Coast. Mar. Sci. 6: 569-582.

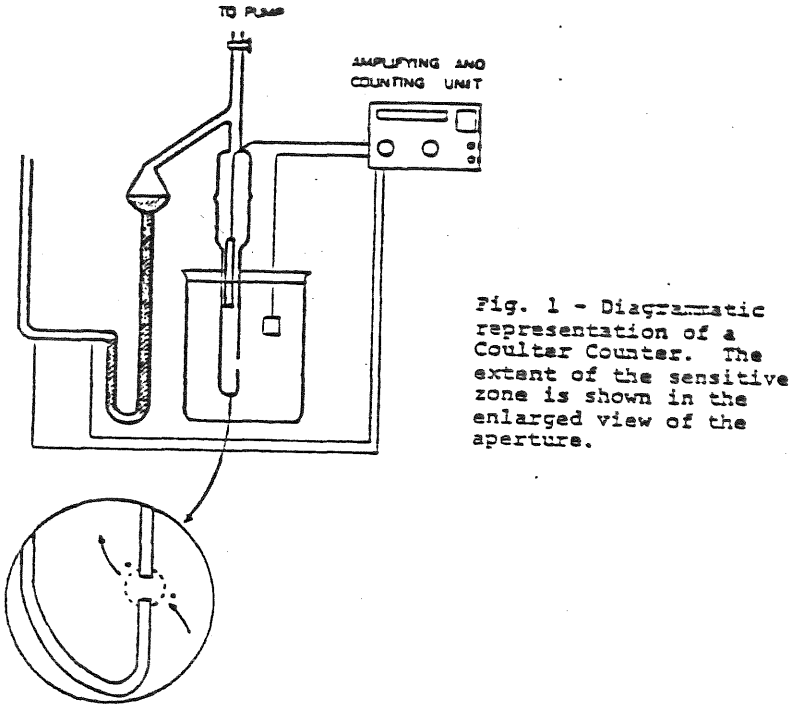

Figure 1. Schematic diagram of the Coulter Counter principles of operation (Sheldon and Parsons, 1967).

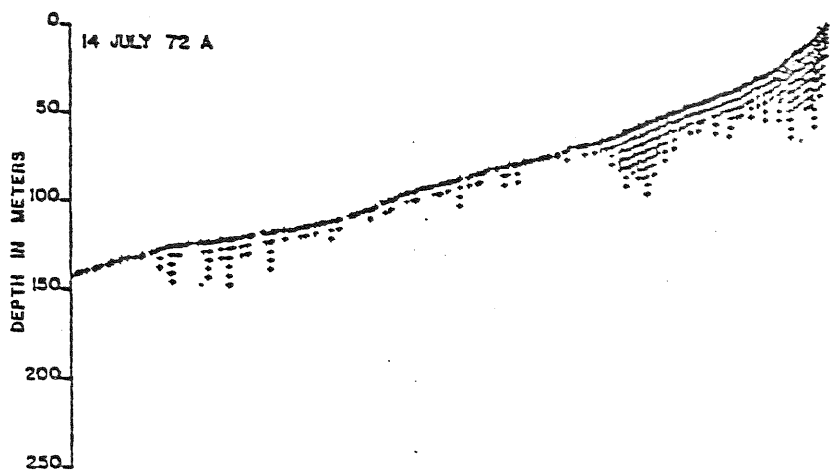

Fig.3. A plot of individual zooplankters detected on tow 14 July 72 A, drawn on the line showing depth (m) of the instrument. Each cross represents a single animal; if more than one animal was detected in a second the cross representing that animal (and others if more were caught in that second) was drawn below the previous cross.

Figure 2. Plot of the concentrations detected by a towed zooplankton sensor (Boyd, 1973).

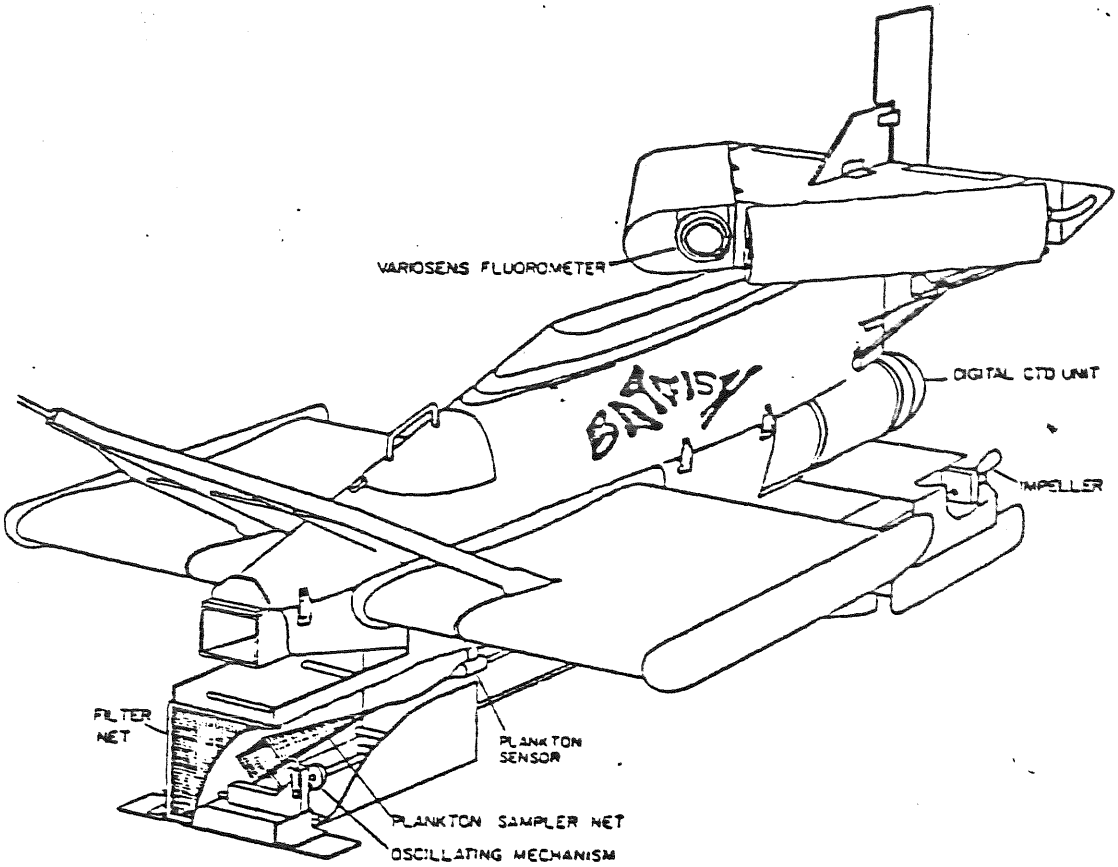

Fig. 1. The Batfish system with its complement of sensors consisting of a Variosens fluorometer, digital CTD unit, and the zooplankton sampler. A plankton net with a 6.4-cm aperture filters and feeds samples to the electronic sensor for counting and sizing.

Figure 3. Diagram of the Batfish vehicle (Herman and Dauphinée, 1980).

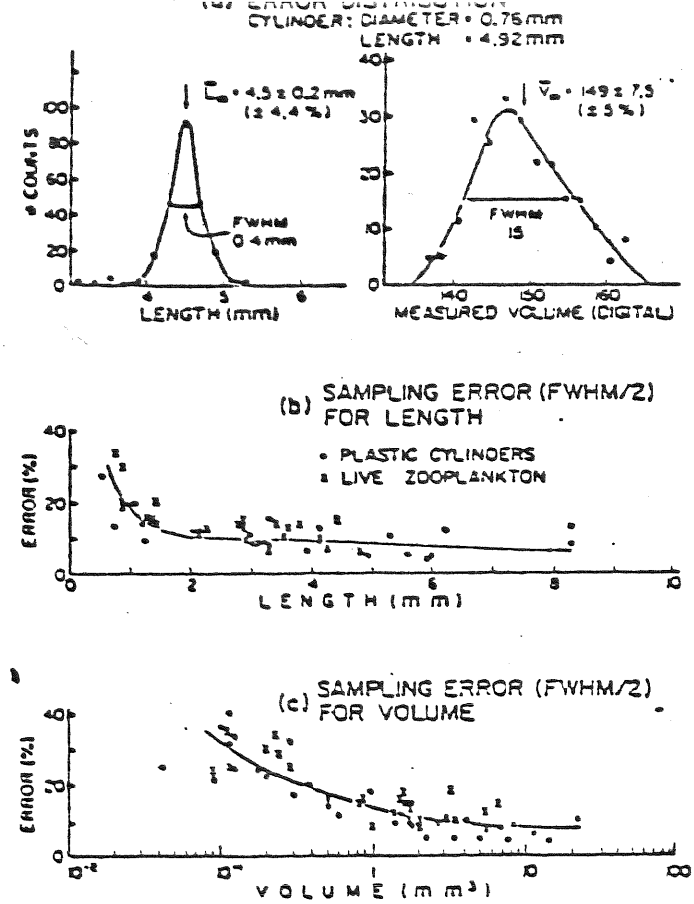

Fig. 4(a) The measured error distribution of length and volume for a cylinder of known geometry. The data were obtained from ~200 passes through the electronic sensor. (b) and (c) Sampling error as a function of length and volume for a wide range of cylinder sizes. Errors are also indicated for live zooplankton.

Figure 4. Error distribution of length and volume of cylindrical beads and zooplankton measured by Batfish zooplankton sensor (Herman and Dauphinée, 1980).

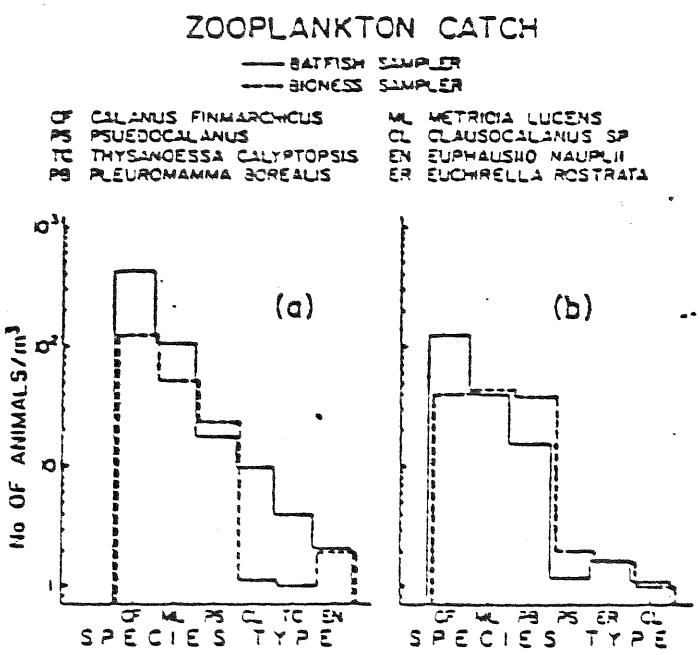

Fig. 2 Comparison of zooplankton catches from the Batfish sampler and a high performance plankton net, the Bioness. The cross-correlation coefficients for the variation of six species for a and b are $r = 0.94$ and $r = 0.57$, respectively.

Figure 5. Comparison between results of Batfish and Bioness zooplankton collections (Herman and Dauphinée, 1980).

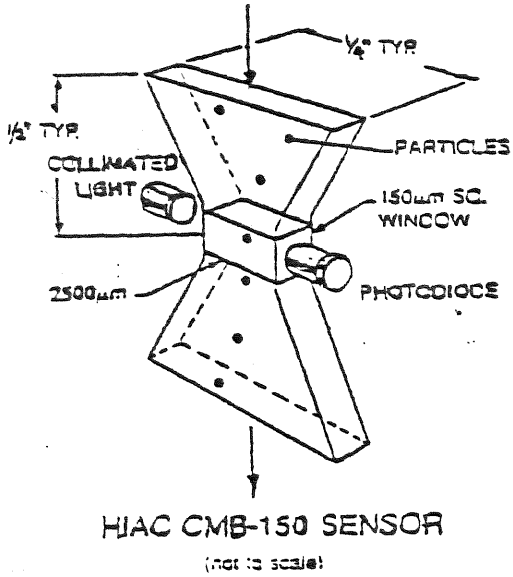

Figure 6. Schematic diagrams of a HIAC sensor (HIAC Instruments, Div. of Pacific Scientific, Bulletin No. 7901, 1979).

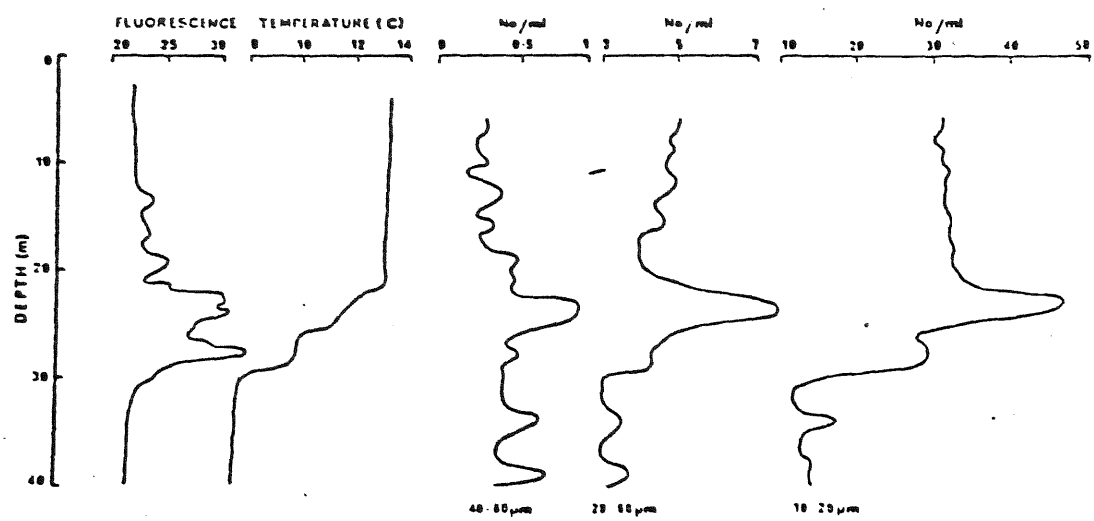

Figure 6. Vertical profiling at a station in the North Sea. Fluorescence, temperature and particle counts/ml in three size ranges.

Figure 7. Particle size distributions measured by HIAC (Pugh, 1977).

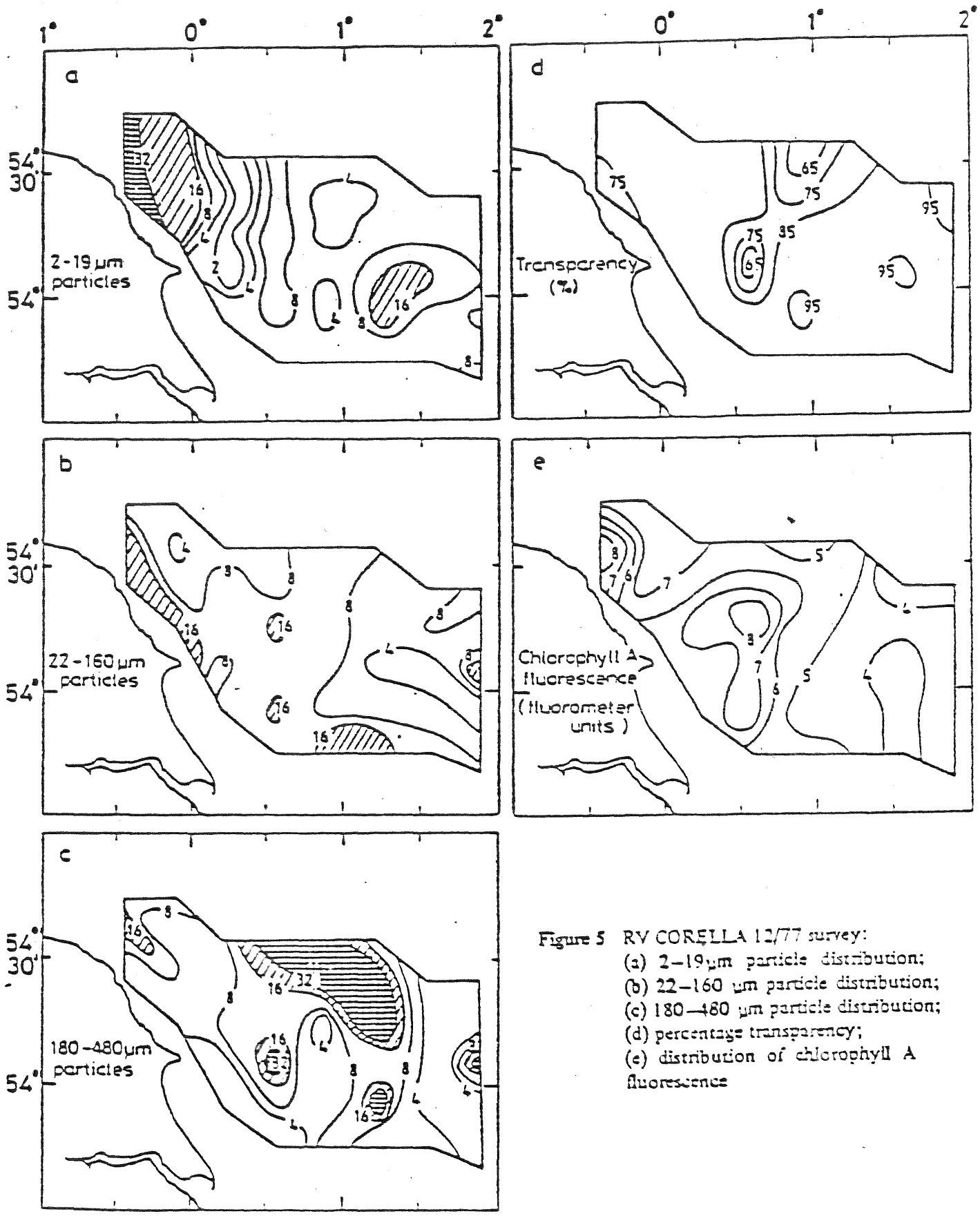

Figure 5 RV CORELLA 12/77 survey:
(a) 2–19 µm particle distribution;
(b) 22–160 µm particle distribution;
(c) 180–480 µm particle distribution;
(d) percentage transparency;
(e) distribution of chlorophyll A fluorescence

Figure 8. Particle size distributions measured by HIAC (Tungate and Reynolds, 1980).