

AN *IN SITU* PARTICLE COUNTER¹

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ABSTRACT

Knowledge of the microdistribution and of the microecology of plankton is an important aspect of biological oceanography. Work in this area has been hampered by observational inadequacies. Operational considerations are discussed and a specific technique is presented which offers an improved solution for some of the problems involved in this field of study. A device is described which can measure the number and sizes of individual particles such as zooplankton organisms *in situ* and record their spatial distribution as it is towed along transects through the sea.

INTRODUCTION

Virtually all primary production in the seas takes place in the mixed layers above the thermocline. A large fraction of the total life in the oceans is to be found in this upper few hundred meters of water. For instance, Grice and Ilulsemann (1965) found minimum zooplankton concentrations above 200 m in northeast Atlantic Ocean stations to be from 4 to 7 times as high as those found at any depth below 200 m. Their data indicate that from 12 to 37% of the total zooplankton in a water column greater than 4,000 m deep was found in the upper 200 m. The surface mixing region may be considered the most important environment in the sea's ecology.

Inhomogeneity or "patchiness" in the distribution of plankton has been recognized and investigated by a number of workers (Hardy 1936; Cassie 1959; Cushing 1961). Cassie found evidence of nonrandom distribution of organisms on a scale of less than 1 m. Rates of equilibration that are rapid from the point of view of the physical oceanographer studying the behavior of large water masses may have very different significance in their effects on planktonic organisms. Phytoplankters with reproduc-

tive cycles measurable in hours can experience profound variations in light intensity due to the motions of wind-wave orbitals, convection cells, (for example, as in Langmuir circulation), internal waves, and turbulent current boundaries. Much is made of the "constancy" of the marine environment, but the relatively small range of environmental variations is reflected in the high sensitivity of many marine organisms to such variations. Local variations of temperature, light, salinity, and chemical composition on the temporal-spatial scale of mixing in the near surface regions may well have as much importance for the ecology of plankton as greater microenvironmental variations have for terrestrial ecology.

Important questions about the ecological nature of the mixing surface layer are: 1) what recognizable structures does it have in space and time; 2) is the distribution of organisms related to mixing patterns; 3) what are the causal relationships involved; and 4) what are the operational means to answer these questions?

The study of the sea's ecological microstructure presents serious experimental difficulties: The turbulent fluid environment lacks permanent reference points in the horizontal plane and even in the vertical dimension there often are, on the scale concerned, large relative motions between a shipboard observer and the object of interest; ranges of chemical and physical parameters are generally narrow, requiring highly sensitive methods for their measurement; and finally, the visualization of the small scale distribution of the organisms

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themselves is beyond the capacities of hitherto available experimental methods.

An ecologically useful description of the mixed layer's structure may be achievable by statistical characterization of the spectra of dimensions of circulation cells defined by measuring such variables as temperature or electrical conductivity of the water along sections drawn by *in situ* detectors. Electrical conductivity is an ambiguous property since it is a function of both temperature and salinity, but it offers the possibility of higher measurement speed than is obtainable with most thermal detectors, such as thermistors, that may be too slow in approaching equilibrium with rapidly changing water temperatures. In many cases conductance would be primarily a function of temperature because of the magnitude of the variations of the two variables that normally occur in the oceans. High response speed is, of course, required for spatial resolution along the tow. The determination and analysis of the "grain" or structural patterning of the mixing region can be aided greatly by the use of modern information handling and computing techniques. Temperature measurements are particularly useful because heat exchange at the sea-surface can "label" water that has recently been at the surface. Inhomogeneities in concentrations of specific chemical constituents are not as easily measurable as are patterns of temperature, but the continued development of polarographic and selective-membrane techniques may eventually yield suitable *in situ* detectors.

Conventional plankton net techniques do not offer the required temporal and spatial resolution, nor does the Hardy plankton sampler principle (Hardy 1926) appear promising for the detection and localization of organisms relative to structural features on a small scale. Using a $\frac{3}{4}$ -inch (1.9-cm) square opening at the front, the Hardy sampler filters $0.67 \text{ m}^3/\text{mile}$ ($0.36 \text{ m}^3/\text{km}$), with each mile (1.8 km) represented on a 2-inch (5.1-cm) length of 4-inch (10.2-cm) wide gauze (Hardy 1939). A thousand times as much gauze length for the same distance towed would be needed

to resolve occurrences of organisms to a scale of meters. More importantly, in most of the ocean, only a few animals of copepod size, or larger, could be expected within the 0.67 m^3 filtered. An aperture at least 0.1 m^2 in area would be necessary to overcome this sampling inadequacy, increasing the amount of gauze required still more. Even if it were mechanically feasible to collect them, the analysis of the gauze records and their correlation with the circulation pattern records would present severe data reduction problems and require an impractical amount of human labor.

Because of the relatively sparse occurrence of organisms in seawater, an ideal biological detector for microdistribution studies should have the largest effective sampling aperture area that is possible consistent with the required spatial resolution in the direction of its largest dimension. The detector should respond rapidly enough to provide the desired spatial discrimination along the direction of the tow. It should provide a measure of size or biomass. The factors of aperture size, speed, and proportionality of response limit the exactness with which plankton microdistribution can be observed and described in a manner analogous to the respective roles of camera aperture, shutter speed, and film exposure linearity in determining the quality of a photograph. In addition to the foregoing requirements for resolution, an ideal detector should provide an electrical output signal that would be compatible with machine data handling. A truly ideal device would determine the nature of each organism and provide species discrimination and identification.

We have developed a technique possessing many of the desired features described above. The basic principle employed is the same as that used in the Coulter Counter®, a laboratory instrument widely used for counting blood cells, bacteria, and other small particles (Coulter 1957). Our device is quite different in form and electrical operation, with features that adapt it to *in situ* operation under field conditions.

The basic detector consists of three an-

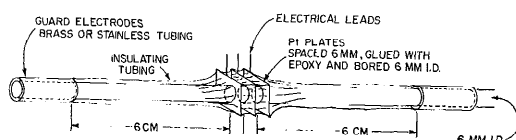


FIG. 1. Construction and dimensions of a detector electrode system, as used in the smaller of the two devices described.

nular platinum electrodes imbedded in the wall of a tubular passage through which water and suspended particles flow (Fig. 1). The volumes of water between the electrodes serve as two arms of an a-c bridge circuit. When a particle whose impedance differs from that of the seawater it displaces passes through the tube, the electrical balance of the bridge changes first in one direction and then in the other. If the specific impedance of the particle is large compared to that of seawater, the effect on the bridge is essentially proportional to the volume displaced. Two outer guard-electrodes isolate the detector from external electrical fields such as those produced by electrical equipment on the ship carrying the device.

DESCRIPTION OF AN ACTUAL DEVICE

In a simple design for a field instrument, the bridge is operated slightly off balance. This preserves desirable rejection of com-

mon mode disturbances such as conductivity changes in the water due to salinity variations or temperature change, but permits simplified electronics with no need for a phase-sensitive detection system (Fig. 2). The a-c system eliminates polarization problems with the electrodes and allows tuning of the first amplifier stages with consequent reduction of noise acceptance band-width. The high specific impedance of living organisms is not significantly reduced by their capacitive susceptance at the 100 kc/sec frequency used.

The output of the simple detector and low-pass filter is bi-phasic, showing a positive and negative pulse as a particle passes through the halves of the bridge. These pulses can be faithfully recorded on an ordinary audio frequency range tape recorder.

The transistorized electronics, consisting of a 100 kc/sec oscillator, tuned 100 kc/sec amplifier, detector, filter, and low-frequency amplifier with a low-impedance emitter follower output stage, are arranged around the tubular detector and cast in epoxy resin plastic to form a streamlined capsule. Direct current power is supplied from the surface and signals are transmitted to the surface by multiconductor cable. The capsule is mounted at the rear of a fiber glass frame bearing a conical plankton net whose small end is attached to the detector (Fig. 3). A

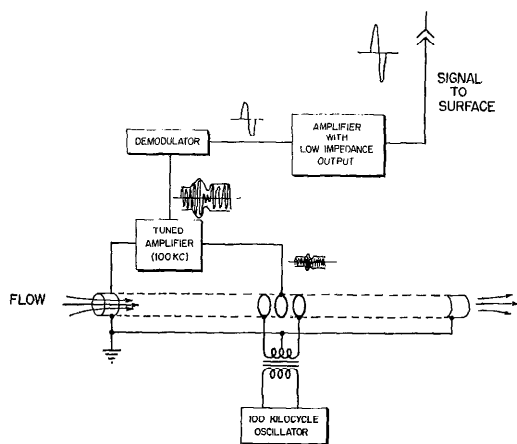


FIG. 2. Electrical scheme of the *in situ* particle detector.

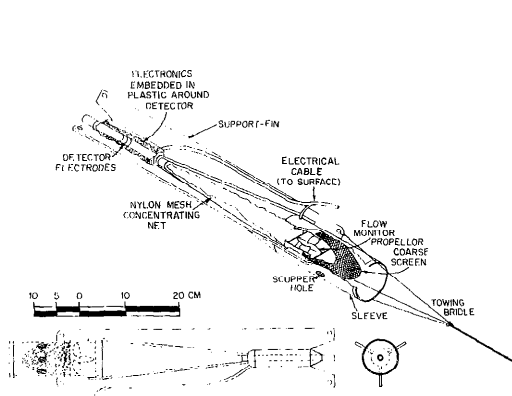


FIG. 3. Arrangement of detector and concentrating net for a small sampler.

coarse wire-mesh screen in the sleeve at the front of the apparatus prevents the entrance of large objects which might clog the detector aperture. Scupper holes in the sleeve provide an exit for objects deflected by the coarse screen. The side supports serve as stabilizing fins when the apparatus is towed. If greater concentration of the organisms is required than is provided by the small net built into the device, the flanged sleeve at the front may be clamped in the cod end of a conventional plankton net in place of a collecting jar.

A plastic propeller mounted in the sleeve at the front end of the apparatus rotates a magnet which actuates a magnetic-reed switch (not shown in Fig. 3). This monitors the flow into the front orifice of the net. Pulses from the flowmeter may be used to control an electronic counter so that particles passing through the instrument are represented as counts per volume.

Performance

An electrode and aperture structure with the dimensions shown in Fig. 1 is sensitive to 0.001 mm^3 particles with the output pulse five times the voltage of background noise. The response is proportional to volume for particles up to about 2 mm^3 . In practice, the upper size limit is set by the dynamic range of the amplifiers which use a low-voltage power supply. Another limit is encountered when any dimension of the particle approaches a value of about one-half the length or diameter of the detector cells. Increase in particle size beyond this limit causes disproportionate increases in signal. In practice, the 3-mm mesh screen rejects such large particles.

The use of the instrument involves a number of assumptions about the nature of particle populations in the water. Bubbles appear as electrically "solid" particles and care must be taken when using the device at shallow depths to avoid towing in the ship's wake or at depths penetrated by bubbles produced by breaking waves. Except for bubbles, nonliving particles of zooplankton size which might be misinterpreted are very rare in the sea. The large flocculent

TABLE 1. Comparison of visual counts of particles caught in a No. 6 retaining net with the electrically determined counts of the in situ device ahead of the net. The electrical size threshold used was 0.04 mm^3 . These Buzzards Bay samples appeared to be made up mostly of immature calanoid copepods. Some Phacocystis was present

Tow no.	Counts		Differences	% difference
	visual	electrical		
1	473	464	-10	-2.1
2	395	396	+1	+0.3
3	445	429	-16	-3.6
4	342	352	+10	+2.9
5	318	486*	+168	+52.8

* This value probably includes false counts resulting from air bubbles trapped in the net during lowering. This view is supported by an excessive initial counting rate on the magnetic tape record of this 3-min tow.

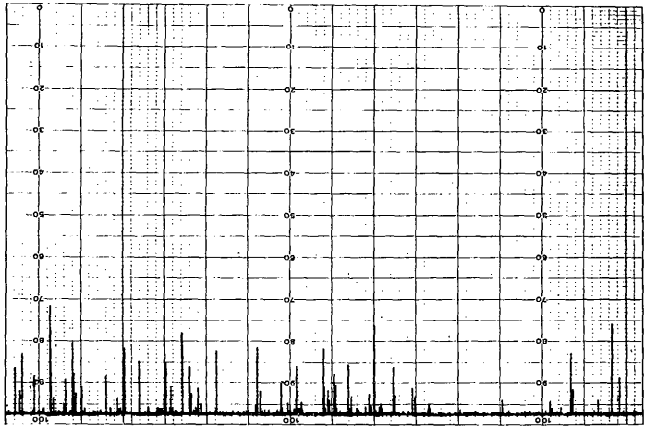
aggregates reported by Riley and others (Riley 1963; Nishizawa, Fukada, and Inoue 1954) seemingly are too electrolytically permeable to cause difficulties in the zooplankton size range.

Conventional net tows made while using the instrument aid in interpreting the results. If the sampled population is comprised of a few species of different sizes, analysis of the electrical pulse heights will permit species identification of individual organisms.

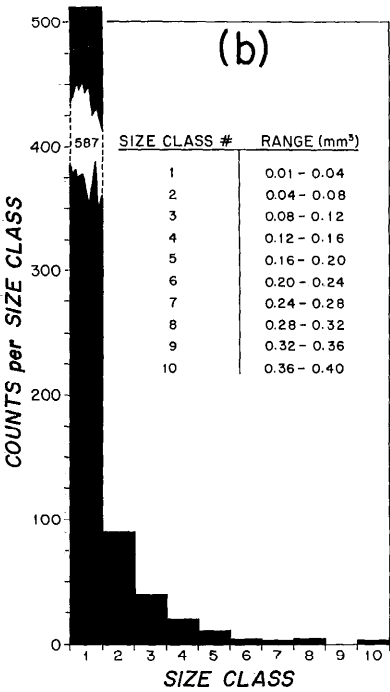
A small retaining net may be placed at the rear of the device to catch all particles counted during short tows. Direct examination of the contents of the net yields counts that agree within a few per cent with those obtained by electronic counting. Table 1 shows the results of five such comparisons.

Coincidence of particles passing through the detector is unlikely in most field situations because zooplankters rarely occur at sufficiently high densities. If coincidence does occur, statistical compensation may be possible. The more serious problem is the scarcity of zooplankton in most oceanic regions. Work has been done in the Sargasso Sea using a $\frac{1}{2}\text{-m}^2$ plankton net with the particle counter in the cod end. This raised the count rate to approximately 1/sec when towing at 2 knots (3.7 km/hr). In comparison, several counts per second were

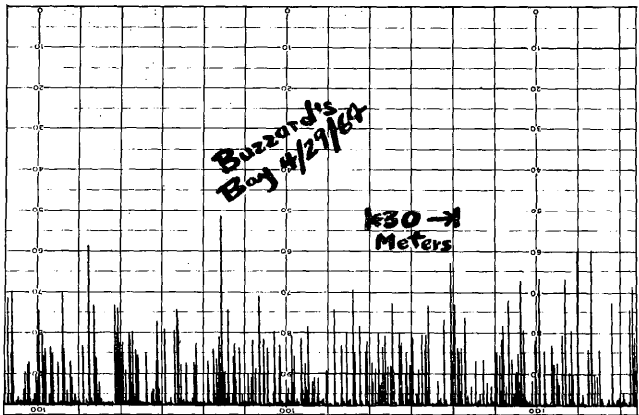
(a)



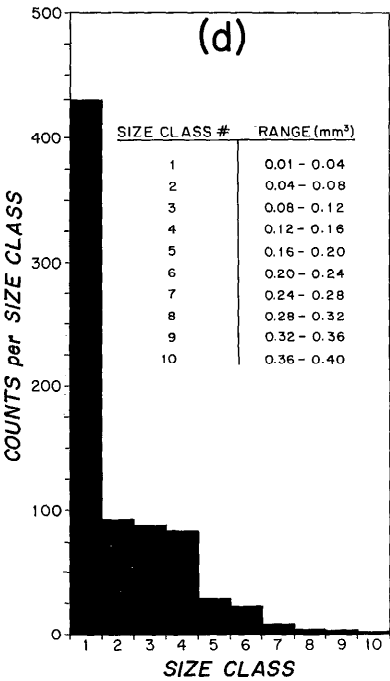
(b)



(c)



(d)



obtained in Buzzards Bay (Massachusetts) using the particle counter alone. Fig. 4 shows portions of paper chart records made for the two locations and also shows the observed size distributions for samples from each location. The paper chart records were made from magnetic tape recordings and suffer from the limited response speed of the paper chart recorder. The Buzzards Bay population shown in the Fig. 4c, d was also sampled by six successive 4-min Clarke-Bumpus sampler (Clarke and Bumpus 1950) tows, and the mean wet volumes of the six samples collected were estimated by the mercury-flotation method (Yentsch and Hebard 1957) to be $62 \pm 14 \text{ mm}^3/\text{m}^3$. If the mean volume per particle for the sample shown in Fig. 4c, d is taken as approximately 0.1 mm^3 and this figure is multiplied by the $654/\text{m}^3$ counts $> 0.04 \text{ mm}^3$, the estimated wet volume for this sample as determined electrically would be $65.4 \text{ mm}^3/\text{m}^3$. These results and the "kneec" in the histogram (Fig. 4d) apparently are influenced by the fact that both the *in situ* counter's concentrating net and the Clarke-Bumpus sampler's net were of No. 2 mesh netting which has a mesh size in close agreement with the linear dimensions of 0.04 mm^3 particles of normally compact form. Both the plankton net and *in situ* counter's netting were of No. 6 mesh when the Sargasso Sea sample was taken. The concentrating net is the most troublesome part of the apparatus, and the present arrangement could probably be improved upon.

The device, as described, has been used for a year in zooplankton studies (to be reported elsewhere). Evaluation of the results suggests that a larger effective sampling aperture would have improved the effectiveness of the statistical analysis of

the data, especially for observations made in the Sargasso Sea where mean concentrations of organisms are low. The use of too large a concentrating net ahead of the detector presents the risk that particles may be retained within turbulent, slow-moving water trapped in the net so that a time lag occurs that reduces resolution. Increasing the diameter of the detector tube to increase the flow rate through it can improve this condition and thus increase the effective sample aperture that can be realized.

A recently constructed version of the device has a 45-cm long detector tube of venturi form which is 5 cm diameter at the entrance and exit, and constricts smoothly to 2.5-cm diameter in the central portion where the electrodes are located. The high flow capacity of this design not only improves performance with a concentrating net, but it also allows operation without any net for full ship speed synoptic surveys of large areas when reduced resolution is acceptable. To test the latter mode of operation, the detector was mounted in a tow body as shown in Fig. 5. This configuration has been tested at sea at speeds up to 12 knots (30 km/hr) (maximum for the ships used). The flow-velocity through the electrode passage was 1.7 times the towing velocity throughout the speed range, which means that 1.6 m^3 of water was sampled per mile ($0.86 \text{ m}^3/\text{km}$). The lower useful limit of particle size was less than 0.03 mm^3 . This limit was set not by electrical noise but by the effect of conductivity gradients apparently associated with turbulence in the water. Since the effect of this noise source increases as the ratio of detector volume to particle volume is increased, further enlargement of the detector's diameter does not appear profitable.

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FIG. 4. Comparison of particle counter records from Sargasso Sea and Buzzards Bay. (a) Paper chart record of electrical responses during a 450 m portion of a tow made in the Sargasso Sea off Bermuda. Filtering rate was approximately $0.3 \text{ m}^3/\text{m}$. (b) Size-frequency plot for the Sargasso Sea sample. The 750 total counts are represented from a volume of 170 m^3 filtered. (c) Paper chart record from tow made in Buzzards Bay. The linear scale is the same as for (a) but filtering rate was only $0.001 \text{ m}^3/\text{m}$. (d) Size-frequency plot for the Buzzards Bay sample. The 754 total counts shown came from a sampled volume of about 0.5 m^3 .

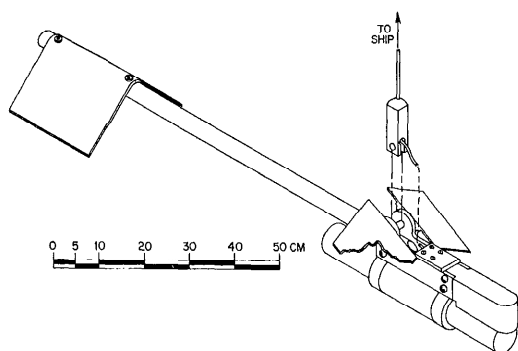


FIG. 5. Embodiment of the *in situ* detector suitable for high-speed towing.

OTHER APPLICATIONS

The detector can be used as a laboratory tool and gives satisfactory responses with formalin-preserved materials (for example, copepods) that have been resuspended in seawater or NaCl solution. The device can also be used to count laboratory populations without injuring them.

In situ detection of particles in the phytoplankton size range is quite feasible with this technique. Narrow-band electronics permit acceptable signal-to-noise ratios for phytoplankton-sized particles with detector apertures approaching 1 mm diameter. Such apertures could be prevented from clogging by using screens. Many of the small-size particles in the sea are nonliving. Unlike solid particles, cellular structures display a large capacitive reactance component of impedance. Therefore, if a high and low frequency measurement were made simultaneously, comparison of the impedances of the same particle at the two frequencies

could discriminate between living and non-living particles. This approach offers the hope of expanding the usefulness of the *in situ* detector to include study of nanoplankton and leptonel.

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