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**SONIC SCATTERING LAYER STUDIES
— AN INTERIM REPORT**

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REPORT I

SONIC SCATTERING LAYER STUDIES - AN INTERIM REPORT

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This report contains results of work carried out for the Office of Naval Research of the Navy Department under research project NR 165-195, Contract Nonr - 1135 (01).

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summary

1. A submarine photometer sufficiently sensitive to measure light intensity at the depth of the scattering layers has been designed. The lower threshold of this instrument was reached in California waters at a depth of 185 meters whereas in Bermuda this threshold was not attained until the instrument had been lowered to a depth of at least 400 meters.
2. Photosensitive pigments have been extracted from mixed invertebrate plankton collected from the scattering layers. These pigments absorb maximally at intervals between 425 and 585 m μ .
3. Infra-red image converters have been adapted to obviate the use of visible light in dissections and extraction procedures. The results obtained indicate that even the dim red light ordinarily used for illumination in such work destroys or modifies certain of the photosensitive pigments.

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introduction

Background: The occurrence of more or less well defined sonic-scattering layers in the sea is widespread. Their definition and the depths which they attain during daylight hours vary with weather and latitude. In a given area the layer is more prominent when the weather is clear than when the sky is overcast. The layers in transparent tropical waters occur at much greater depths than those in turbid coastal waters. Under polar conditions of permanent daylight the layer is seldom noticed or at best weakly defined. When day-night conditions are restored, however, the layer is again noticeable. Although some have been reported to maintain constant depth, a pattern of diurnal migration is commonly associated with the layers and is most often toward the surface as the sun sets and toward greater depth as the sun rises.

Collections have shown that the concentration of living organisms is greater in the layers than above or below them. Recent work by Herdman (1) on the R.R.S. DISCOVERY II has revealed the presence of a non-migrating shallow layer in areas where a well-defined thermocline is noted. It is suggested that this may be caused by phytoplankton or detritus arrested in its descent by a density interface. Several investigations have been made of the biological composition of the deep layers, and at present it seems that both plankton and nekton are involved. Whether the plankton and nekton inhabit individual components of the scattering layers, as suggested by Tucker (2), and whether the nekton are present as predators on the plankton are questions which are as yet unresolved.

Regardless of the composition of the layers, however, the close correlation between their behavior and the degree of submarine illumina-

tion would seem to indicate that the primary stimulus is photic. Whether all the organisms in the layer are responding to light or whether only a few phototropic species are followed by predators is yet to be determined.

The initial step in any phototropism--that is, response to light--must involve absorption of all or part of the incident stimulating radiation. In vertebrates the light sensitive retina has been shown to contain pigments which are changed reversibly by exposure to light. Certain of these have been isolated and their chemical constitution and metabolic relationships have been established. Visual spectral sensitivity--that is, sensitivity to various wave lengths of light--in the animals concerned has been shown to depend on the spectral absorption of these pigments.

Little work has been done with photosensitive substances in invertebrates, but those which exhibit phototropisms, whether or not they possess true eyes, must contain substances which are sensitive to light.

Acknowledgements: The submarine photometer, on which the success of this investigation depends so heavily, was designed by James M. Snodgrass of the Special Developments Division, Scripps Institution of Oceanography. We are especially indebted to him for his interest and the time spent in devising this sensitive and ingenious instrument. Its construction was supervised by G. Frank Hetzel, of the same Division.

We should like to thank Roger R. Revelle for the hospitality of the Scripps Institution over a period of several months and for the use of the research vessels CHEST and E. W. SCRIPPS on numerous occasions.

Denis L. Fox very kindly made available the use of his spectrophotometer.

eter and the other facilities of the Biochemistry Division of the Scripps Institution.

The time-depth recorder used in California was lent by Gordon H. Tucker, Navy Electronics Laboratory, San Diego. The closing net and release mechanisms were borrowed from the U. S. Fish and Wildlife Service.

The first dissections to be carried out in total darkness were with the aid of an infra-red image converter (Snooperscope) lent by Frederick Crescitelli, University of California at Los Angeles.

Technical advice and assistance were given freely by the Bermuda SOFAR group of Columbia University, directed by Gordon Hamilton.

The friendly co-operation and interest of William H. Sutcliffe, Jr., Director of the Bermuda Biological Station, made the work we were able to accomplish in Bermuda a very agreeable task.

present work

Work of the past year has been devoted mainly to instrumentation, which has been costly and slow. Some preliminary results of interest have emerged, however, and are presented here. Originally, it was intended that all the work should be done in Bermuda, but a combination of factors prevented this.

The development and final calibration of the special submarine photometer were carried out in California, and demanded our presence there. While there, opportunity was given us of embarking on other phases of the enquiry, and this was done. This was particularly fortunate since there was considerable delay in commissioning the R. V. PANULIRUS

in Bermuda and, indeed, the fathometer was not installed until October.

The project is to be continued in 1954 at the Scripps Institution of Oceanography under contract with the Geophysics Branch of the Office of Naval Research. This report, therefore, while essentially a progress report, is presented as the final report for the contract between the Bermuda Biological Station and the Biology Branch of the Office of Naval Research, which was effective from April 15, 1953 to February 14, 1954.

METHODS

Work at Sea

Light measurement: For the measurement of the intensity and spectral characteristics of light at depth, a special submarine photometer has been designed at the Scripps Institution of Oceanography. It is a self-contained, recording instrument which may be attached to the hydrographic cable near the net in order that light measurements may be made simultaneously with collection from the scattering layer communities.

In the work reported here a survey, non-recording model, constructed during the development of the self-contained instrument has been used. The survey model consists of two units, one on deck and the other submerged. They are connected by demolition cable on which the submerged unit (Fig. 1) is lowered. The wiring diagram for this photometer is shown in Fig. 2. The instrument was calibrated with a Weston footcandle meter through seven decades of light intensity. A direct relationship exists between light intensity and millimeter readings. The lower threshold of the instrument is at 3.25×10^{-9} footcandles.

Although, by virtue of the extreme sensitivity of its photomultiplier

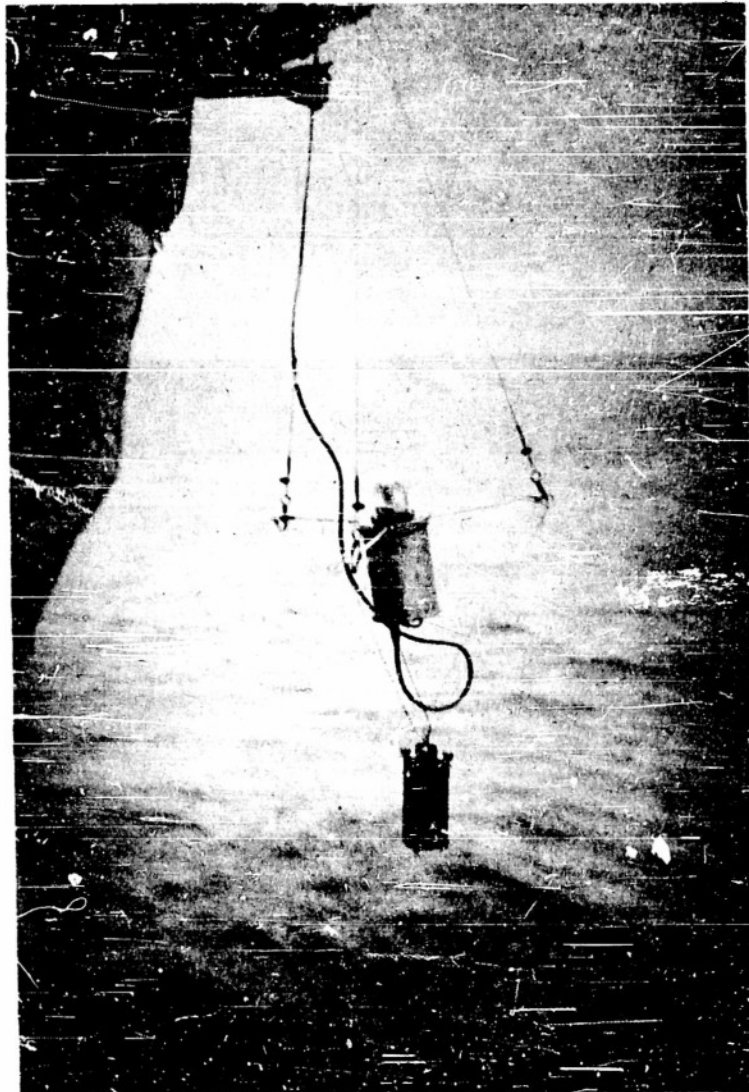
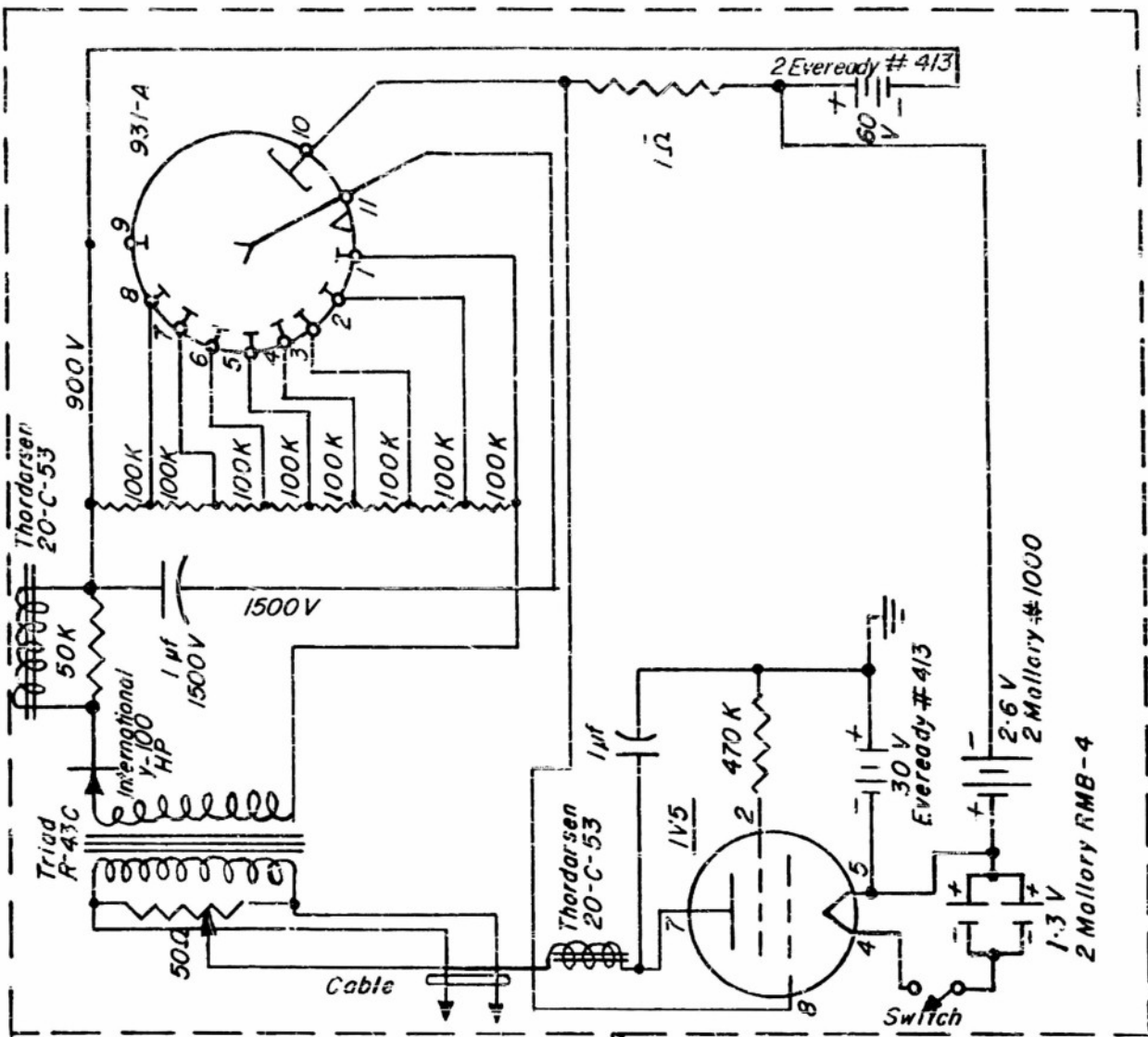


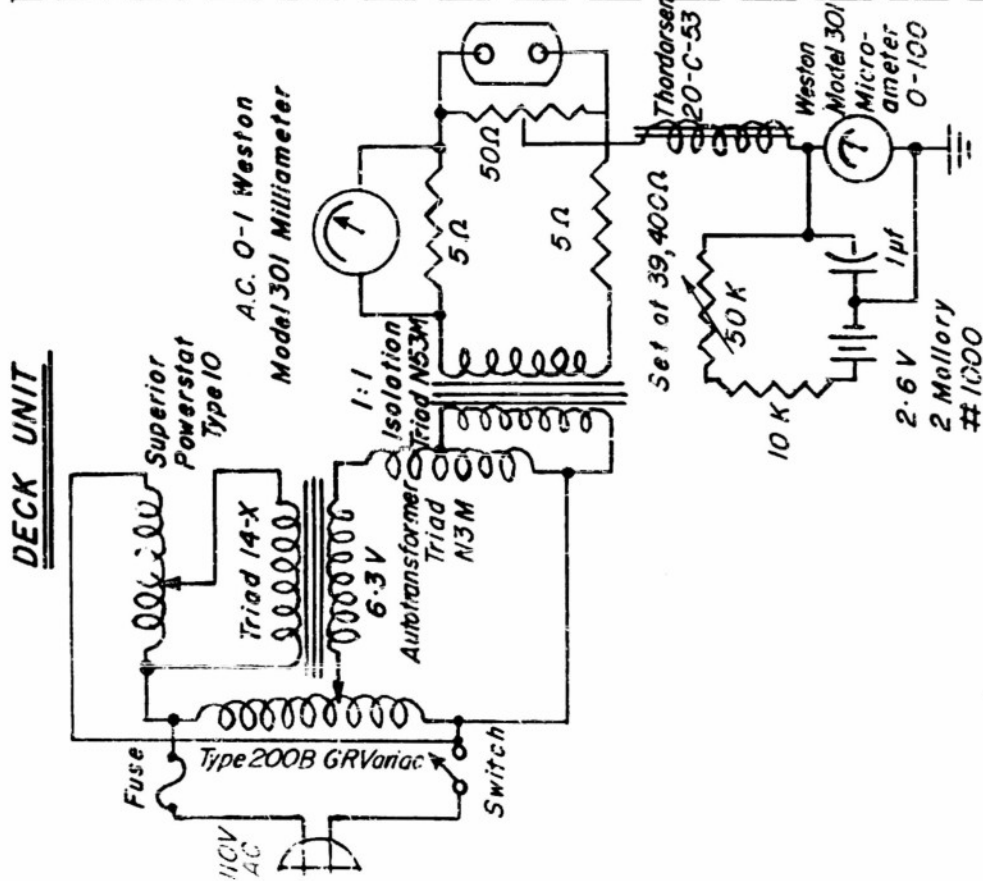
Figure 1. Submerged unit of photometer rigged for lowering on demclition cable. Time-depth recorder attached below photometer.

tube, the exploratory model has yielded interesting preliminary information, the necessity for building a self-contained instrument is obvious, if light measurements and plankton collections are to be made simultaneously.

Plankton Collections Standard plankton techniques were used in both the Bermudian and Californian phases of the work.



SUBMERGED UNIT



**SURVEY PHOTOMETER
FOR BERMUDA — MODEL E-266**

SPECIAL DEVELOPMENTS DIVISION
SCRIPPS INSTITUTION OF OCEANOGRAPHY

Figure 2

In Bermuda a stramin net with a mouth diameter of two meters was used. The last meter of the net was lined with a sleeve of OXX (38 meshes to the inch) bolting silk which led into the cod-end proper. This was to prevent destruction of the more delicate planktons by tumbling action

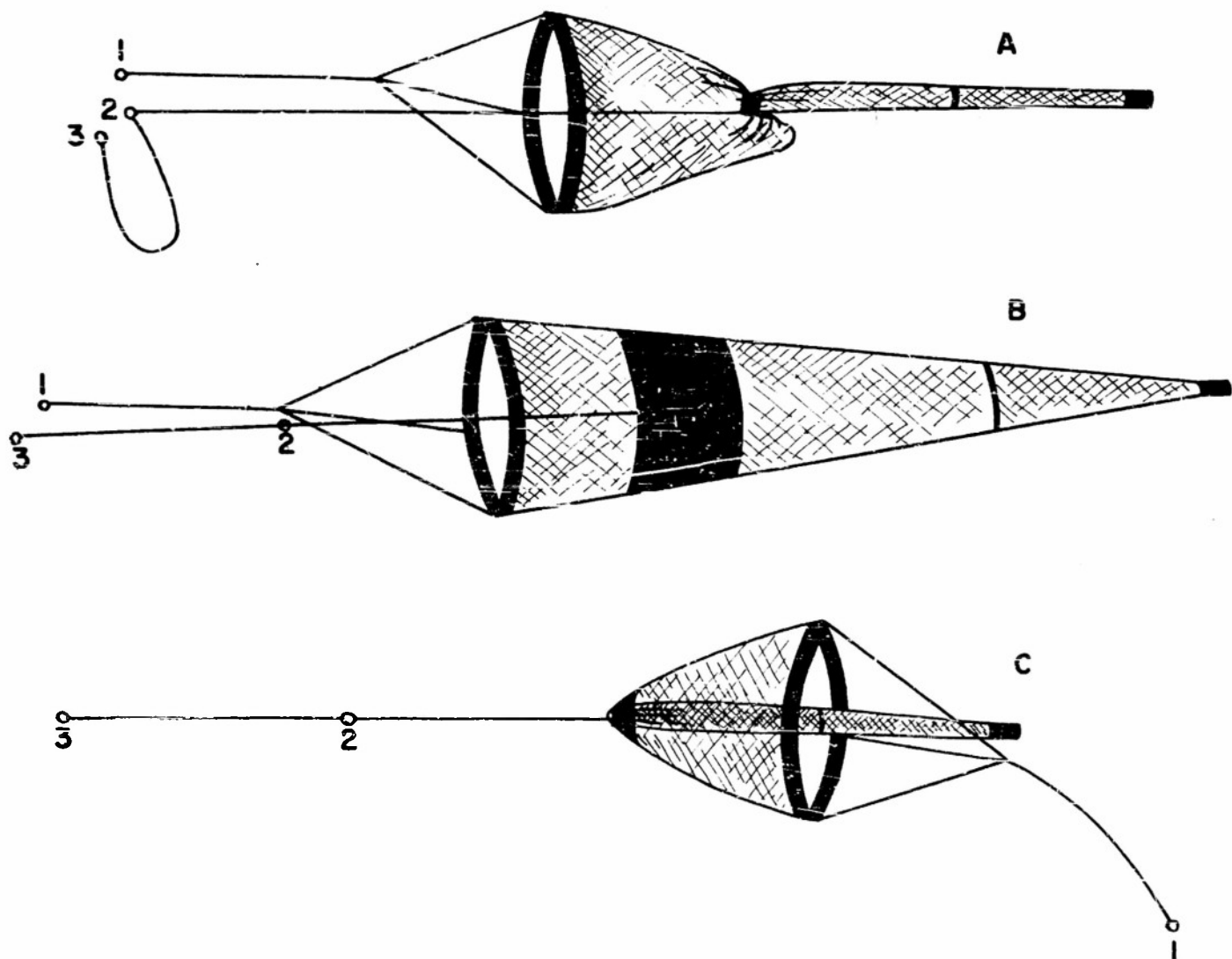


Figure 3. Opening and closing net in closed (A), open (B) and closed (C) positions.

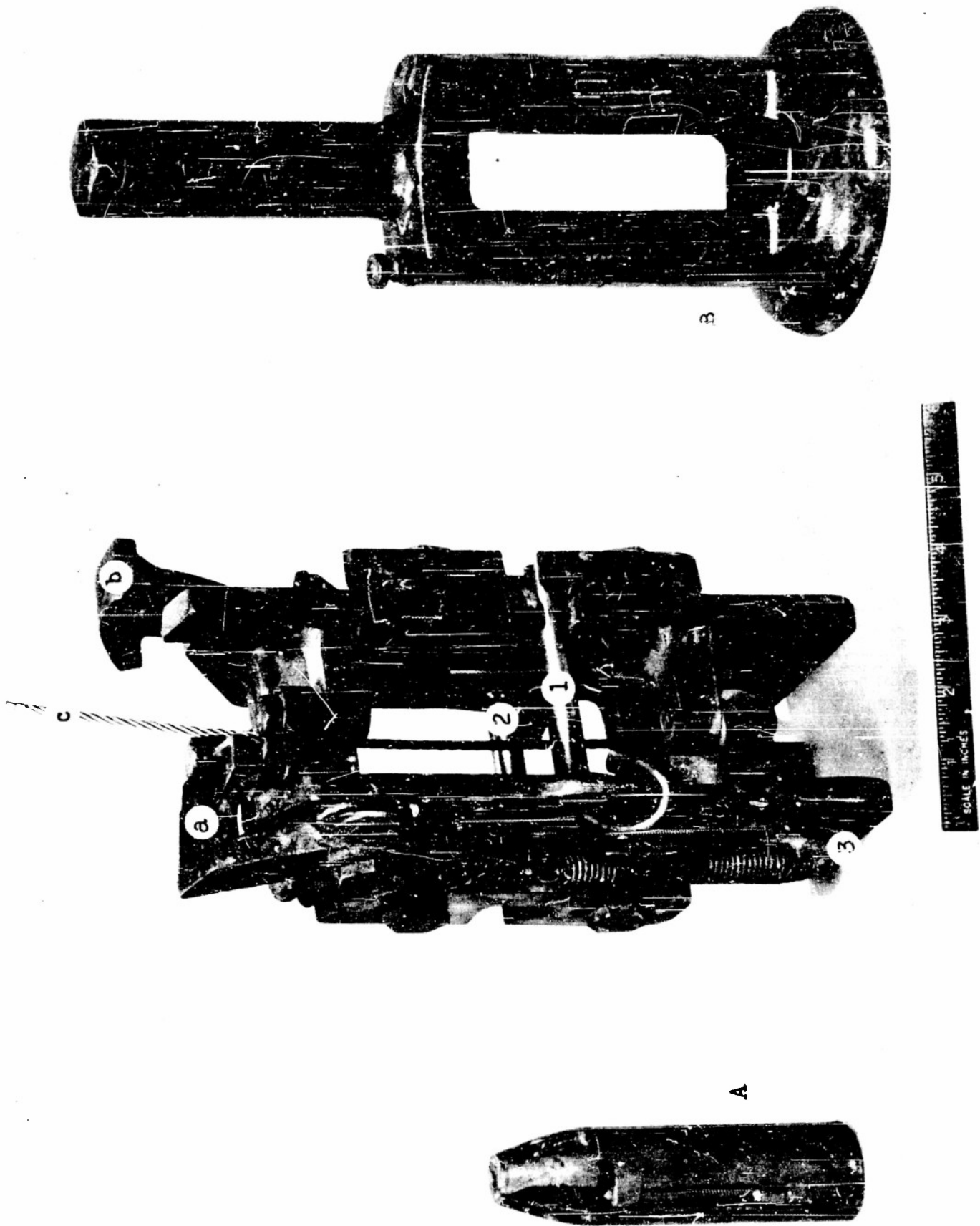


Figure 14. Opening and closing mechanism

along the coarse stramin walls.

In California a cotton net was substituted for the stramin net. This net corresponded approximately to 0000 bolting silk (about 18 meshes to the inch) and it was unnecessary to use a bolting silk sleeve.

Both nets were rigged for opening and closing at depth, and the method used in California is shown in Figures 3 and 4. The net is sent down closed (Fig. 3, A) with the two rings (2 and 3) on the pucker line attached respectively to the first release bar (Fig. 4, 2) and point of ultimate attachment (Fig. 4, 3) on the release mechanism. The ring (Fig. 3, 1) on the bridle of the net is attached to the second release bar (Fig. 4, 1) of the release mechanism.

When the net has reached the desired depth (this is estimated from the wire angle and length of wire out) it is opened by sending down the first messenger (Fig. 4, A). The messenger strikes the platform (a) which releases the catch holding the first release bar (2). The ring of the pucker line (Fig. 3, 2) is thus released, and the water forces the net open and takes up the slack in the pucker line. The net is now fishing (Fig. 3, B).

When it is desired to close the net at the end of the haul the large nesting messenger (Fig. 4, B) is sent down the wire. This fits over the messenger A and strikes the platform b. This releases the second release bar (1) and the ring on the bridle (Fig. 3, 1) is thus freed. The pucker line closes the net and the ring falls back into the position shown in (Fig. 3, C). The closed net is then brought to the surface with the pucker line ring (Fig. 3, 3) attached to the small hole in the release mechanism (Fig. 4, 3).

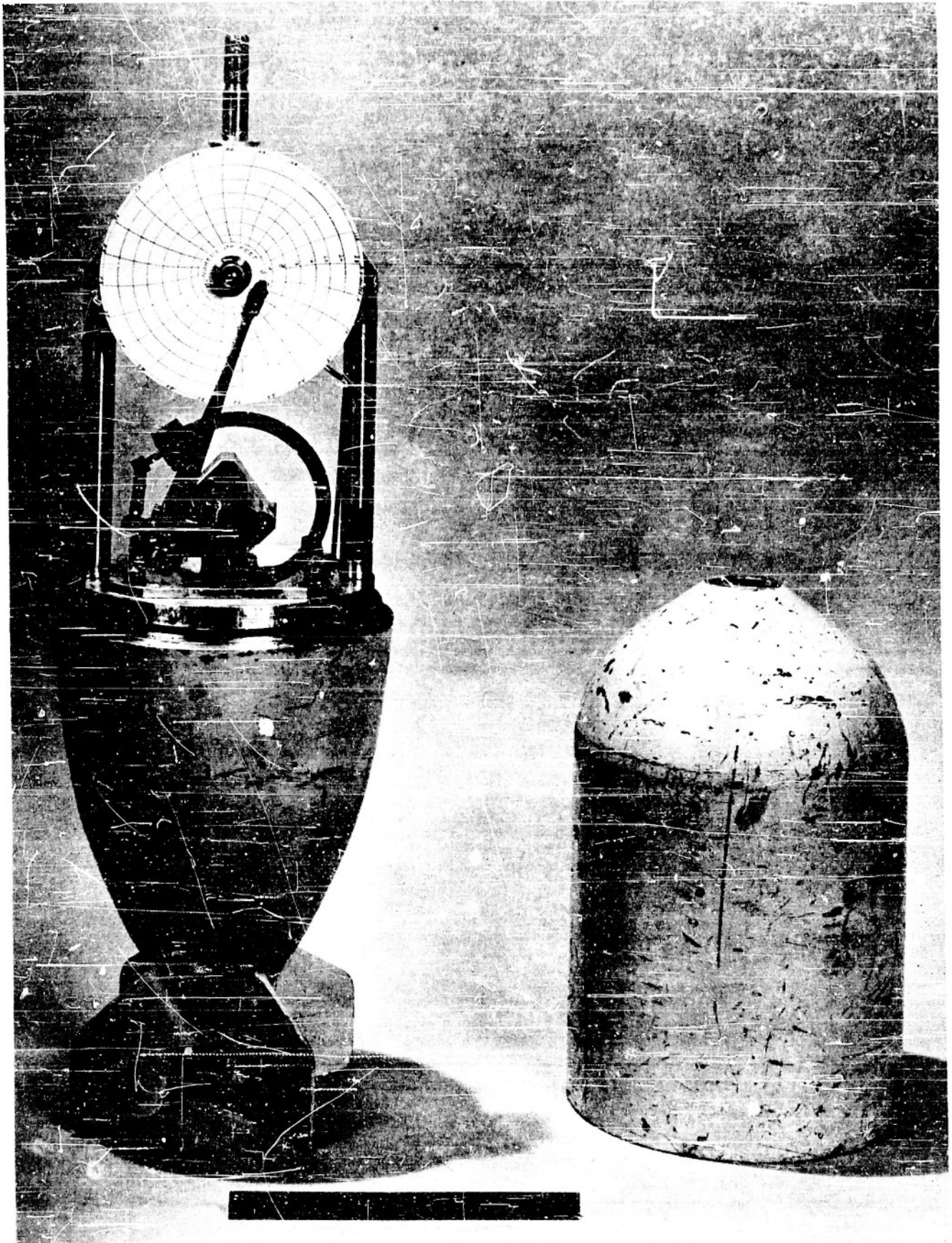


Figure 5. Time-depth recorder disassembled.

position causes such turbulence at the mouth of the net that the plankton is prevented from entering.

The method used in Bermuda for opening and closing the net differs in minor respects from that described above, but is essentially similar. Two pucker lines of different lengths are used to close the net in the two closed positions. Provided that a fairly small wire angle is maintained both methods work with a high degree of success. The complication of the second pucker line in the method used in Bermuda renders it a trifle more prone to failure.

Hauls were generally of an hour's duration and were oblique through the depth of the scattering layer as shown on the fathometer.

Depth recordings: A time-depth recorder of the type shown in Fig. 5 was attached to the cable five meters below the plankton net. This instrument proved extremely satisfactory and did not fail on any occasion. A depth record of the type shown in Fig. 6 (a tracing of an actual record) was obtained. The instrument was calibrated with the aid of reversing thermometers, and it was found necessary to apply a correction factor of 0.86 to the depths indicated on the disc. A smaller and more compact instrument was constructed and calibrated for the Bermuda operation by the Woods Hole Oceanographic Institution. This worked on precisely the same principle (a Bourdon tube and a 24-hour clock) but had the disadvantage that the recording-disc was extremely small.

Detection of scattering layers: In this preliminary work we have been confined in our detection of the sonic-scattering layers to the use of conventional echo-sounders. In California the R. Vs. CREST and the E. W. SCRIPPS are equipped with NMC-2 fathometers with 17 k.c. sound-

pulsers. In Bermuda the R. V. PANULIRUS is fitted with an older model NMC fathometer. This type of instrument has proved satisfactory in previous investigations (Boden, 3), but, unfortunately, it has been installed on the PANULIRUS in such a way that it will operate only when the ship is hove to.

Handling of plankton: It was thought desirable, as a first step, to determine what types of photosensitive pigments are present in whole plankton samples. When the live sample is brought on board, therefore, it is subjected to only a cursory examination while still in the cod-end. This is usually sufficient to reveal the dominant components of the plankton and note the presence of others. Specific identification and accurate counts are impossible and, indeed, unnecessary at this stage.

Dark adaptation of live plankton: Exposure to light of intensities greater than those normally encountered has been shown to reduce the concentration of photosensitive pigments in living animals (Hagins and Rushton, 4). Surface light intensities are certainly abnormally high for those planktons associated with the deep sonic-scattering layers, and it is necessary to keep them in total darkness for a period long enough to allow regeneration of their photosensitive materials before extraction of these substances is attempted. It is known that a two-hour dark-adaptation period is adequate for this regeneration in vertebrates, and it was found that the majority of animals associated with the sonic-scattering layers would survive this period in the dark-adaptation chamber.

Each plankton sample was transferred from the cod-end of the net to a metal cylinder the ends of which were tightly covered with bolting silk. This cylinder was placed in a small light-proof container fitted with

opaque inlet and outlet tubes to permit circulation of sea water during dark adaptation.

After two hours the sea-water supply was disconnected, and the dark-adaptation chamber was drained quickly and placed in a film-changing bag. Inside the bag, the cylinder containing the plankton was transferred from the dark chamber to a dry, light-proof can. This can was sealed, removed from the bag, and deep-frozen until such time as the sample could be treated in the laboratory darkroom.

Work in the laboratory

Extraction procedure: Extraction of the light-sensitive materials was carried out either in dim red light or in total darkness with the aid of an infra-red image converter.

A Waring Blendor was used to macerate the plankton sample, and the resultant homogeneous, semi-fluid mass was poured into a graduated cylinder. An equal volume of extractive (saturated aqueous digitonin solution) was added, and the mixture, after vigorous stirring, was left in the dark for four to six hours. At the end of this period the mixture was centrifuged. The clear supernatant extract was pipetted off, and the residue was re-extracted in the same manner.

Detection of photosensitive substances: The absorption of the combined extracts from each plankton sample was measured with a Model DU quartz spectrophotometer throughout the visible spectrum. The absorption cells were filled in the darkroom and transferred, inside the film-changing bag, to the spectrophotometer.

A variety of procedures has been employed to detect photosensitive materials in the extracts:

1. The absorption of the extract was measured directly. The standard cell was filled with extractive and the test cell with the extract which had been protected from all visible radiation or, in those cases where red light was used during extraction, exposed only to wave lengths greater than 620 mp. After the absorption of the unbleached solution had been measured, both cells were exposed to bright white light for ten minutes, and the measurement was repeated.

A loss of absorption in any part of the spectrum after bleaching indicates the destruction by light of some component of the extract. Conversely, an increase in absorption indicates the formation of a bleaching product. The absorption curve of a photolabile substance and any bleaching product, the "difference spectrum," may be obtained by subtracting the absorption values obtained for the bleached solution from those for the unbleached at each wave length.

2. The difference spectrum was obtained directly. The standard cell was filled with a sample of the extract which had been bleached with white light, and the test cell with an unbleached sample.

Although this method diagnoses photosensitivity most rapidly, it gives no clue to the chemical nature of the pigment. Consequently, it was used only in conjunction with method 1.

3. The effect of exposure to red light (of wave length greater than 620 mp) was measured. This method was applied only to those extracts which had been prepared in total darkness. The standard cell was filled with extractive and the test cell with extract which had been shielded entirely from light. After the absorption spectrum of this solution had been measured, the prism of the spectrophotometer was set at 630 mp, the

slit width was adjusted to yield a 10-m μ wave band, and the test cell was exposed to this radiation for 30 minutes. The absorption spectrum was then re-determined, and a difference spectrum indicating the loss that could have occurred had the extraction been prepared in dim red light was obtained.

RESULTS

Light Measurements

The survey photometer used in the work reported here was designed primarily to determine whether the system to be employed in the final recording model was sufficiently sensitive to respond to the very low light intensities encountered at the depths inhabited by the deeper scattering layers.

Calibration of this survey instrument showed that a straight-line relationship between millimeter readings and log footcandles persisted only through the intensity range between 3.25×10^{-6} and 3.25×10^{-9} footcandles. Therefore, although this instrument is highly sensitive, it can give reliable information on light extinction only after it has been lowered to considerable depth.

Observations were made from the E. W. SCRIPPS in California coastal waters in the region of the San Diego Trough. At 1440 hours on November 27, a clear, sunny day with cloud cover less than 10%, the reliable range of the photometer was attained when it had been lowered to 75 meters. Threshold sensitivity (3.25×10^{-9} footcandles) was reached at 125 meters. At this time distinct scattering layers were detected with the NRC-2 fathometer at depths of 150-200 meters.

As we had expected, the oceanic waters around Bermuda proved to be much more transparent. For example, at a station five miles south of Castle Roads at 1500 hours on January 13, with a cloud cover of 100%, the reliable range of the photometer was not reached until it had been lowered to 150 meters, and its threshold sensitivity was observed at 400 meters. Although no discrete layers of sonic-scatterers could be detected at this time with the NMC echo-sounder on the PANULIRUS, it has been shown previously that they occur at about 320 meters in Bermuda waters in February (Moore, 5).

Observations of this nature indicate the adequacy of the system's sensitivity. The self-contained recording model retains this sensitivity and is modified to respond reliably to the higher intensities encountered in surface waters.

Photosensitivity of extracts

The results obtained with extracts of whole plankton samples are most promising. To our knowledge these constitute the first successful experiments of their kind with planktonic invertebrates.

The dark-adaptation methods employed here appear adequate to allow regeneration of photosensitive substances in the living animals, for in no case was a light-stable extract obtained. The degree of photosensitivity, as evidenced by the concentration of pigment, and the color, as indicated by the position of the absorption maxima in the spectrum, varied from sample to sample, depending on the composition of the plankton.

The results obtained from a collection composed mainly of calanoid copepods, larval and juvenile euphausiids, and a few amphipods, chaetognaths and ctenophores are shown in Figure 7. This extract was prepared in total

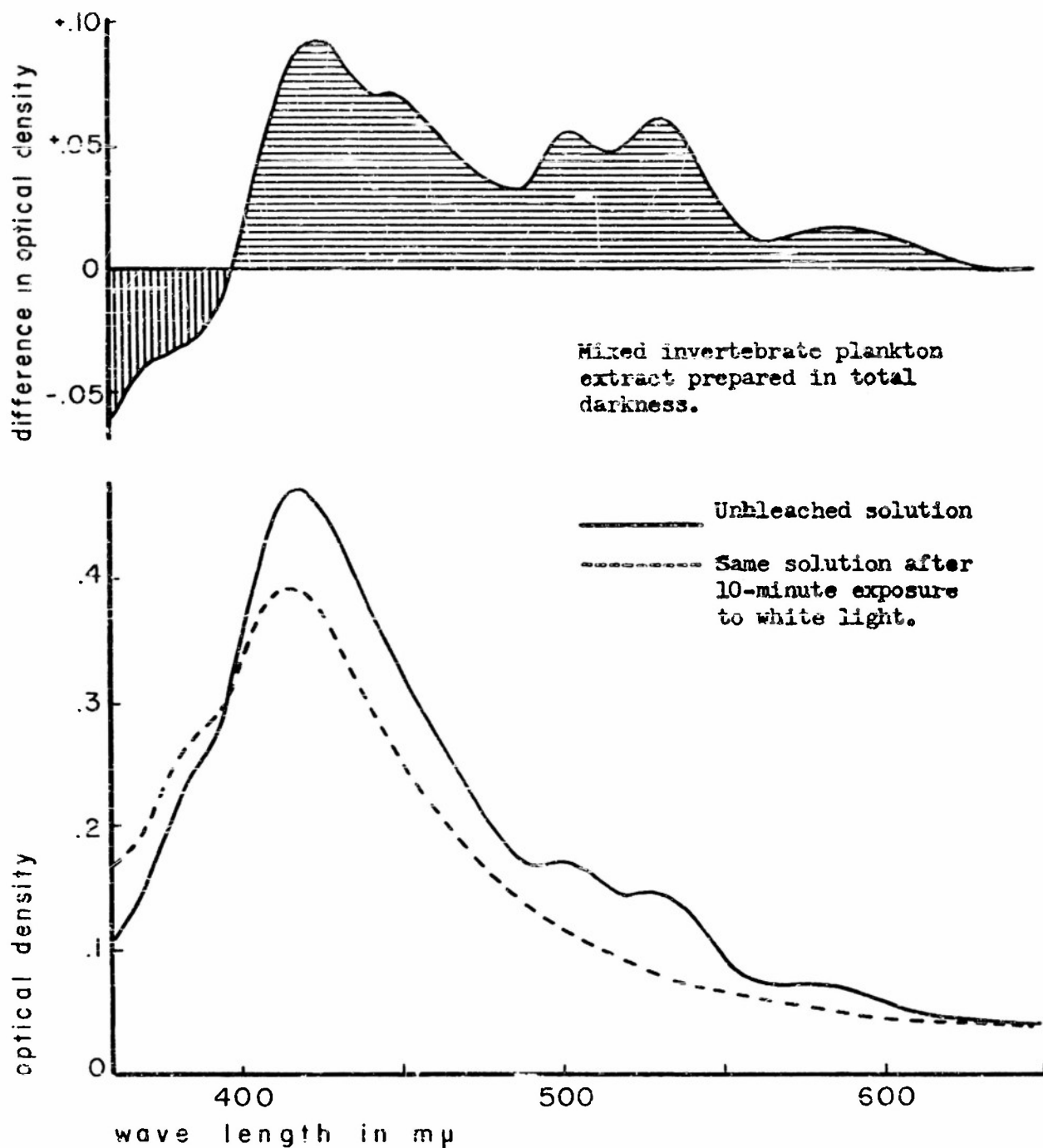


Figure 7. Bottom: Absorption spectra of an aqueous digitonin extract of mixed invertebrate plankton. Top: Difference spectra obtained by subtracting optical density values for the bleached solution from those for the unbleached. The area above the zero-line (horizontal shading) represents materials destroyed by light; the area below the line (vertical shading) indicates the formation of a new substance by exposure to light.

darkness. Spectral absorption of the unbleached extract is indicated by the solid curve in the lower section of the figure. Absorption after a 10-minute exposure to white light is indicated by the broken line.

At wave lengths longer than 400 μ , absorption is decreased after exposure to light, evidence that light has destroyed some substance or substances. At wave lengths shorter than 400 μ absorption is increased by exposure to light. This indicates that some new material has been formed. Although there were no vertebrates in this collection, the behavior of the photosensitive material (s) is not unlike that of the known vertebrate visual pigments, which on exposure to light break down to other colored substances.

The upper section of Figure 7 shows the difference spectrum obtained by subtracting values for the bleached solution from those for the unbleached solution at each wave length. The absorption of the light sensitive material which was destroyed by bleaching appears above the zero line (horizontal shading); that of the material formed by bleaching appears below the line (vertical shading).

The shape of the difference spectrum indicates that at least five separate photosensitive pigments absorbing maximally at 425, 450, 500, 535 585 μ respectively may be present.

It is interesting to note that although no vertebrates were present in this collection, the maxima at 500 and at 535 μ in the difference spectrum are typical of the difference spectra obtained with rhodopsin, the retinal pigment of marine fishes (500 μ) and with porphyropsin (535 μ) from fresh-water fishes.

No technique has been described previously whereby extracts of

materials which are destroyed by light can be prepared in total darkness. The classic approach has been to use deep-red illumination of minimal intensity and to curtail, as far as possible, the periods of exposure.

Utilization of the infra-red image converter--a form of photocell with a silver-caesium oxide photocathode and a fluorescent screen as the anode--obviates the need for visible light during extraction procedures. The object to be observed is "illuminated" with invisible infra-red rays

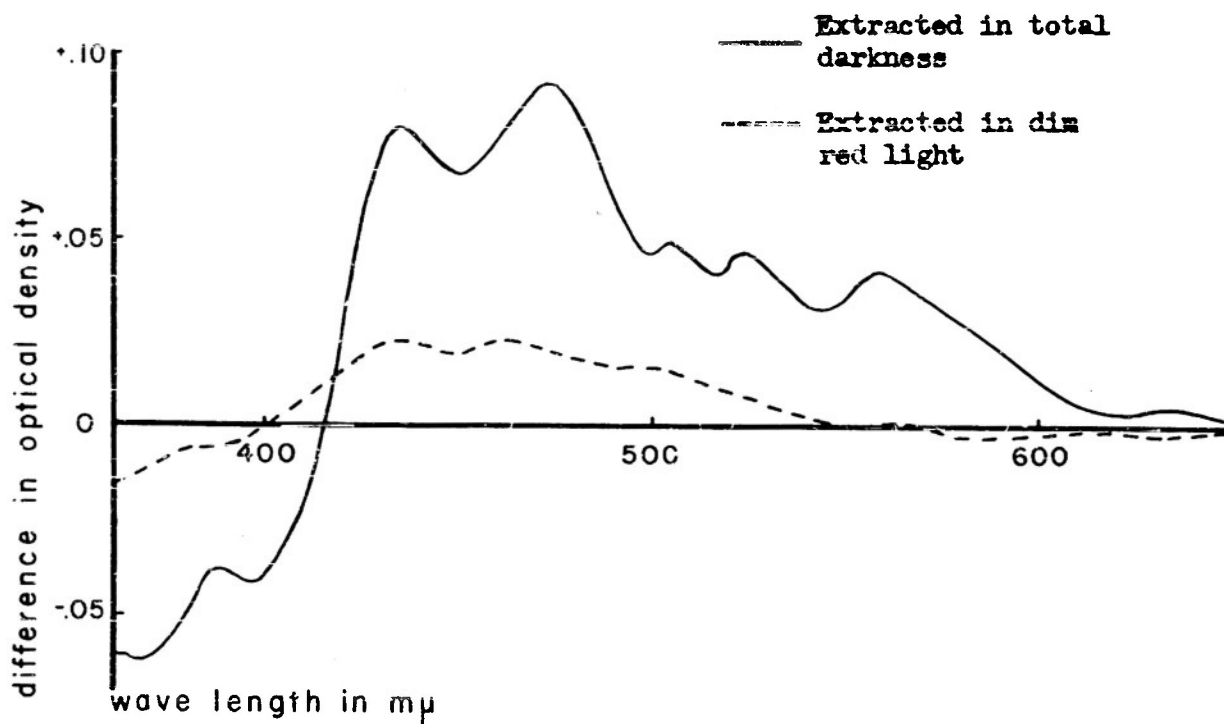


Figure 8. Difference spectra obtained from extracts of aliquots of a mixed invertebrate plankton collection.

obtained from an ordinary white incandescent lamp equipped with an infra-red filter. The rays are reflected by the object and, falling on the photocathode, will release electrons which, accelerated by application of a high voltage, form, on the anode, a fluorescent green image corresponding to the initial infra-red image.

A number of converter tubes have been tested, and the Mullard ME 1202 seems the most satisfactory. It employs a magnetic focusing coil, and, on a 28 millimeter screen, resolutions of 200-500 lines per centimeter can be obtained.

The advantages of the technique to the work with plankton are substantiated by a comparison of the curves in Figure 8. These difference spectra were obtained from parallel extractions of aliquots from a single plankton collection. In one case extraction was carried out with dim-red illumination; in the other only infra-red radiation was used. This plankton sample contained only invertebrate forms.

Since little is known of the light-sensitive materials in planktonic invertebrates, similar parallel extractions of retinas of the Californian surf perch, Amphistichus argenteus, were made. A comparison of the results of this experiment (Fig. 9) makes it apparent that even dim red light destroys a component of the light-sensitive substance in the vertebrate retina. This component, in the case of the surf perch, is undoubtedly a mixture of the total concentration of iodopsin, the "bright light" photosensitive pigment of vertebrates which absorbs maximally at 565 m μ , and a portion of the concentration of rhodopsin, the "dim light" pigment which absorbs maximally near 500 m μ .

Until the photosensitive systems of the planktonic invertebrates are

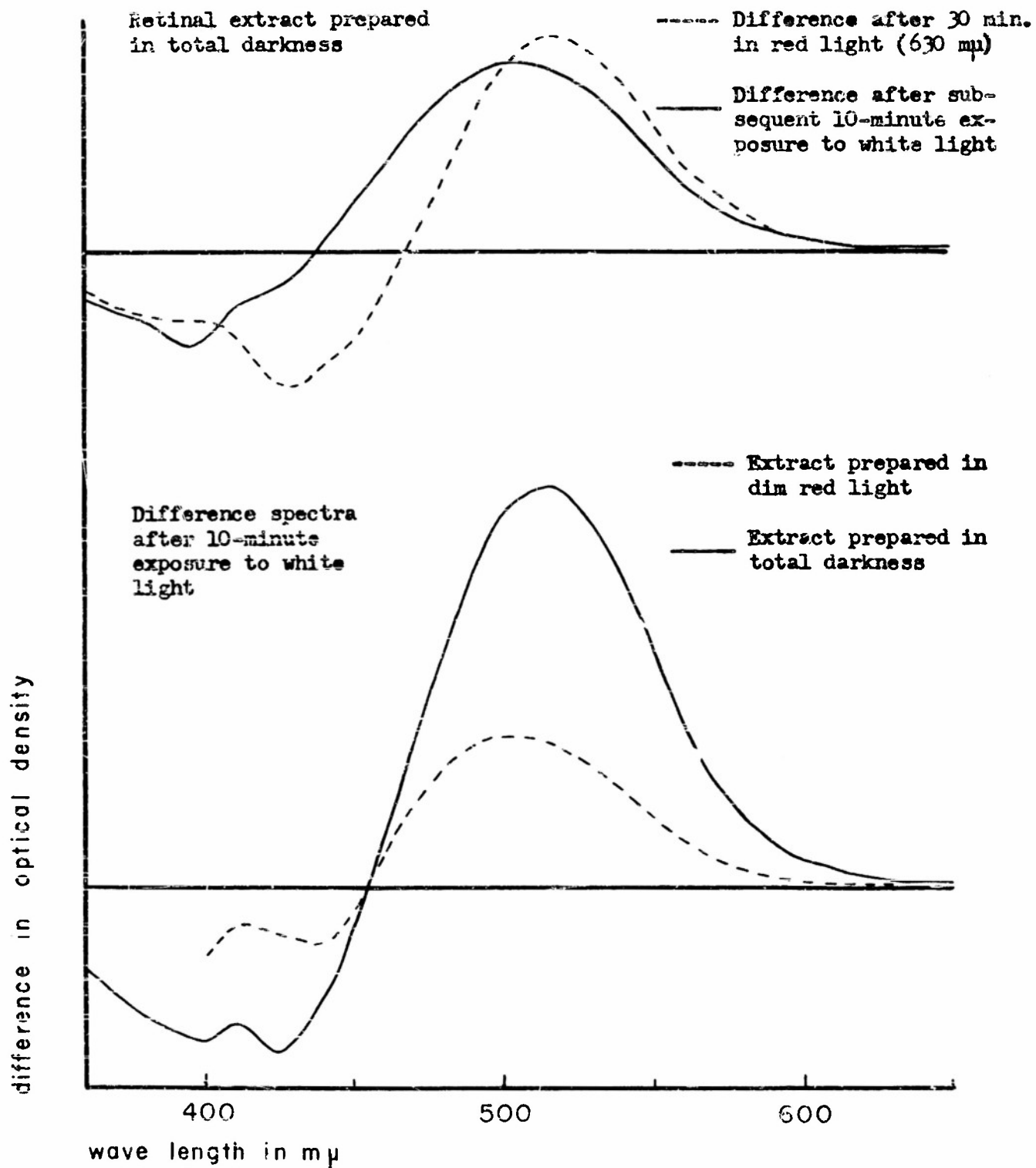


Figure 9. Difference spectra of retinal extracts of the surf perch, Amphistichus argenteus. Top: Difference after exposure of a single solution to red and then white light. Bottom: Differences obtained from two solutions prepared identically except for illumination.

understood, it is imperative that such incidental destruction of light sensitive materials be avoided.

future plans

At the present stage of the investigation, a number of necessary modifications in technique are foreseeable, and an enlargement of scope is contemplated. Much of the success of the project depends on obtaining more precise physical data than has been possible thus far, and considerable effort will be devoted to this. Collection of such data will be much more feasible with the facilities of the Scripps Institution than it has been in Bermuda. The following changes in methods are anticipated now, and, doubtless, as these are incorporated the need for others will become obvious.

Light measurement

The desirability of making simultaneous light measurements and collections at the depth of a scattering layer has been pointed out previously. This has not been possible with the survey submarine photometer used so far. Little difficulty is anticipated, however, in carrying out such measurements with the self-contained recording photometer which can be attached to the wire near whichever net is in use for collections.

The sensitivity of the phototube in the light meter is, of course, directional, and it is proposed to overcome this in obtaining information on total, non-directional illumination at depth by fitting the head of the photometer with a translucent integrating sphere.

Conversely, this directional sensitivity will be made use of in determining the relative degrees of directional light at depth. This will

be done by changing the rigging of the photometer on the cable so that the sensitive element will point successively up, down or sideways.

Information on the spectral characteristics of this directional light will be obtained by screening the photosensitive element with interference filters which allow the transmission of light in very narrow spectral bands.

As more precise data on the characteristics of submarine illumination accrue it will become of interest to determine the causes of transparency fluctuations particularly as these fluctuations affect the depth and migration of the scattering layers. For the measurement of turbidity of sea-water a fluorescence attachment has been purchased for the Beckman spectrophotometer. With this instrument differences up to 86% in Tyndall effect between filtered and unfiltered sea-water have been obtained.

The equipment necessary for micro-filtration with millipore membranes-- a technique described by Goldberg et al. (6)--have also been purchased. It is hoped that this technique will reveal the components of the microflora and -fauna in samples whose turbidity has been determined, and which have been taken from the water-column above the scattering layer.

With millipore membranes it will also be possible to obtain information about the inorganic content of the colloiddally dispersed material in the water column above the scattering layer. For organic analysis adsorbent filters (Fox, et al., 7) will be used. In these analyses, special attention will be paid to detect those substances which absorb light selectively and whose concentrations in any given sample may influence the spectral transmission of the water column.

Plankton Collection

Since we feel that the nets we have used so far are rather selective we intend using a wider variety of gear for collecting from scattering

layer communities. One net of considerable promise, if suitably modified, is the Isaacs-Kidd midwater trawl. A closing mechanism has been devised for this.

Hydrographic casts made immediately before or after the plankton collection will yield temperature data at layer depths. It will also be of considerable interest to investigate the oxygen profile in the water column.

Detection of scattering layers

The main objection to the method used for detecting the scattering layer thus far in this project is the insensitivity of the NMC type fathometers to large portions of the sound spectrum. This has been overcome in part by Galler (8) who used a battery of fathometers operating at 14, 18 and 50 kilocycles, and succeeded in picking up multiple layers not apparent on a single instrument.

The method devised by Hersey et al. (9) utilizes an explosive "white" sound source and filters the reverberation. This eliminates the blind spots on the spectrum, particularly at lower frequencies, and reveals the frequent presence of layers which scatter sound of frequencies as low as 5 kilocycles.

Instruments of the type used by Galler already exist at Scripps, and those necessary for Hersey's technique have been purchased for this project.

It is intended, therefore, to use both techniques, one supplementing the other.

It is hoped that the information thus gained on the acoustic cross-section of the scatterers and their probable size will enable us to design adequate net-hauls.

Handling of plankton

Several refinements are highly desirable here and will be devised as the problem progresses. It is, for instance, proposed to retain a portion of the sample for preservation and subsequent examination and counting in the laboratory.

Of prime importance will be the development of techniques for separating the main constituents of the plankton while still alive. In the work reported here, whole plankton samples have been extracted. Absorption spectra obtained from these extracts have indicated the presence of mixtures of photosensitive pigments. Whether the components of these mixtures are species-specific or whether an individual group may contain several or all of them can only be determined by extracting uncontaminated samples of the various planktons.

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