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The MAFF on-line particle counting system

D.S. TUNGATE and
E. REYNOLDS

LOWESTOFT, 1980

1. Introduction

Marine biologists have long been aware of the horizontal and vertical patchiness of planktonic organisms and the variations in densities of populations, particularly in the vicinity of hydrographic discontinuities e.g. oceanic frontal systems. Progress in the study of these phenomena has been limited by the lack of suitable equipment to sample the complete range of particle sizes found in the sea, particularly the smallest particles (< 10 μm). Most studies in this range have been made using fine nets or waterbottles but, as most known preservatives destroy the smallest cells, microscope counts have to be made at sea, which is a difficult task even in relatively calm sea conditions, and leads to unreliability in the data. A number of biologists have made contributions to this problem. Sheldon (1967) used a Coulter counter (Coulter, 1957) to study the particulate matter in suspension in the Straits of Georgia, British Columbia. He measured densities and size distributions within the range of 1.58 – 256 μm and found that there were modes of 7, 25, 48 and 90 μm in the size frequencies of particles collected from a depth of 5 m. Lee and Folkard (1969) used a Coulter counter to measure sediment size and load in tidal streams in the North Sea during 1957 and 1967.

Further advancement came with the development of an *in situ* particle counter which recorded densities and size distributions as it was towed along transects in the sea (Maddux and Kanwisher, 1965; Boyd and Johnson, 1969). Their equipment employs the same basic principles as used in the Coulter counter but has a different form of electrical operation. Boyd's counter measures particles in the 0.531 – 2.55 mm range, which are mainly zooplankton.

A large proportion of the smallest particulate matter in the sea has not been identified, but it is thought to be of organic origin, possibly derived from the breakdown of phytoplankton cells and bacteria. It has been demonstrated that particles, which oceanographers call organic aggregates, can form in sea water under favourable conditions and that they contain bacteria. Baylor and Sutcliffe (1963) have shown that organic aggregates will form readily in the presence of minute air bubbles. Sheldon (1967) made laboratory observations and demonstrated that particle formation could occur in sea water in the absence of any external influence. He allowed sea water which had been passed through a 0.45 μm filter to stand undisturbed for 24 h and on observation found that it contained considerable numbers of particles in the 1.5 – 4.0 μm size range and that this phenomenon of particle formation occurred in both coastal and oceanic filtered sea water, but that higher concentrations formed in the coastal water.

This report describes an on-line particle counting and sizing system developed at The Ministry of Agriculture, Fisheries and Food (MAFF), Fisheries Laboratory, Lowestoft and illustrates its capability, using data collected in the course of its development.

2. Description of particle counting equipment and principle of operation

The particle counting equipment developed by MAFF, Lowestoft, is based on the HIAC Criterion PC 320 system which is used extensively in the oil and pharmaceutical industries when testing for impurities. In its original configuration it will count and categorise particles into twelve size ranges which are preselected by the operator. It is an optical system which operates on the principle of light blockage. Particles in fluid suspension flow through a channel and past a window of which the area is known accurately. Collimated light beamed through the fluid at right angles to the direction of flow passes through the window to fall onto a photodiode. The output from this goes to a pre-amplifier in the sensor and then to the main particle counter. There, a peak level detector generates a signal controlling a servo loop which maintains the output level of the sensor pre-amplifier at -10 V when there is no particle passing the sensor window, thus ensuring that the sensor calibration is valid, even if the optical density of the carrier fluid should change. When a particle passes the window it partially blocks the light beam causing a reduction in voltage. A pulse is produced of which the peak amplitude is proportional to particle area and the width proportional to the velocity of the particle as it passes the sensing zone. The varying voltage signals are then sorted into twelve selected size ranges, the numbers are displayed in sequence on the front panel of the instrument, and a permanent record is produced on a paper printer.

3. The on-line sampling system

For shipboard operation, an on-line sampling system has been developed. It consists of three sensors (Figure 1) which have been selected to cover the size range of 1-2500 μm. Water is pumped from a depth of 5 m and run continuously through each of the sensors at the calibration flow rates. The flow rates selected are 8 ml min^{-1} for the 1-60 μm sensor, 100 ml min^{-1} for the 10-600 μm sensor and 1000 ml min^{-1} for the 50-2500 μm sensor. These flow rates can be altered within limits specified by the manufacturer, but re-calibration is necessary. To ensure that flow rates to each sensor remain constant, automatic flow regulators and flow meters are located in the system. These will maintain water flow within 2% of a selected rate, providing that a minimum pressure of 0.703 kg cm^{-2} is maintained in the main pumping system.

To prevent blockage of the sensors by large particles, pre-filters are located up-stream of each sensor. These are standard 50 mm diameter Sartorious filter holders in which the filter supports have been replaced by discs of 58, 85 and 2300 μm nylon filter material.

Each sensor is sampled in sequence by an automatic switching unit which can be programmed to count periods of time, which can be varied from 1 to 99 s. The interval between each operation can be adjusted between 1 and 99

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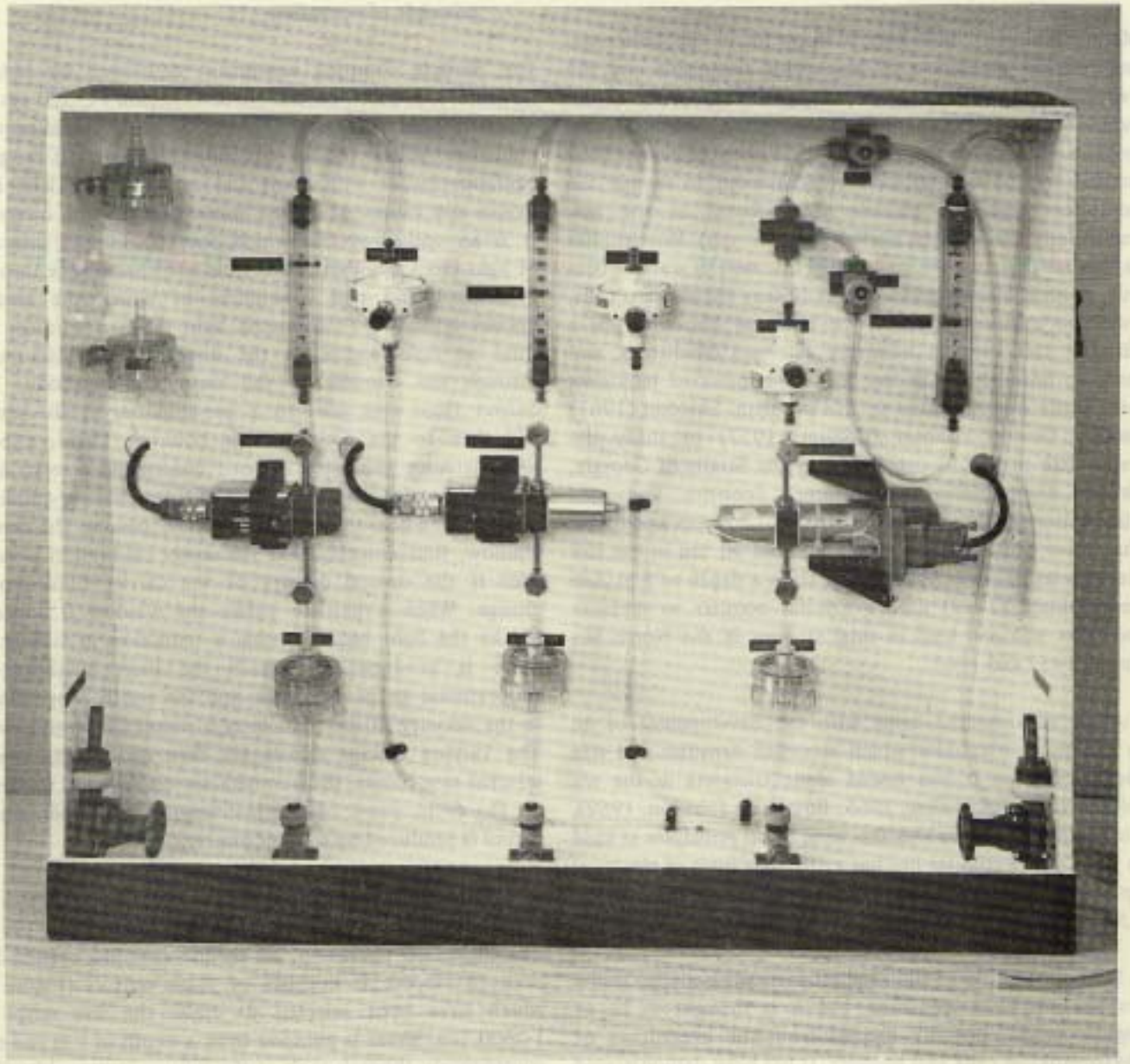

Figure 1 The on-line flow system

min. At this stage of development the most practical timing sequence is to sample for 1 min and have 1 min intervals between each sensor operation. The system will sample at more frequent intervals if needed.

The twelve channels in the HIAC counter unit are inadequate for studying mixed plankton populations, so a pulse height analyser (PHA) has been interfaced. This records and stores the particles into either 512 or 1023 channels and at the same time gives a visual display of the size spectrum. The numbers in each PHA channel can then be transmitted either to appropriate data storage systems, e.g. chart recorder, cassette magnetic tape, data logger, or directly into a computer (Figure 2). After each sensor operation the data collected in the PHA is automatically transferred, its memory is destroyed and it is re-armed to receive the results of the next operation.

4. The HIAC counter unit and pulse height analyser modifications

The HIAC range of sensors produces output pulses proportional to the particle size. As detection methods depend upon the interruption of a light path, the output pulses are essentially proportional to the side view or area of the particle; due to rotational effects as the particle passes through the detector aperture, it is likely that the maximum side view area will be measured.

Each of the HIAC sensors has a nominal particle size range

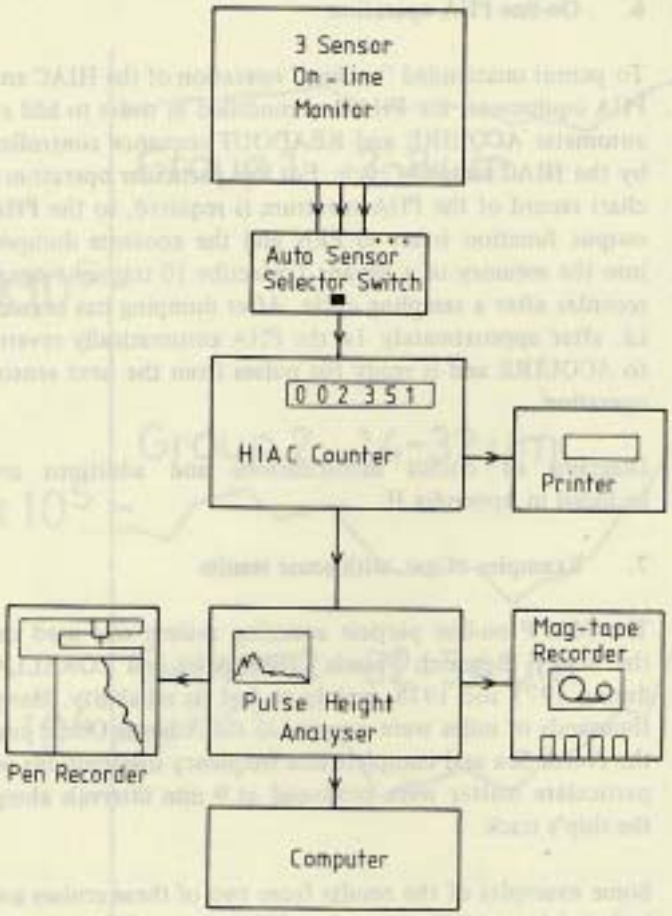

Figure 2 The MAFF on-line sampling system, showing the available data output options.

ratio of 60:1. For example, the small sensor will measure particles in the range 1–60 μm: the output pulses corresponding to this range of sizes have heights within the range 10 mV – 10 V, i.e. a pulse height range ratio of 1000:1. These pulses are sorted by the HIAC main unit, using a ladder of voltage comparators, each of which can be set within the range 10 mV – 10 V. The relationship between particle size and comparator setting is obtained from calibration curves supplied with each sensor. The number of comparators used (up to twelve) defines the number of channels available for storing a particle size spectrum, the lower limit of any one channel being determined by its threshold setting and its upper limit by the threshold setting of the next comparator in the ladder. The function of each comparator is simply to produce a fixed output level when its threshold has been crossed. The output levels of the comparators are sorted by logic circuits so that the number of particles within the operating limits of any one comparator can be routed to an appropriate storage circuit.

The command to store is generated after a particle has been sized by the comparator ladder so as to ensure that the peak height of the output pulse has been attained. This command is designated STROBE in the HIAC logic sequence.

After storage a RESET pulse is generated which resets the logic circuits in readiness for the next pulse measurement. Thus a twelve-element histogram of particle size and frequency can be obtained. For some applications this is perfectly adequate, particularly when the expected particle size range is known and the individual channel settings can be pre-set for the best resolution of the total spectrum. Often the size distribution is not known and the frequency of the smaller particles may be very high in relation to the larger particles. To deal with these situations an analogue output socket is provided on the back of the HIAC counter unit which can be connected to an external multichannel pulse height analyser and, provided that the HIAC analogue pulses are properly interfaced to the PHA equipment, this mode of operation can provide data covering a wide frequency distribution.

5. Multi-channel analysis of HIAC sensor pulses

Most multi-channel PHAs are designed for nuclear pulse spectrometry and the input signal specification is designed around this application. Particle sizing sensors produce pulses with very different characteristics so for the best results some degree of signal processing and control is necessary. These additional functions include:

(i) pulse shaping and gain control;

(ii) pulse height compression;

(iii) pulse height integration;

(iv) pulse conditioning logic.

5.1 Pulse shaping and gain control

A standard nuclear pulse amplifier was modified to accommodate pulses with rise times of not less than 5 μs and with durations of up to 500 μs. The precise times are a function of the particle shape and the flow rate of the pumping system, but the band width characteristics of the modified amplifier will usually cover the spread of pulse shapes from a properly operated sensor. The coarse and fine gain controls are part of the standard amplifier and are necessary to match the dynamic range of the HIAC sensor pulses to that of the PHA.

5.2 Pulse height compression

The HIAC sensor has a useful pulse output ranging from 10 mV to 10 V, a ratio of 1000:1. As the useful range of pulses which can be accepted by a PHA is about a ratio of 100:1, some form of amplitude

compression is necessary if the total HIAC particle spectrum is to be acquired. To provide this a Tracor logarithmic amplifier is used which converts an input signal range of 10 mV – 1 V into a three cycle log output with a range of 1 V – 10 V. To facilitate PHA calibration and setting up of the logarithmic amplifier, a calibration pulse generator has been designed which will simultaneously mark the start and end of each log cycle on the PHA display.

5.3 Pulse height integration

PHA's have recommended rise times of about 1 μs for nuclear pulse spectrometry, but they can accept more slowly changing inputs by sampling the input at an appropriate time. As the HIAC sensor pulses have slower rise times and do not necessarily reach peak height at the same time, the output pulses from the log amplifier are applied to a capacitor integrating circuit which stores the peak value of any one pulse event. This integrated pulse is applied to the PHA input circuits and sampled for 2 μs at peak level by the PHA GATE pulse. After sampling, the capacitor is discharged in readiness for the next pulse event by the INTEGRATOR GATE pulse. (see Appendix I).

5.4 Pulse conditioning logic

5.4.1 PHA GATE pulse

This pulse is initiated by the HIAC STROBE pulse and it is further conditioned by associating its generation with one or more of the HIAC's comparator channels. This enables the operator to restrict the PHA analysis to particular HIAC channels, which is useful when the operator wishes to identify quickly a portion of the total PHA spectrum which is associated with a particular HIAC channel. Additional switches marked UPPER CHANNEL and LOWER CHANNEL are fitted to the HIAC counter main frame to select this function. For normal operation of the equipment these switches are adjusted so that all HIAC channels command the generation of a PHA GATE pulse.

5.4.2 INTEGRATOR GATE pulse

As soon as the lowest channel threshold setting on the HIAC counter is crossed, the INTEGRATOR GATE pulse is generated and this removes a short circuit from the integrator capacitor, so that the peak level of the log amplifier output pulse can be stored. The INTEGRATOR GATE pulse is terminated after the peak level has been sampled by the PHA GATE pulse and the short circuit is restored until the next pulse event.

6. On-line PHA operation

To permit unattended "on line" operation of the HIAC and PHA equipment, the PHA was modified in order to add an automatic ACQUIRE and READOUT sequence controlled by the HIAC sampling cycle. For this particular operation a chart record of the PHA spectrum is required, so the PHA output function is set to PEN and the contents dumped into the memory of a Bryans Transcribe 10 transient signal recorder after a sampling cycle. After dumping has ceased, i.e. after approximately 1s, the PHA automatically reverts to ACQUIRE and is ready for pulses from the next sensor operation.

Diagrams of circuit modifications and additions are included in Appendix II.

7. Examples of use, with some results

The MAFF on-line particle counting system was used on the MAFF Research Vessels CIROLANA and CORELLA during 1977 and 1978, mainly to test its reliability. Many thousands of miles were steamed in the Atlantic Ocean and the North Sea and complete size frequency distributions of particulate matter were produced at 9 min intervals along the ship's track.

Some examples of the results from two of these cruises are included here, to illustrate the effectiveness of the system. Figure 3 shows a transect of 129 km crossing the Ushant oceanic frontal systems situated off Cape Finisterre. The particulate matter recorded ranged in size from 2 – 480 μm. The size ranges 2–8, 14–32, 32–54, 54–120, 180–480 μm were selected to correspond with the peaks in size distribution shown in Figure 4. Figure 3 also shows the chlorophyll A fluorescence values which were measured with an on-line Turner fluorometer. From Figure 3 it can be seen that there is a good relationship between size groups 1, 2 and 3 and the chlorophyll A fluorescence, whereas groups 4 and 5 do not show this, mainly because the numbers sampled were relatively small and there was the possibility that some of the larger particles could have been zooplankton, probably copepod nauplii.

Figure 5 illustrates particle size distributions (numbers per litre), transparency and chlorophyll A measured on RV CORELLA 12/77 during surveys off the north-east coast of England. Relatively high concentrations of the smallest particles (size range 2–19 μm) extended northwards from Flamborough Head to about 28 km from the coast (Figure 5a). The majority of these small particles were in the 2–5 μm size range. The distribution of medium sized particles (22–160 μm) was similar to that of the small particles, with high numbers found in the coastal regions (Figure 5b). The bulk of the phytoplankton would be distributed in this group. The relatively high numbers at the southern boundaries of the survey grid were probably due to the outflow of the River Humber, whilst the concentration on the eastern boundary of the survey area was very near to the shallow water at the south-west edge of the Dogger Bank. The

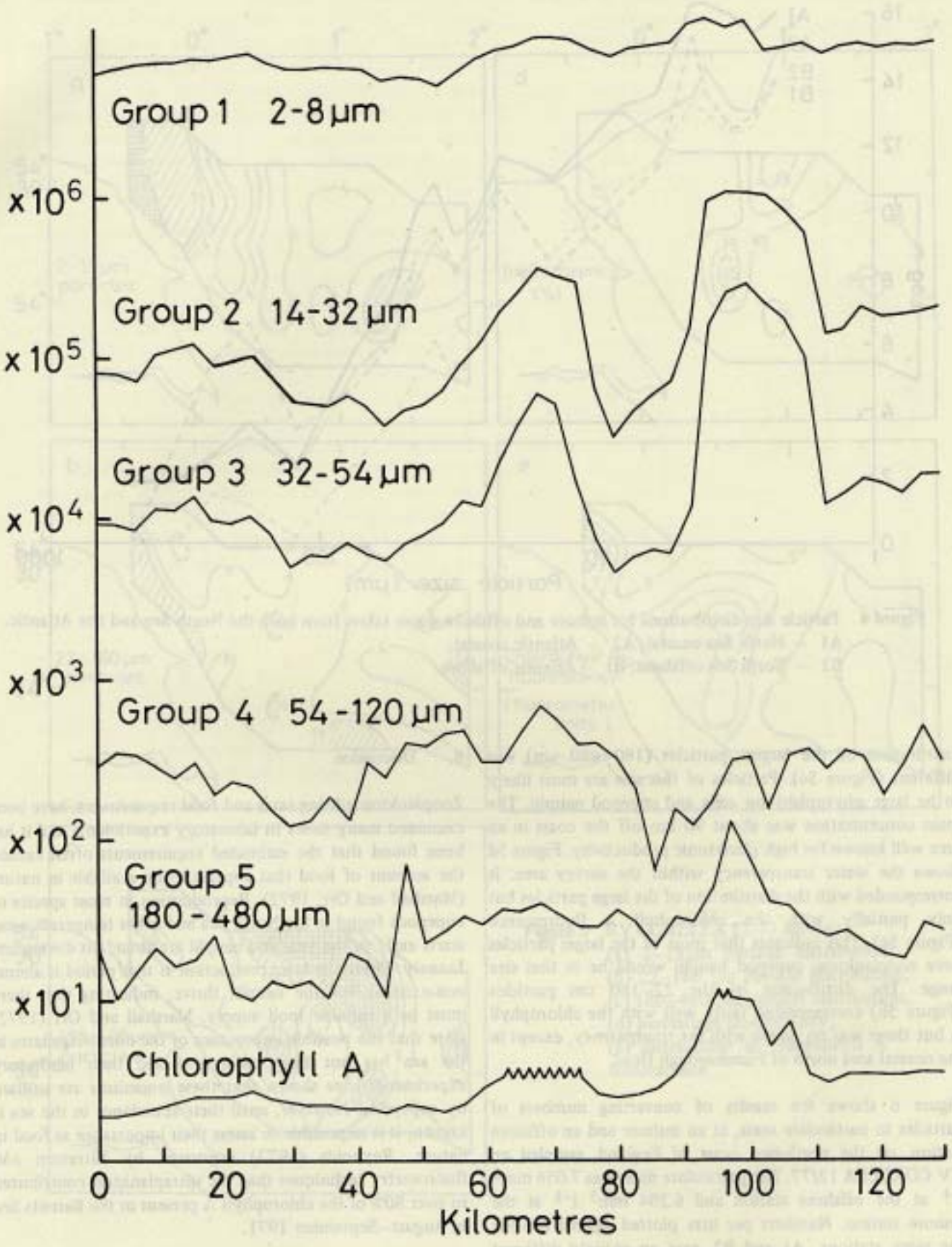

Figure 3 A transect over the Ushant oceanographic frontal systems, showing distribution of the five groups of particles in relation to chlorophyll A values

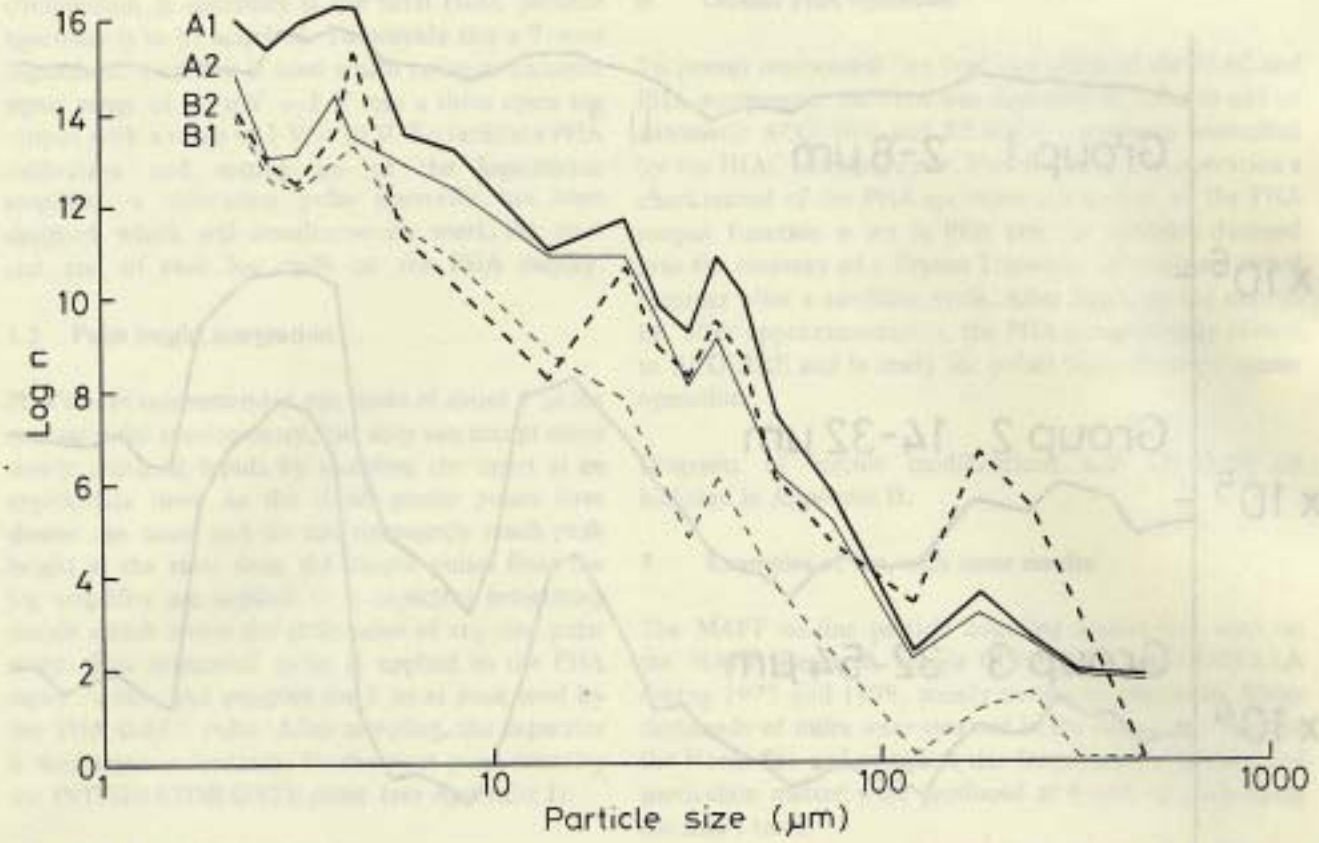

Figure 4 Particle size distributions for inshore and offshore water taken from both the North Sea and the Atlantic.
A1 – North Sea coastal; A2 – Atlantic coastal;
B2 – North Sea offshore; B1 – Atlantic offshore.

distribution of the largest particles (180–480 µm) was different (Figure 5c). Particles of this size are most likely to be large phytoplankton cells and copepod nauplii. The main concentration was about 90 km off the coast in an area well known for high planktonic productivity. Figure 5d shows the water transparency within the survey area: it corresponded with the distribution of the large particles but only partially with the chlorophyll A fluorescence (Figure 5e). This indicates that most of the larger particles were zooplankton; copepod nauplii would be in that size range. The distribution of the 22–160 µm particles (Figure 5b) corresponded fairly well with the chlorophyll A but there was no match with the transparency, except in the coastal area north of Flamborough Head.

Figure 6 shows the results of converting numbers of particles to particulate mass, at an inshore and an offshore station, on the north-east coast of England, sampled on RV CORELLA 12/77. The particulate mass was 7.056 mm^3 l^{-1} at the offshore station and 6.384 mm^3 l^{-1} at the inshore station. Numbers per litre plotted against size for the same stations, A1 and B2, gave an entirely different representation, mainly due to the presence of large particles at the offshore station.

8. Discussion

Zooplankton grazing rates and food requirements have been examined many times in laboratory experiments and it has been found that the estimated requirements often exceed the amount of food that appears to be available in nature (Marshall and Orr, 1972). Reproduction in most species of copepods found in the North Sea and other temperate areas starts early in the year and nauplii are abundant during late January. Phytoplankton production at that period is almost non-existent but the nauplii thrive, indicating that there must be a suitable food supply. Marshall and Orr (1972) state that the possible importance of the microflagellates in the sea has not been recognised and their laboratory experiments have shown that these organisms are utilised by copepods. However, until their abundance in the sea is known, it is impossible to assess their importance as food in nature. Reynolds (1973) estimated by filtration and fluorometry techniques that the ultraplankton contributed to over 80% of the chlorophyll A present in the Barents Sea in August–September 1971.

The data collected so far show that there are large numbers of particles less than 10 µm in size present in inshore and

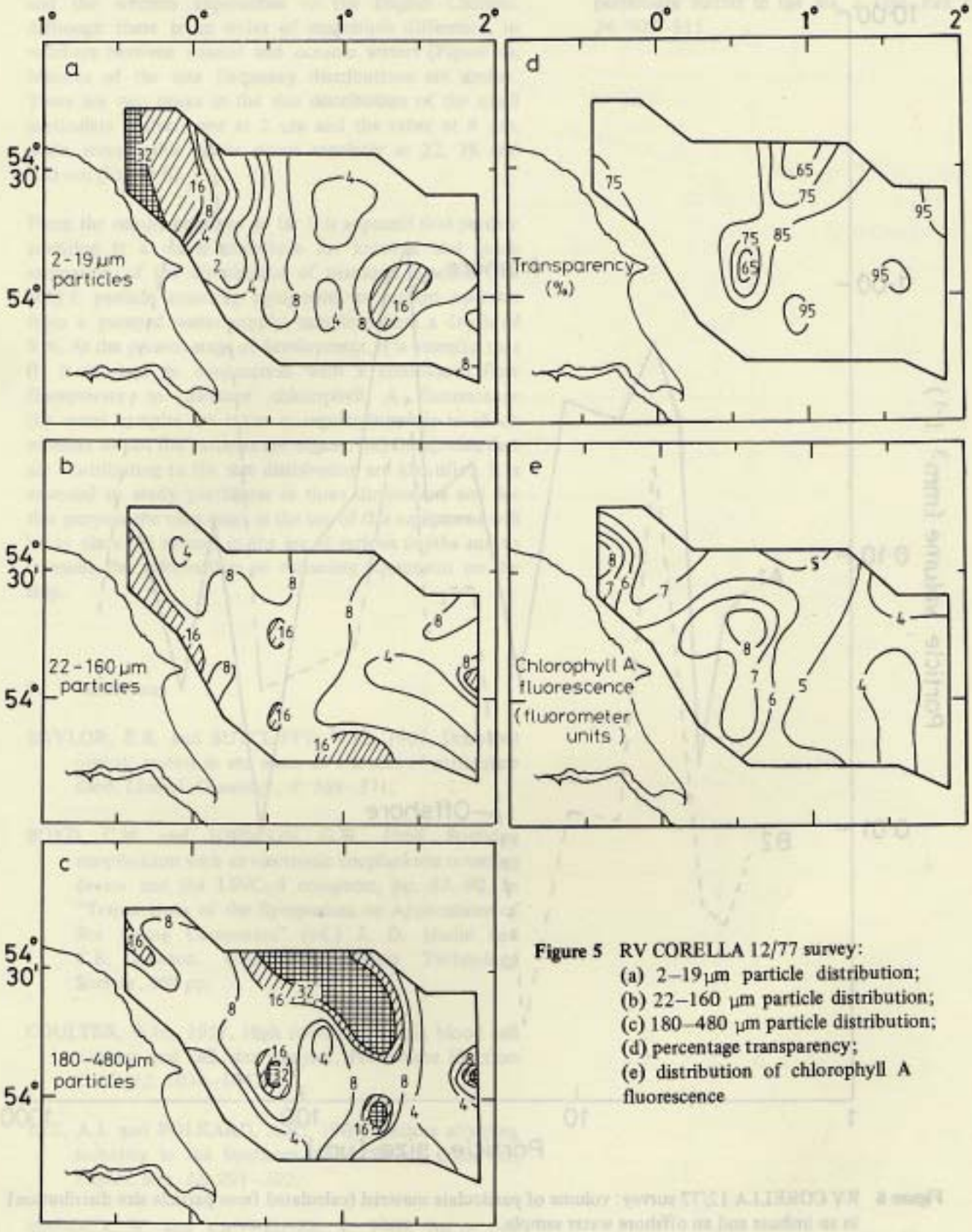

Figure 5 RV CORELLA 12/77 survey:
(a) 2–19 µm particle distribution;
(b) 22–160 µm particle distribution;
(c) 180–480 µm particle distribution;
(d) percentage transparency;
(e) distribution of chlorophyll A fluorescence

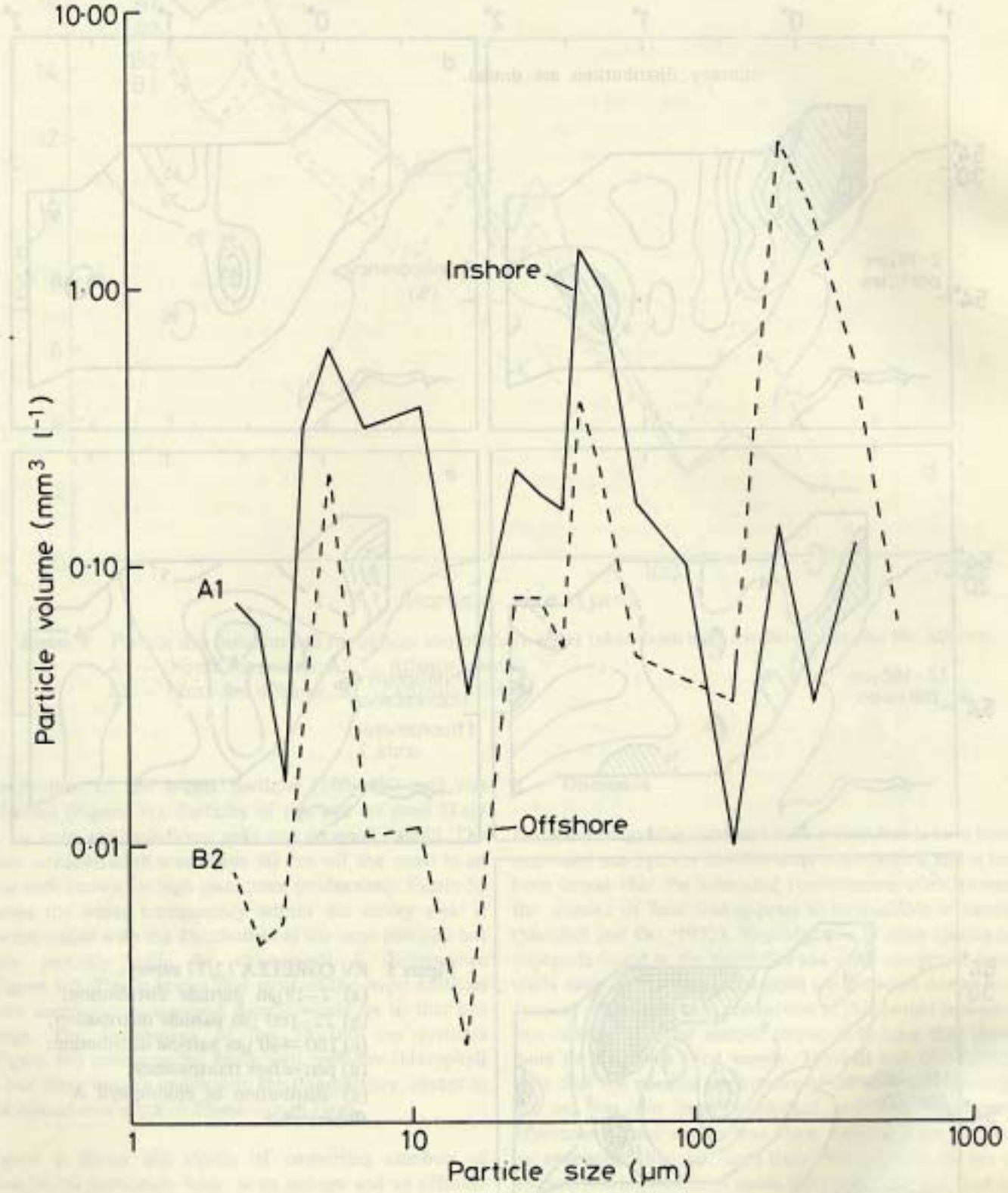

Figure 6 RV CORELLA 12/77 survey: volume of particulate material (calculated from particle size distribution) in an inshore and an offshore water sample.

offshore waters of the North Sea, the Atlantic shelf area and the western approaches to the English Channel. Although there is an order of magnitude difference, in numbers between coastal and oceanic waters (Figure 4), features of the size frequency distributions are similar. There are two peaks in the size distribution of the small particulate matter, one at 2 μm and the other at 4 μm, while recognisable peaks occur regularly at 22, 38 and 120 μm (Figure 6).

From the results obtained so far it is apparent that particle counting is a viable technique for location and quick estimation of the distribution of plankton patches. The MAFF particle counting equipment at present operates from a pumped water supply, sampling from a depth of 5 m. At the present stage of development, it is essential that (i) it is used in conjunction with a continuous flow fluorometer to measure chlorophyll A fluorescence (ii) water samples are taken at regular intervals to check whether or not the particles are organic (iii) the species that are contributing to the size distribution are identified. It is essential to study patchiness in three dimensions and for this purpose the next stage in the use of this equipment will be to place the sensors in the sea at various depths and to transmit the information to recording equipment on the ship.

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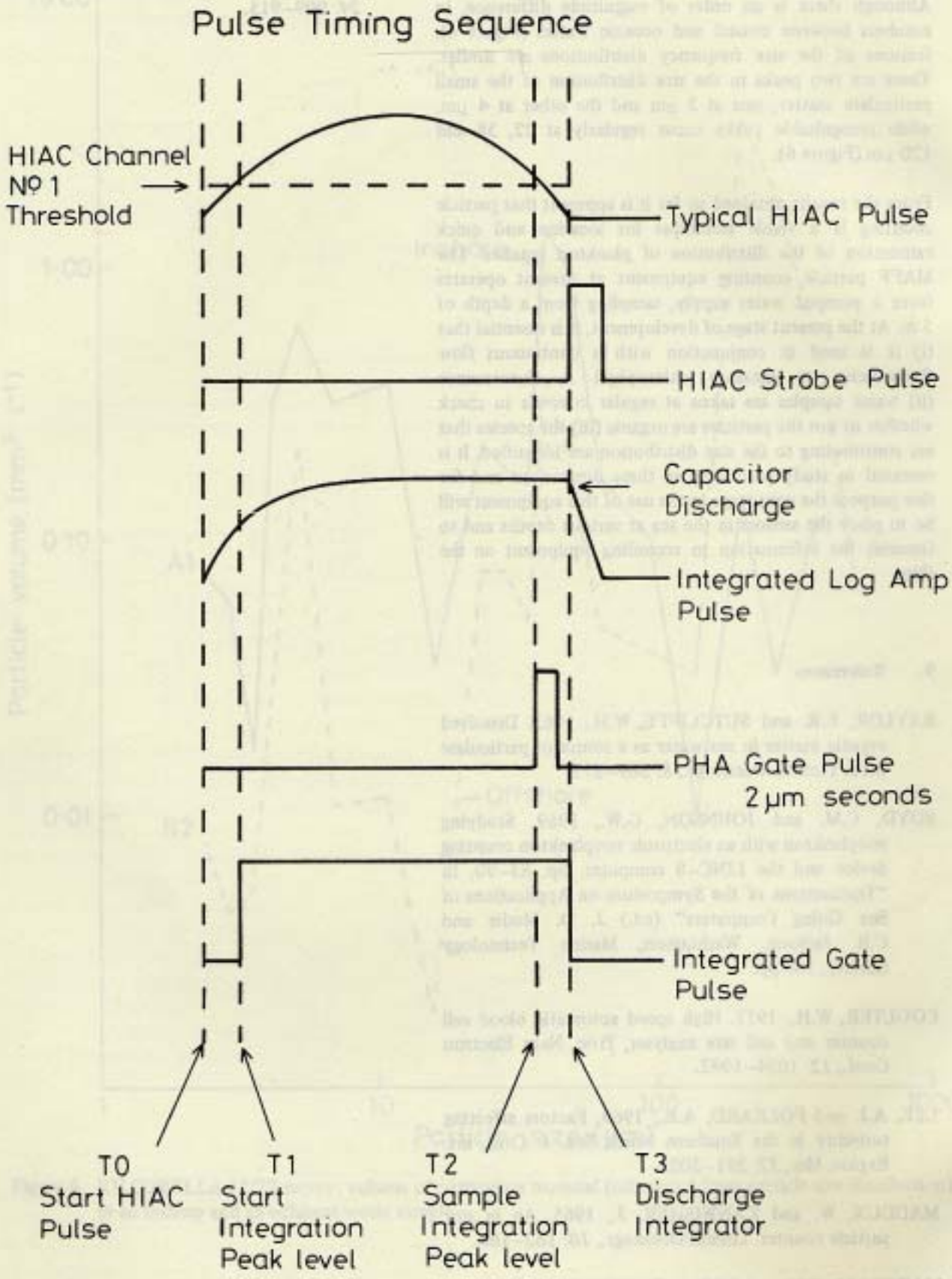
Appendix I Pulse timing sequence diagram



Appendix II HIAC and PHA circuit modifications