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NAVAL OCEANOGRAPHIC OFFICE TECHNICAL REPORT

A PROTOTYPE BIOLUMINESCENCE PHOTOMETER

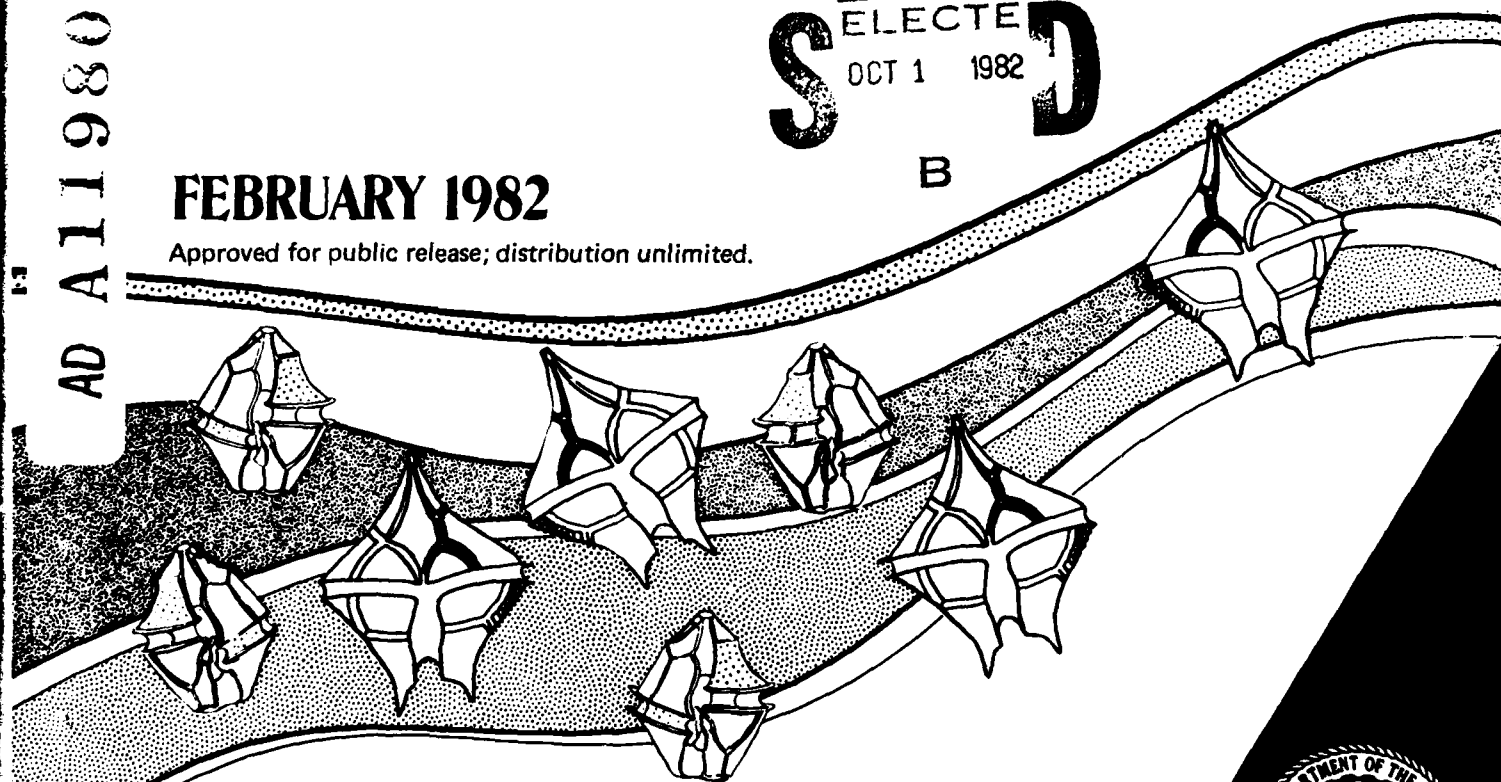
MARK L. GEIGER

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NAVAL OCEANOGRAPHIC OFFICE
NSTL STATION, BAY ST. LOUIS, MS 39522

FOR
COMMANDER,
NAVAL OCEANOGRAPHY COMMAND
NSTL STATION, BAY ST. LOUIS, MS 39529



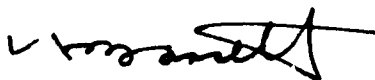
FOREWORD

Data describing how marine bioluminescent organisms distribute themselves through time and space in the global ocean is limited. However, we do know that, on a coarse scale, a major portion of the bioluminescent light produced in the upper ocean comes from large numbers of dinoflagellates that live within drifting phytoplankton communities. Another sizeable contribution of bioluminescence is made by larger and more mobile organisms that live within zooplankton and nekton communities.

Dinoflagellate luminescence can be studied using a pumping photometer, a device that pumps sea water and tiny planktonic organisms (initially in laminar flow) into a viewing chamber. In the chamber, a suddenly-turbulent flow regime triggers dinoflagellates into emitting flashes of light which are electronically detected and recorded. We assume that pumping photometers will not sample some of the larger, more mobile organisms; but, the extent to which this is true is a variable entity that requires further study. Measurement variabilities depend on photometer intake dimensions, on pumping rates, and on several aspects of the organisms involved.

The U.S. Naval Oceanographic Office (NAVOCEANO) has been engaged in a "pilot" program designed to develop competence in measuring bioluminescence at sea. Because a satisfactory approach has yet to be devised to circumvent the measurement problems created by large-animal instrument avoidance, this effort has, thus far, concentrated on using a pumping photometer.

This report describes the NAVOCEANO Bioluminescence Photometer System and documents the history and performance of this device.



C. H. BASSETT
Captain, U.S. Navy
Commanding Officer

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER N00 TR-280	2. GOVT ACCESSION NO. AD-A119807	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) A PROTOTYPE BIOLUMINESCENCE PHOTOMETER		5. TYPE OF REPORT & PERIOD COVERED
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) MARK L. GEIGER		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. NAVAL OCEANOGRAPHIC OFFICE CODE 7114 NSTL STATION, MS 39522		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. NAVAL OCEANOGRAPHIC OFFICE CODE 7002 NSTL STATION, MS 39522		12. REPORT DATE FEBRUARY 1982
		13. NUMBER OF PAGES 59
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) BIOLUMINESCENCE PHOTOMETER MARINE BIOLUMINESCENCE PUMPING PHOTOMETER		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This document describes the NAVOCEANO Bioluminescence Photometer System, a vertically-lowered device that records flashes of light produced when small bioluminescent marine organisms are pumped into a turbulent flow regime chamber where they are viewed by a photomultiplier tube. The history and performance of this device are documented in this report. The combined results of experience at sea and engineering		

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performance evaluations suggest that:

1. The present instrument is a useful tool for mapping dinoflagellate bioluminescence capabilities within the upper ocean.
2. A recent modification (based on a twelve-conductor set of winch slip rings) allowed continuous profiles of dinoflagellate light production to be recorded without significant decreases in sensitivity or signal degradation by noise,
3. The present instrument is not useful for underway work or for measuring light produced by organisms of approximately 400 microns in length and larger (thus excluding the larger copepods and practically all euphausiids, fishes, squids, etc.).
4. Several improvements can be made on the present device by experimenting with underway systems and with chamber sizes, shapes and flow regimes; by altering rates of pumping, and by deviating from the present chart record counting of flashes to an automated data retrieval system based not on individual organism performance but on the maximally-stimulated bioluminescent light produced within a unit volume of sea water.
5. Extrapolation beyond the improvements suggested implies that different kinds of measurement systems will eventually be required to study the large-animal luminescence that is now subject to instrument avoidance problems. Although they are subject to animal avoidance problems, pumping photometers are, nevertheless, likely to play important roles in future schemes for measuring bioluminescence at sea. This is true because pumping photometers do yield information on dinoflagellate luminescence, and this is more important than it would be if only the global ubiquitousness of dinoflagellates was considered. Specifically, dinoflagellate luminescence is an indirect index of a region's potential for large-animal luminescence 1) through the dinoflagellates' link with regional fertility, and 2) through the dinoflagellates' ability to trigger secondary luminescence in larger organisms.

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ACKNOWLEDGEMENTS

Dr. Richard V. Lynch, III, of the Naval Research Laboratory provided information on the early history of the photometer system. Mr. Adron Hall of NAVOCEANO provided information on the recent history of the instrument along with several helpful suggestions which led to the improvement of this manuscript.

The NAVOCEANO Biology Section, the NAVOCEANO Engineering Department, and the Visibility Laboratory of the Scripps Institution of Oceanography collaborated in testing and evaluating the photometer system described.

This document was edited by Dr. Ben J. Korgen.

I. INTRODUCTION

Bioluminescence has been an area of intense curiosity and awe since the beginning of man's association with the marine environment. Written accounts date back to the Roman Empire and there have been many reports and investigators since that time. Harvey (1952, 1957) provides an extensive review of this early work. It has only been in the last three decades, with man's technological advances in electronic instrumentation, that the ubiquitous nature of bioluminescence has been demonstrated.

The evolution of the photomultiplier tube and development of bathy-photometers by Clark et al., (1956); and by Boden (1957); and Kampa (1956) resulted in a quantum leap in the field of bioluminescence research. These instruments provided records which allowed the quantitative estimation of bioluminescent organisms in all oceans and at all depths. These original instruments have been redesigned and improved over the last thirty years by both the original developers as well as other investigators (including Backus et al., 1961; Seliger et al., 1961; Hardy and Kay, 1964; and Rudyakov, 1968). Tett and Kelly (1973) provide an excellent review of this era. Bathyphotometers remain the basic research instruments in the field.

The purpose of this technical report is two-fold. Primarily, it documents an instrument system used by various investigators and referred to in many reports (Hall and Staples, 1978; Lynch, 1978; Lynch et al., 1979; Hall, 1980; and Willett et al., 1982 a,b). Secondly, it provides a vehicle by which other investigators developing new photometer systems can benefit from our experience and ideas.

II. HISTORY

The U. S. Naval Oceanographic Office (NAVOCEANO) bioluminescence photometer was built at NAVOCEANO's Maury Center by Dr. Abraham Muhlbaum in the mid-1960's. This instrument (a duplicate of a design reported by Seliger et al., 1962) provided maximum mechanical stimulation to dino-flagellates, and incorporated light baffles to permit continuous measurement without interference from ambient light. Associated electronic equipment was designed to measure D. C. photomultiplier output in two modes simultaneously: a) single flash events using an oscilloscope, and b) electronically integrated light intensity using a RUSTRAK strip chart recorder. The operational depth limit of the instrument was 50 m. The NAVOCEANO instrument system, in its original configuration, was never deployed at sea.

The first modification of the instrument involved substituting the RUSTRAK recorder with a high speed Sanborn 299 strip chart recorder to record integrated light 'packages'. The photometer system was first used by Dr. Richard V. Lynch, III, of the Naval Research Laboratory (NRL) in June, 1975. This cruise, as well as several more cruises by NRL, indicated deficiencies in the instrumentation.

The U. S. Naval Oceanographic Office, in 1976-77, funded a redesign of the photometer system, which was performed at NRL. Major modifications included: a) placement of the photomultiplier tube 10 cm. from the observation chamber to reduce the effect of organism (light source) placement in the chamber on the intensity recording; b) a more powerful pump; c) a pressure transducer (depth sensor); and d) new pressure housings with an operational depth of 200 m. The instrument was used in this configuration for three NAVOCEANO cruises (Hall, 1981) and one NRL cruise (Lynch, 1978).

In 1979, a multi-conductor, single-component cable was purchased to replace the old multi-component cable. A Hydro-streamer winch was modified and slip rings incorporated to ease and expedite deployment (previous deployment/retrieval operations employed a capstan and manual stowage). A solid state power supply was added to the circuitry to replace the batteries, and the non-integrated output circuit was reconnected. The instrument at this stage of its evolution was deployed on two NAVOCEANO cruises (Willett et al., 1982 a, b).

III. GENERAL DESCRIPTION

The prototype bioluminescence photometer consists of four basic components: the underwater sensor, the sea cable, the winch/slip ring assembly, and the deck control unit (figure 1).

A. UNDERWATER SENSOR

The underwater sensor is composed of two separate water-tight pressure housing assemblies (figure 2). The lower assembly contains a 1/4 HP electric drive, 3450 RPM, D.C. motor that is magnetically coupled to a centrifugal impeller. The impeller provides a flow of water through the sample chamber and provides a source of organism stimulation. A pressure transducer is also housed in the lower unit providing a remote depth sensor.

The sensor pressure housing consists of an amplification circuit, an RCA 1P21 photomultiplier tube (PMT) (figure 3, table 1) which views the sample chamber through a glass pressure port and a 70-mm focal length, 60-mm diameter, biconvex lens (figure 4).

The area between the sensor and the pump motor pressure housings contains the sample chamber, the impeller, and the intake baffle plate (figure 5). Water containing organisms is fed to the sample chamber through

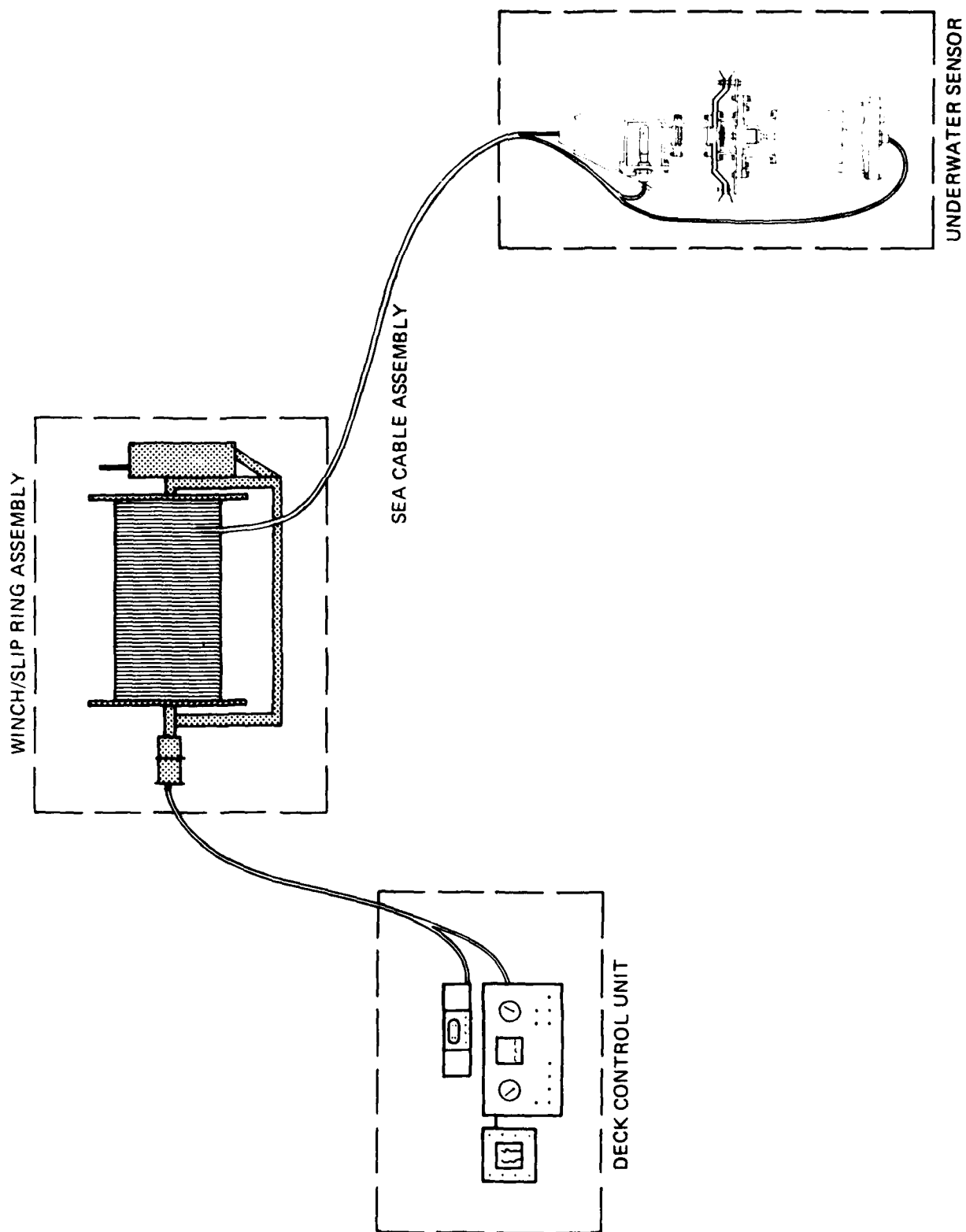


Figure 1. Basic Components of NAVOCEANO Photometer System

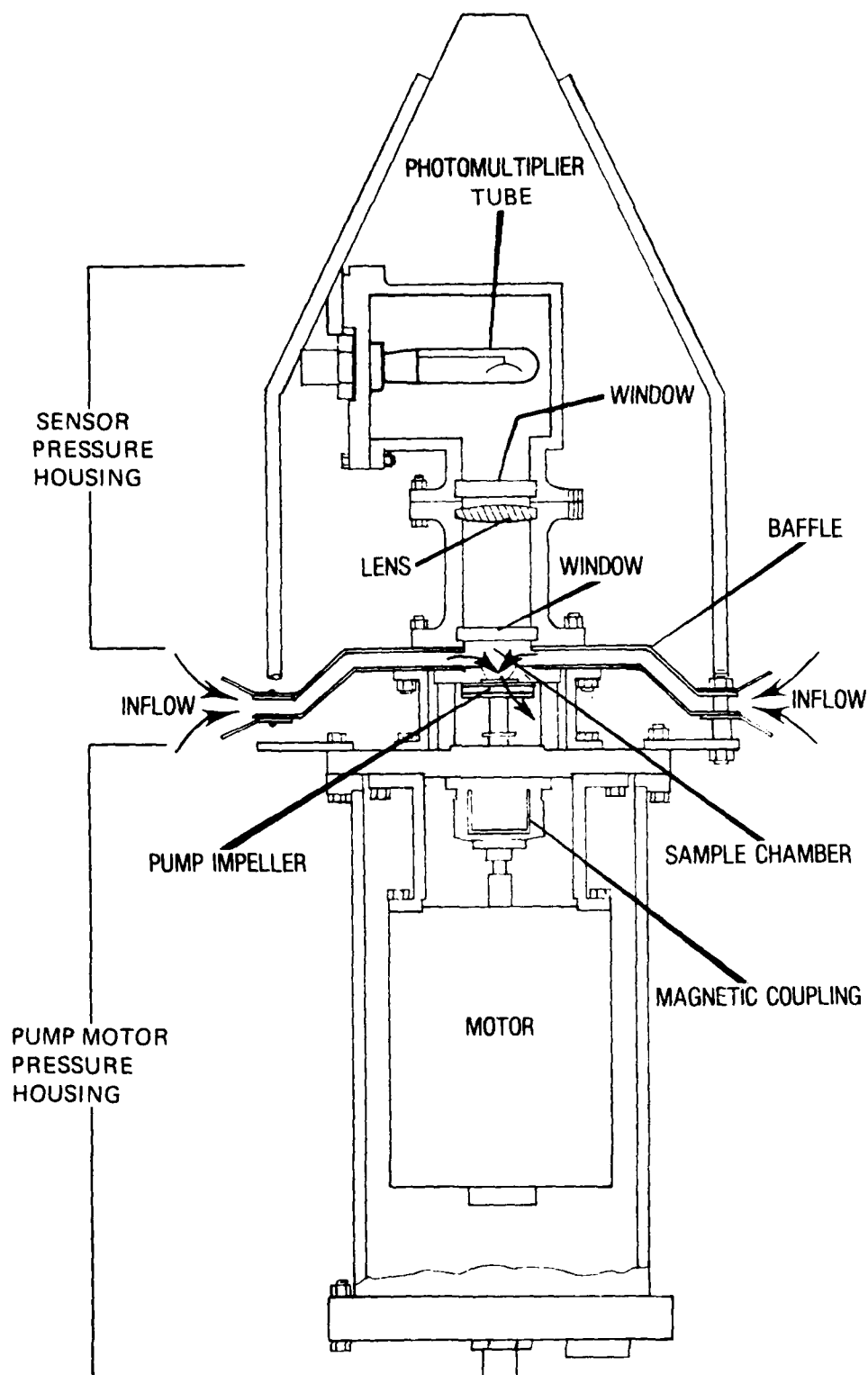


Figure 2. NAVOCEANO Bioluminescence Photometer (No Scale)

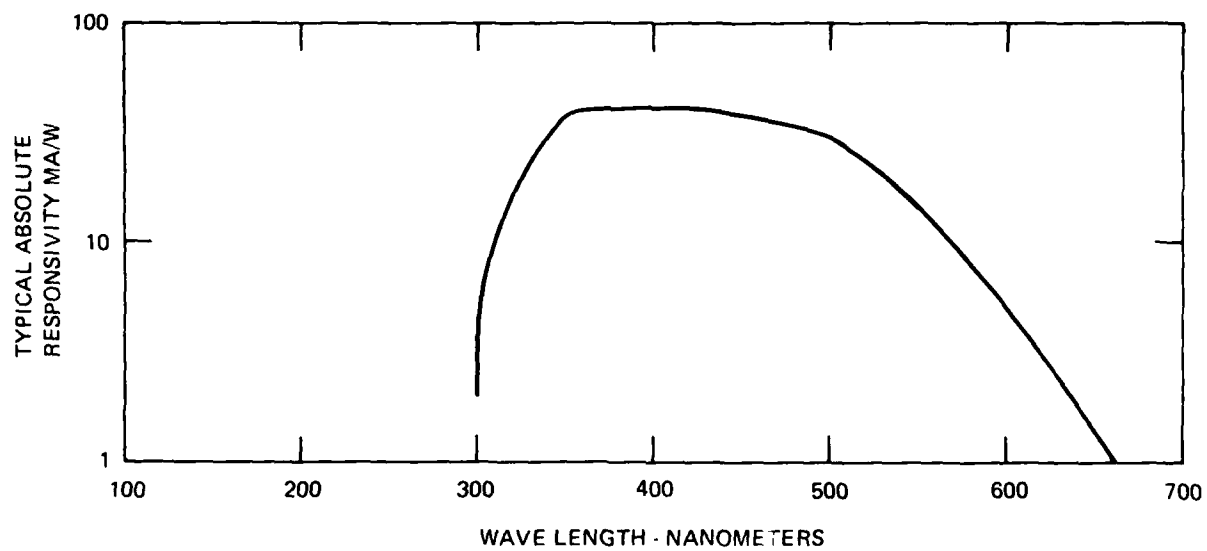


FIGURE 3. Spectral Response Curve of the 1P21 Photomultiplier Tube.
Modified from RCA (1976).

Number of stages and cage structure	Supply voltage [V]	Typical characteristics at specified operating supply voltage, voltage distribution, and 22°C								Wavelength of Maximum Response [nm]
		Responsivity				Anode Dark Current @ Anode Luminous Responsivity [nA@A/1m]	Anode Pulse Rise Time [ns]			
		Radiant ^a		Luminous ^b						
		Anode	Cathode	Anode	Cathode					
		Typ. [A/W]	Typ. [mA/W]	Min. [A/1m]	Typ. [A/1m]			Min. [uA/1m]	Typ. [uA/1m]	
9/Circular Cage	1250	1.3x105	42	40	120	20	40	1 @ 20	2	400

- a. At wavelength of maximum response of the spectral responsivity characteristic.
- b. With a tungsten filament lamp having a line glass envelope. Lamp is operated at a color temperature of 2856K.

Table 1. 1P21 Photomultiplier Rating and Characteristics.
Modified from RCA (1976)

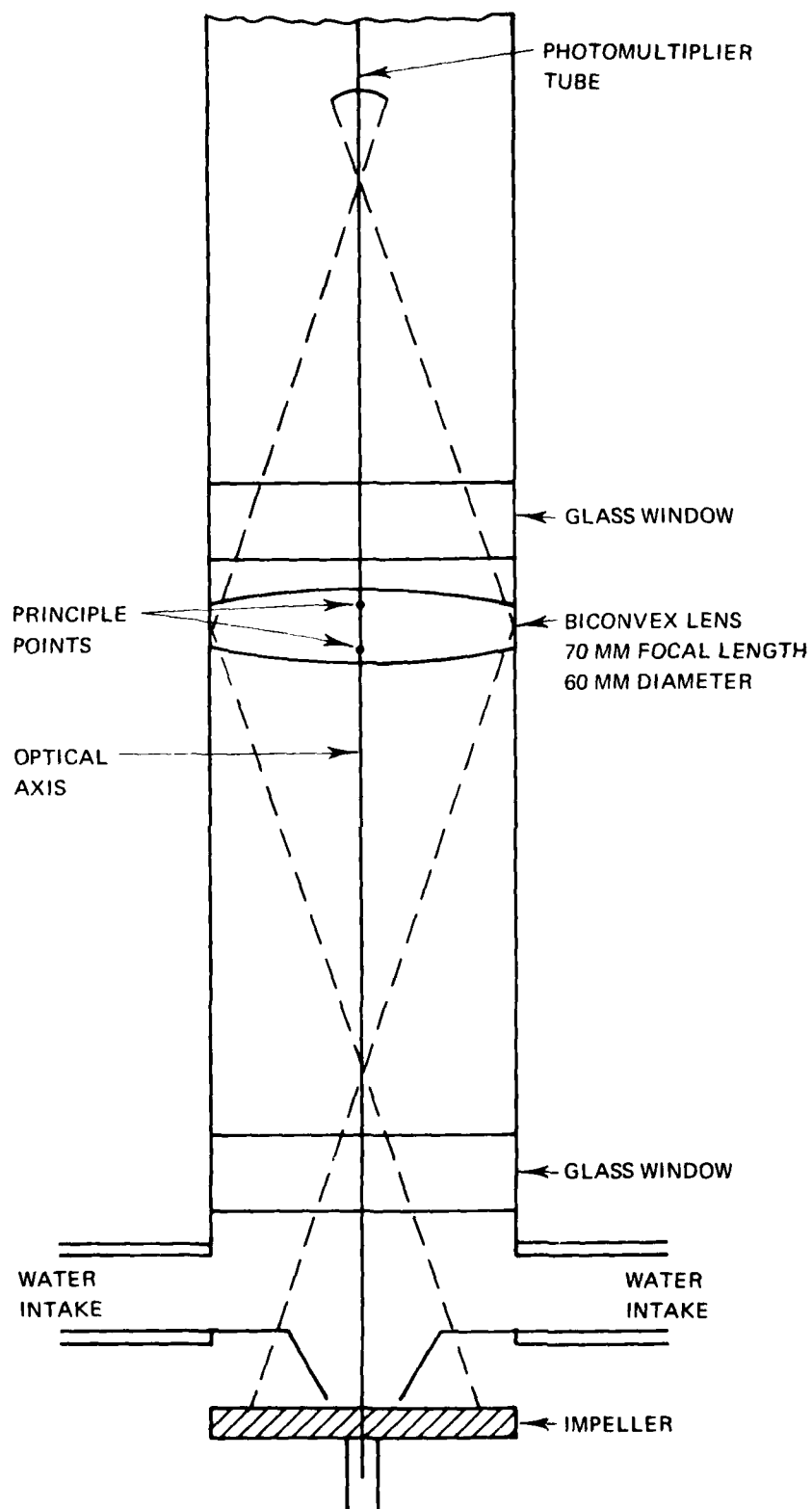


Figure 4. Bioluminescence Photometer Optical Diagram

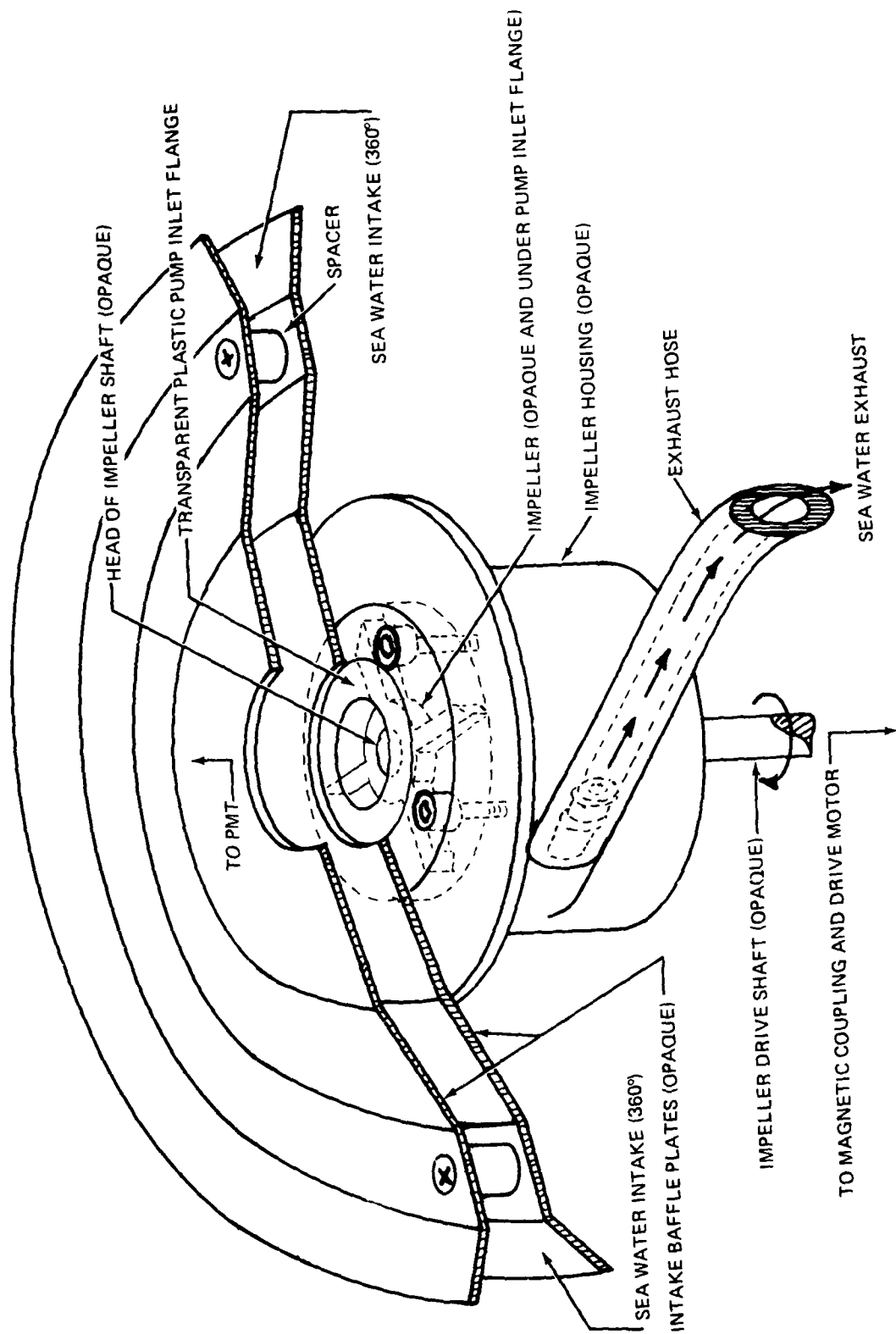


Figure 5. Bioluminescence Photometer Baffle System and Sample Chamber -
Conceptual View

a light baffle. The light baffle consists of two inverted, stacked, 40-cm diameter, pie-plate shaped discs with a 13-mm separation. Water intake through the baffle occurs circumferentially, in the horizontal plane. The water flow through the baffle is gradual and laminar due to the large cross-sectional area of the baffle in relation to the area of the sample cell. Decreasing cross-sectional area results in an increased flow with maximum velocity occurring at the transparent pump inlet flange. This flange consists of an opening which tapers to 1 cm just above the impeller which is located in the 29-cc sample chamber. The target organism is stimulated by a combination of both shear and mechanical stimuli. The shear stimulus occurs as the water is drawn into the tapered hole in the pump inlet flange and a vortex is created. The mechanical stimulus is created as the organisms collide with both the tapered walls of the inlet flange and the surface of the impeller. The organisms are then forced out the sample chamber exhaust nozzle through a plastic hose and discharged at a point below the pump motor housing.

The assembled underwater unit is mounted on a pedestal and fitted with a hoisting cage to aid in shipboard deployment and retrieval. Total weight of the unit is approximately 38 kg in air.

B. SEA CABLE

The sea cable assembly is constructed with fourteen electrical conductors, an internal strength member, non-fibrous type void fillers, and an elastomer outer jacket. The cable diameter is 19 mm and the length is 300 meters.

C. WINCH/SLIP RING ASSEMBLY

The sea cable is stored, deployed, and retrieved using a Hydro-streamer winch (Model 26170-02, Teledyne Exploration Co.). The winch was

modified to accept a twelve-conductor set of slip rings to eliminate the repeated connect/disconnect operation during deployment. These slip rings permitted the testing of the instrument in a continuous profile mode previously considered impractical due to a decrease in sensitivity and an increase in noise caused by the use of the slip rings. Additionally, the winch gear ratio was changed to accomodate the weight of the instrument/cable package.

D. DECK CONTROL UNIT

The deck system consists of controls for the light sensor, (figure 6), the pump, depth sensor, (figure 7), and a recorder for signal trace. The deck unit contains a high voltage power supply for the PMT and also a nine-step resistor block which adjusts the amplification/sensitivity of the instrument. There are two signal outputs: integrated (recorder output shunted by capacitor) and non-integrated (direct).

Controls for the forward/reverse, variable speed operation of the pump are also contained in the deck control unit. The depth monitor consists of a simple LED display calibrated for readout in meters. Any high-speed response strip chart recorder can be used with the system; at present a two-channel (recording integrated and non-integrated signals simultaneously) MFE corporation recorder is used.

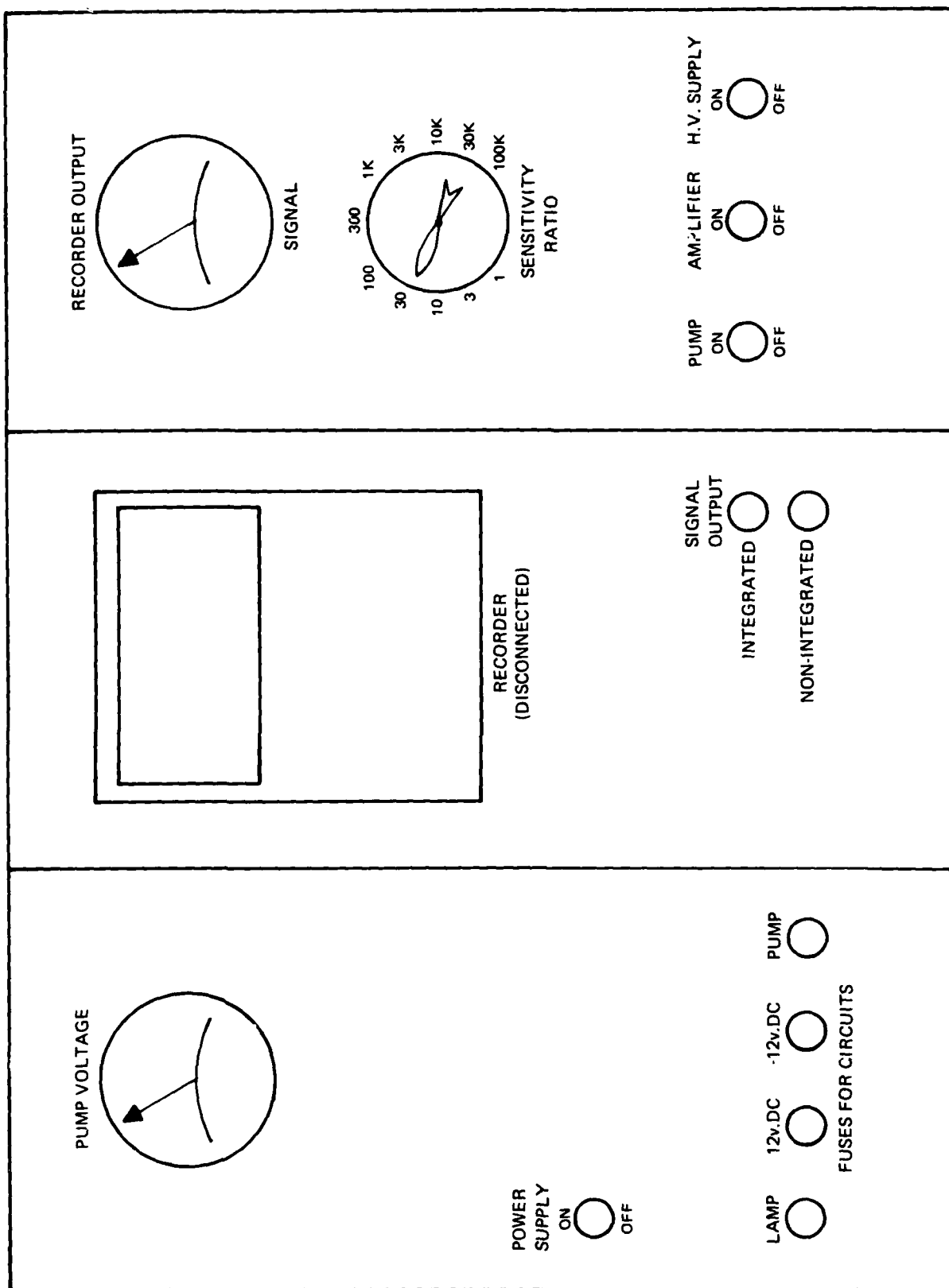


Figure 6. Photometer Deck Control Unit (Amplifier and Control)

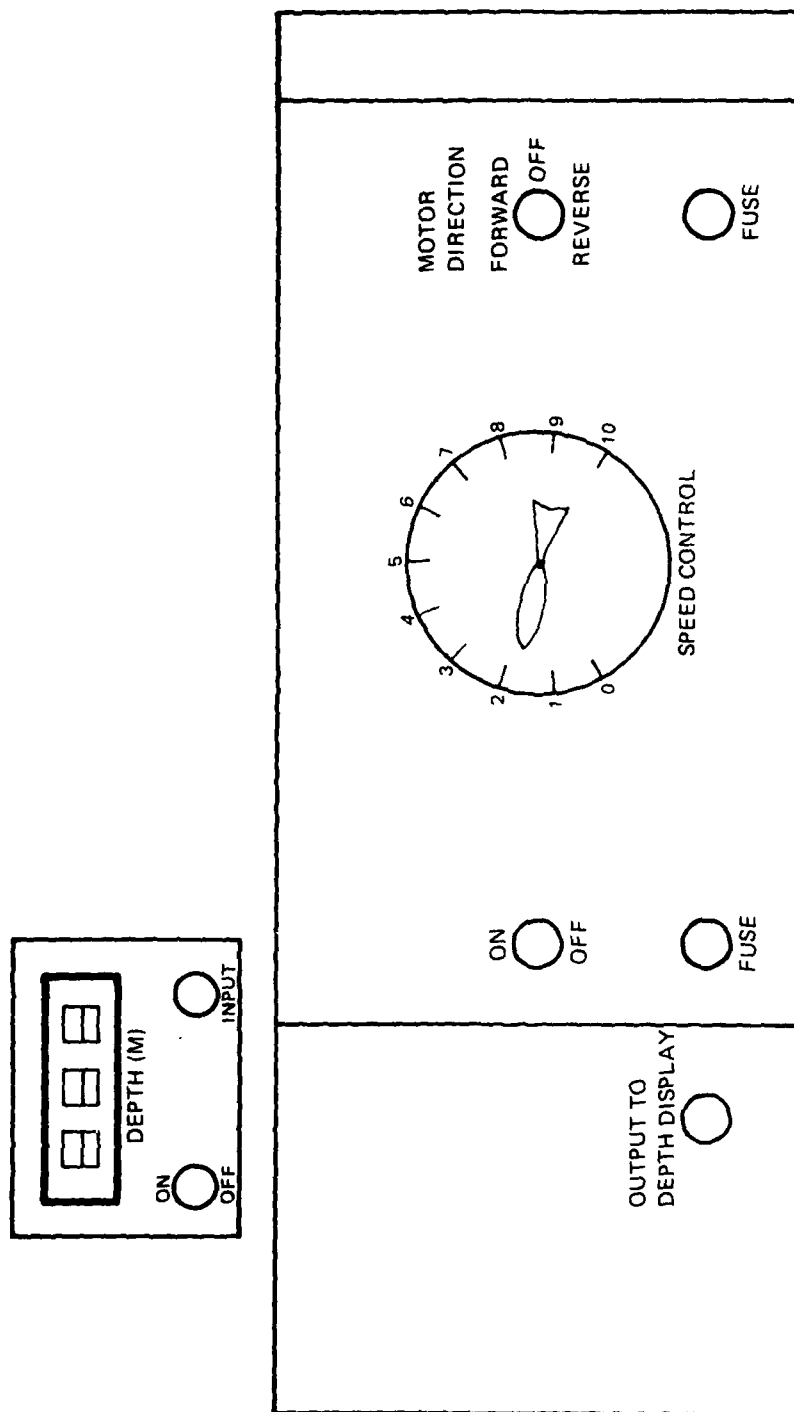


Figure 7. Photometer Deck Control Unit (Pump Motor Control, Depth Display)

IV. OPERATIONAL DESCRIPTION

Deployment of the bioluminescence photometer occurs usually between two hours after sunset and two hours before sunrise. About 2.5 - 3 hours is required to complete a deployment.

Preceding deployment, all watertight connections are inspected and the instrument, including all components, is allowed to warm up a minimum of 30 minutes. All records and logs are annotated with instrument settings and ancillary information such as weather, sea state, and moon phase. After the warm-up period a calibration or dark count is obtained for the photomultiplier and associated amplification circuits on the most sensitive scale (sensitivity ratio 1). This is accomplished by placing a heavy, dark cloth over the sensor, which blocks out any ambient light which may infiltrate the baffle system. Any signal observed is considered inherent in the system and is compensated for on the recording device. The photometer is uncovered and deployed to a maximum depth of 200 meters. A five-minute sample period is obtained at 10-meter increments. Deck lights remain out during the sample periods taken near the surface.

The volume of water sampled in any period can be controlled by the variable speed pump motor. Flow rates range from about one liter per minute to 9 liters per minute at zero head (figure 8) with the forward/reverse control switch in the forward position.

During surveys conducted after 1979 (i.e. Willett et al., 1982 a, b), after the measurement at 200 meters is obtained, the pump remains in operation while the instrument is retrieved, thereby obtaining a continuous profile of the upper 200 meters of the water column.

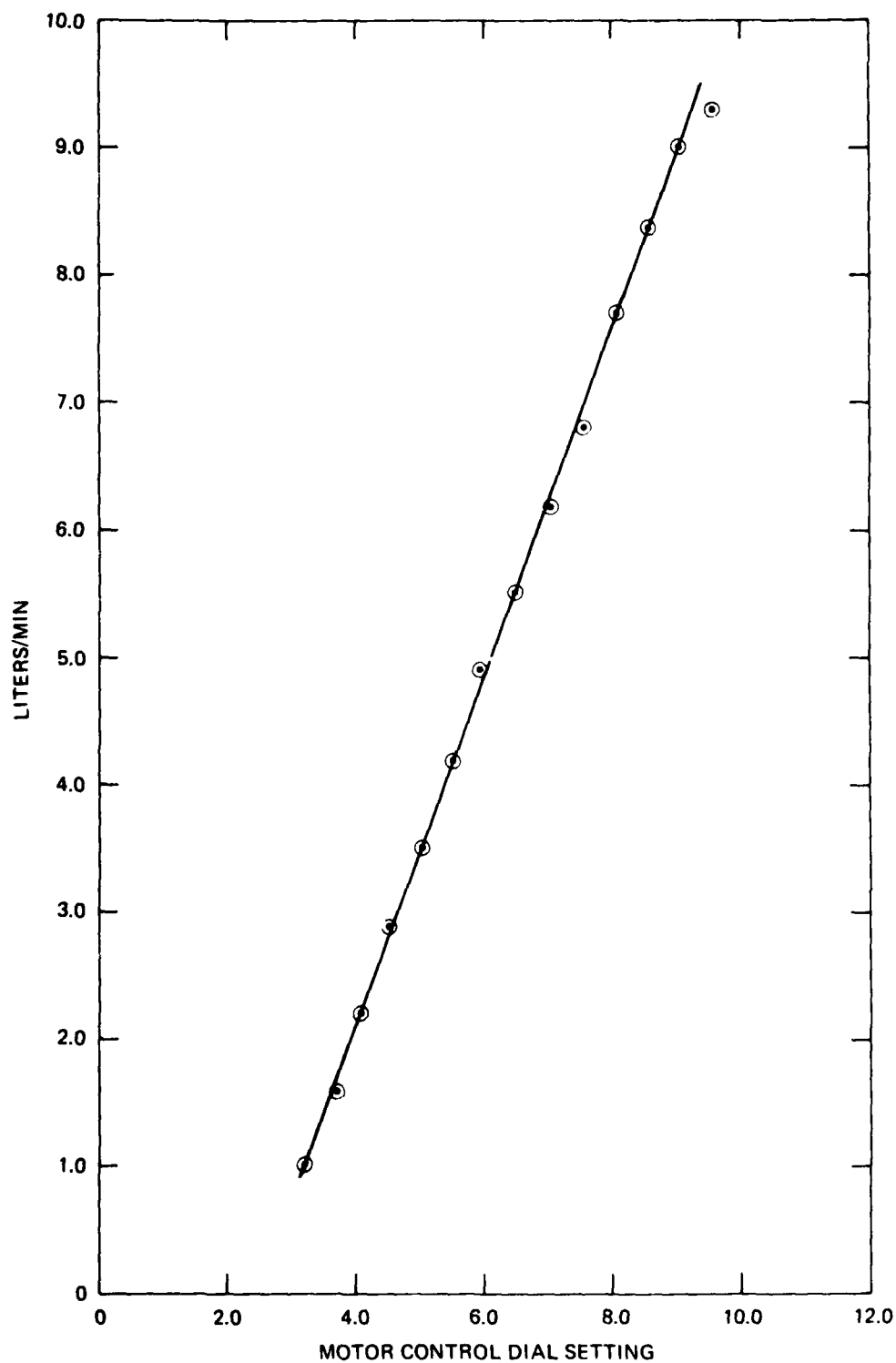


Figure 8. Photometer Motor Control Dial Setting vs. Flow Rate
(Zero Head, Forward)

V. OPERATION PERFORMANCE

The performance characteristics described are derived from both laboratory testing and actual at-sea deployment; they do not necessarily represent design specifications. Portions are adapted from an independent study by Scripps Institution of Oceanography Visibility Laboratory (letter to Mr. Adron Hall, NAVOCEANO, dated 11 May 1979). Speculative performance estimates are also incorporated in areas which are difficult if not impossible to evaluate.

A. SIGNAL

Signal refers to the electron current which is generated and amplified when a photon impinges upon the photocathode of a photomultiplier tube. The source of the photon, the elementary quantity of radiant energy, is the biological organism which is contained in the photometer sample chamber and is stimulated to bioluminesce. Stimulation is accomplished, as described earlier, by a combination of shear and mechanical components.

There are two signal output formats: integrated and non-integrated (figure 9). The integrated output averages the signal or groups of flashes over a time interval using a capacitor, expanding the horizontal (time) and vertical (amplitude) output as represented on the recorder. The extent of the expansion depends on a combination of factors including: the capacitor, the duration of the flash or flashes, and the intensity of each flash or groups of flashes.

The representative deflection recorded on the strip chart is not indicative of total light output or of the time history of the event. Each light package or peak may represent a flash by an organism, or a count of bioluminescent organisms passing through the sample chamber. This would assume, however, that each organism is viewed individually and this could be true only in areas of extremely low population densities at low pumping rates.

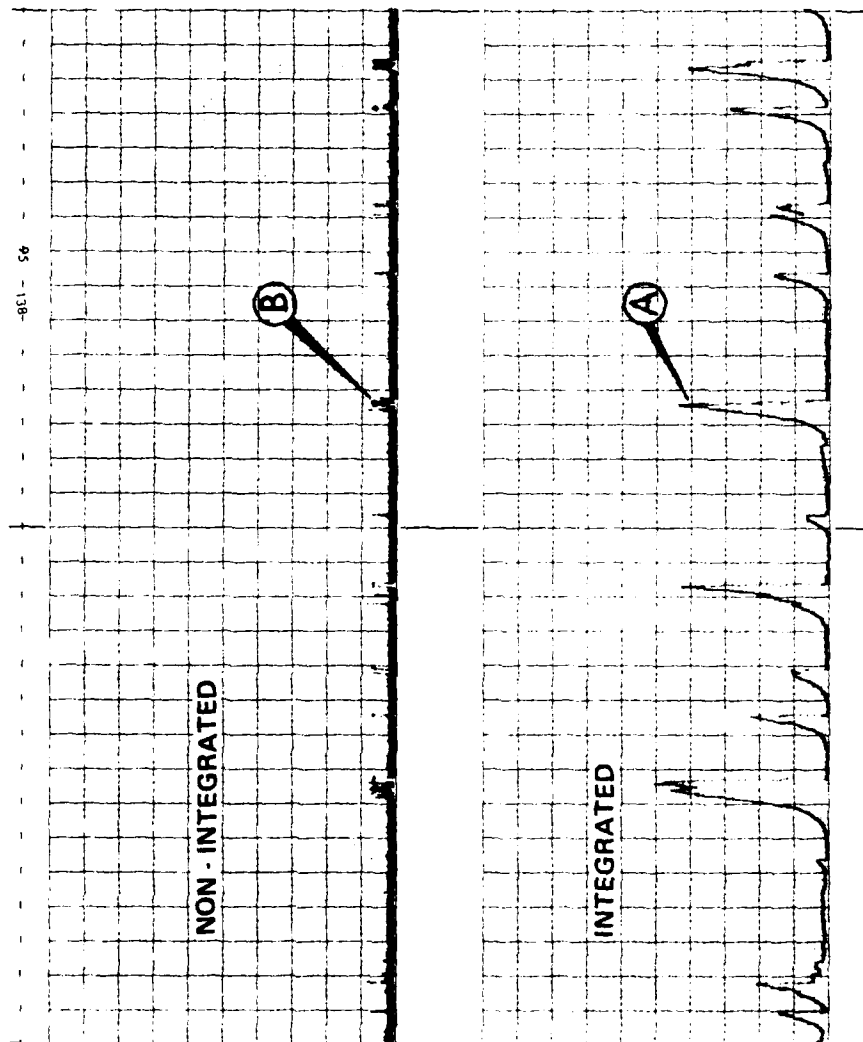


Figure 9. Example of Photometer Output. A-Flash as Recorded on the Integrated Channel, B-The Same Flash as Recorded on the Non-integrated Channel Indicating the Occurrence of Multiple Events.

In typical areas, several organisms could be in the chamber at once and flash simultaneously or nearly simultaneously resulting in a trace at the recorder which appears as a single peak. If two or more such events are 'in phase', the overall deflection will be greater; but it is indeterminable whether one large organism was excited or several small organisms were the source. In a near-simultaneous event situation, the curve on the recorder will contain a small deflection or a change in slope (figure 9-A); however, often this change is so small that it is indistinguishable as a separate event. In such instances of multiple flash events, bioluminescent organism concentration information must be looked at with skepticism because it can be significantly underestimated, especially in areas where several hundred events are recorded during a sample period.

This problem is somewhat reduced by use of the non-integrated output, (N.B. figure 9-B which reveals multiple events). This circuit monitors the signal before the integrating capacitor. This output is representative of the flash time history, which is on the order of hundreds of milliseconds. The temporal resolution, however, of the flash events recorded on the strip chart is poor, because the flashes are of a much shorter duration than is practical to operate a strip chart recorder paper feed. Noise generated by the D. C. pump motor results in a broad baseline (about 1 mm) for the non-integrated signal record. The resolution of flash events producing a vertical deflection less than 1 mm is questionable.

The use of both the integrated and non-integrated recordings during data reduction helps to arrive at a more accurate count, although there is still a highly subjective decision involved which reduces the precision of the measurements. Neither output mode is calibrated; that is, deflections

on the strip chart represent single or multiple events; they are not related to any absolute quantity of light.

The signal recorded during retrieval from 200 meters, providing a continuous profile, revealed no excessive noise created by the slip rings. However, the data collected with the present system is less than optimal due to a mismatch of instrument sample rate (pump speed) and instrument retrieval rate (winch speed). Nevertheless, this technique could reduce ship station time by as much as 50 percent and still provide useful data.

B. FLOW RATES

Flow rates are regulated by adjusting the pump motor speed which can operate in both a 'forward' (clockwise) and 'reverse' (counter-clockwise) direction (the pump is a centrifugal type; therefore, the direction of flow remains unchanged regardless of pump impeller rotation). The flow in the forward direction is about one-half that when the pump is operated at the same speed in the reverse direction. This is presumably due to the design of the impeller chamber (specifically the angle of the discharge port from the chamber) (figure 5) which allows for a less turbulent flow of water, and a higher pump rate, when the pump is operated in the reverse direction.

Organism stimulation (whether it be shear or mechanical) is dependent on the flow of water through the chamber; therefore, an experiment was conducted to determine the effects of both pump speed and direction on the amount of stimulation as measured by the resultant bioluminescence.

The instrument was deployed in a shallow coastal area relatively free of any mixing. The principle bioluminescent plankton were the dino-flagellates, as evidenced by water sample collections made during the deployment. The instrument remained at a fixed depth (1 m) and bioluminescence

was recorded at all pump speeds in both the forward and reverse directions. Replicate samplings occurred in rapid succession and in varying order to reduce the bias introduced by the change in the bioluminescent population and change in light intensity; both can vary with time.

The results of the experiment (figure 10) indicate that at all speeds greater excitation does occur when the pump is operated in forward; the added turbulence when operating in the forward direction creates an added stimulus. Maximum stimulation for this chamber design is not achieved in either pump impeller direction; however, the reverse direction appears to be approaching this maximum. The forward pumping direction undoubtedly creates more stimulus and therefore more bioluminescence, and the slope of the line indicates that this trend would continue if higher pumping speeds were applied. Flow rates, in a closed volume instrument such as the NAVOCEANO photometer, cannot be infinitely increased to obtain maximum stimulation; residence time of an organism in the field of view of the PMT must be long enough to permit detection. Too short a residence time after the organism is stimulated will result in a loss of information; too long residence time after stimulation can result in multiple stimulation and therefore multiple flashes. The residence times of the NAVOCEANO photometer are presented in table 2. These values are calculated using: 1) the maximum chamber volume (29cc) which could be viewed by the PMT and 2) the respective flow rates at each pump impeller direction and speed (assuming the flow is laminar, i.e. the water enters and exits the chamber by the most direct route). The residence times could be significantly different when the various shapes of the organisms interrelate with the hydrodynamic flow regime which occurs in the chamber (which is dependent on flow rate and direction of impeller rotation).

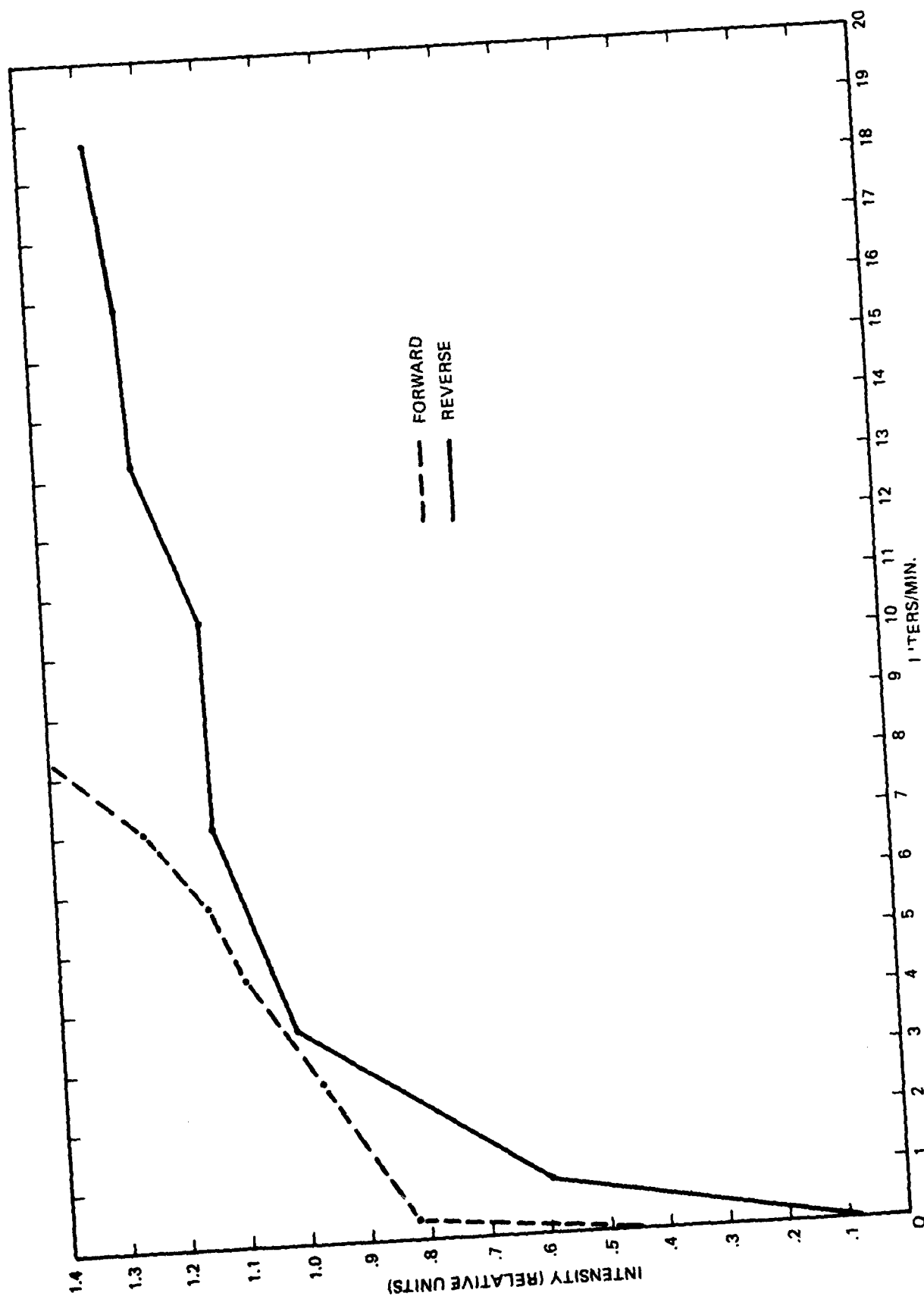


Figure 10. Bioluminescence Intensity vs. Flow (1/min) and Direction of Rotation

TABLE 2. Chamber Residence Times at Various Flow Rates and Direction

DIRECTION	FLOW RATE (l/min)	RESIDENCE TIME
FORWARD	.4	4.35s
	2.7	644ms
	4.5	387ms
	5.7	305ms
	7.0	249ms
	8.0	218ms
	8.3	210ms
REVERSE	.8	2.2s
	3.7	470ms
	7.1	245ms
	10.5	166ms
	13.2	132ms
	15.8	110ms
	18.0	97ms
	18.6	94ms

C. ZOOPLANKTON AVOIDANCE

Avoidance is an inherent problem in any instrument that is designed to quantitatively sample the planktonic environment. The NAVOCEANO photometer is no exception; it was originally designed to measure the luminescence created by dinoflagellates (i.e. making no attempt to solve the avoidance problems associated with the larger organisms) to provide insight into their ecology and physiology. The present requirement has expanded the original use to an open ocean general survey instrument used to estimate total bioluminescence potential of the water column. This task results in the deployment of the instrument in waters that not only contain dinoflagellates, but also larger and more motile copepods and euphausiids, both groups being capable of significant contribution to the bioluminescence field. Therefore, to determine whether the instrument actually records the entire bioluminescent potential field or just a portion, samples of the causative organisms were collected.

A 15-cm diameter, 20-micron plankton net was attached to the discharge hose during deployment of the photometer to capture the organisms actually passing through the sample chamber. The pump was operated in the forward direction at a speed to provide a flow of 3.5 l/min and 9 l/min. At each photometer station a vertical plankton tow was made using 66-cm diameter bongo nets (360-micron and 505-micron mesh nets) to sample the zooplankton component of the water column. The length of the largest organism and the composition for each sample were noted (i.e. the major bioluminescent groups present were dinoflagellates and copepods; no euphausiids were captured in the photometer samples). At the 3.5-l/min pump rate both dinoflagellates and small copepods were encountered, the largest copepods not exceeding 400 microns. At the 9 l/min. pump rate, again dinoflagellates and copepods

were present, the largest copepods being approximately 3 mm long. In all of the bongo hauls copepods and euphausiids were captured.

The increase in organism size with an increase in flow rate (the larger, stronger swimming organisms which escaped the 3.5 l/min flow were overcome at the 9 l/min flow) and the capture of the larger copepods and euphausiids in the bongo nets that were absent in the photometer samples indicate an avoidance problem with the photometer.

VI. SUMMARY AND RECOMMENDATIONS

The NAVOCEANO photometer system has been deployed numerous times, and with each use, information has been gathered concerning both the operation and performance of the instrument and the bioluminescence of the marine environment.

A. INSTRUMENTATION SHORTFALLS

Several factors can contribute to an underestimation of total stimuable bioluminescence in a particular area. These include: less than maximal stimulation in the sample chamber; the multiple flash phenomenon which appears as a single flash on the recorder, and the avoidance of the photometer by larger, more motile organisms. These factors are a function of several instrument design parameters, the most important being the chamber configuration and pumping rate. The NAVOCEANO photometer can be a valuable survey tool as long as the flash counts are not construed to represent any more than what the original design intended - dinoflagellate bioluminescence. When this instrument is deployed in areas containing bioluminescent zooplankton, the total bioluminescence capacity will not be monitored. However, dinoflagellate bioluminescence may be used as an indicator of high bioluminescent areas (both horizontally and vertically). Dinoflagellates form a portion of the primary link of the marine food web; these primary

producers are a necessary component to support the higher order life forms. Therefore, areas with high dinoflagellate bioluminescence provide a food source that could attract predators capable of luminescence. Additionally, one of the stimuli of luminous predators is light: the primary dinoflagellate bioluminescence could stimulate secondary bioluminescence by the predators. Dinoflagellate bioluminescence, then, could be exploited to provide valuable information on the distribution of bioluminescence in the oceans even though total light production information is not available. Conventional net sampling would lend support to this hypothesis, but associated problems can also lead to biased interpretation due to patchiness.

Appropriate electronic filters should be incorporated in photometers which use a D.C. pump motor to insure that a minimum of the low level signal is lost due to noise.

Continuous vertical profiles of bioluminescence can be obtained using slip rings without signal degradation due to excessive noise; however, the instrument sample rate must match the instrument retrieval rate so large enough samples are monitored to provide statistical reliability.

B. RECOMMENDATIONS FOR FUTURE PHOTOMETERS

Future bioluminescence photometers should depart from analog event counts, such as the NAVOCEANO system and incorporate digital techniques as utilized in radiation physics. These very sensitive techniques enable the counting of single photons and delete the signal integration necessary with an analog system. The digital signal can be monitored on a time interval that matches the duration of the flash, thereby providing information representative of the actual flash duration and intensity, rather than a function of electronic circuitry.

Flow rates need to be of sufficient volume to allow capture of larger, more motile organisms. This is a potential problem using the impeller in the chamber as a source of stimulation: larger flows will require larger chambers, which are necessarily limited in size due to the volume that can be viewed by a PMT. If existing chambers are made more efficient, i.e. less turbulent, then less than maximum stimulation will be the result. A flow-through chamber (Losee and Lapota, 1981) is an alternative design which allows higher pumping rates without exceeding the field of view limitation of the PMT. Stimulation is provided by a restriction of the supply line, a grid, or some other apparatus just before the chamber entrance which provides a completely turbulent flow inside the chamber. Information is then gathered in 1-ms time frames and reported as event rates (photons/time/volume). This short sample period reduces the residence problem because the event rate approaches a steady state situation. That is, the elemental quantity of light is being measured rather than a complete flash event.

Multiple PMT's, using appropriate optical band pass filters, viewing the same volume would provide a spectral monitoring capability.

All photometer systems, regardless of type or construction, need to have a well-defined, thoroughly documented, and repeatable source of stimulation and method of detection. This would afford some degree of comparison among various investigators. This stimulus and detection technique must be consistent with the type of bioluminescence measurement required, whether it be total stimuable, background, or otherwise. This technique must also take into consideration the size and shape of the causative organism. These factors are especially applicable in the natural environment where the variability of bioluminescent organisms, along with the corresponding change

in the optimum stimulation source and detector, can be extreme. In light of these factors, it becomes apparent that a single, ultimate, universally applicable bioluminescence photometer may not be practical. Perhaps, a more plausible solution is to employ one or more of a suite of instruments, each of which is capable of satisfying particular sampling objectives. The most appropriate instrument (or instruments) could then be mounted on a tow body to make rapid, large-scale sampling possible.

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