FLORIDA SEA GRANT PROGRAM

A SYSTEM FOR THE DETERMINATION OF CHRONIC EFFECTS OF POLLUTANTS ON THE PHYSIOLOGY AND BEHAVIOR OF MARINE ORGANISMS

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INTRODUCTION

This paper is a description of a laboratory system that was designed for the execution of controlled experiments on the effects of low levels of pesticides such as DDT and Mirex on the behavior and physiology of several species of estuarine organisms.

It has become increasingly apparent that many estuarine and coastal ecosystems are under stress due to various forms of pollution. Pesticides, heavy metals, industrial contaminants, and petrochemicals are often ingested and absorbed by various marine organisms; this can often lead to serious changes in aquatic systems. Lindall (1973) has pointed out that 85 percent of the commercial fish and shellfish in south Florida are estuarine dependent, and that the multimillion dollar industries that depend on such organisms could be threatened if the estuaries are impaired or destroyed by man-related activities. Already there has been considerable alteration of such systems due to thermal addition, dredging, reduction of fresh water runoff, and numerous sources of human, animal, and

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industrial contamination. With an increasing demand for food from the sea, the problem cannot be ignored. Unfortunately, little is known concerning the effects of long-lasting contaminants such as pesticides on estuaries and all too often, the biological significance of pollution is not immediately apparent. The ecological impact of chronic (low level) pollution is difficult to assess, but it is undoubtedly of more significance than the more obvious acute effects. When a number of such sources of stress act together, there can be subtle but serious disruptions of the natural systems that can result in gradual changes in species composition and reduced productivity.

In spite of the incredibly complex nature of near-shore communities and the numerous factors (natural and unnatural) that influence such systems, there is some evidence that behavioral functions can be determined with respect to ecological changes, and that relatively low concentration of pollutants can alter such behavior. The many relationships between behavioral functions and community mechanisms remain largely unknown; this is especially true in marine systems. Klopfer (1973) has pointed out many of the important behavioral factors that are implicated in ecological situations. Feeding behavior, communication, territoriality, learning, and gradient responses are all important in the determination of the distribution of a given species. Foster, Cairns, and Kaesler (1969) have listed the advantages of quantitative behavioral bioassay work. Such functions as sensory responses, reproductive behavior, activity patterns, etc. are just a few of the behavioral functions that can be analyzed with respect to the effects of chronic (subacute) pollution. For example, if bay sediments are contaminated with pesticides such as DDT and Mirex, it is quite possible that some species will avoid such areas. Depending on the ecological relationships of such species, these areas could become useless as nursery or breeding grounds even though there are no overt signs (such as an extensive kill) that anything is wrong. If such contamination is transferred to the organisms in food or directly from the sediments or water, it is also possible that bioconcentration could cause the impairment of important functions such as reproduction without actually killing the organism. This form of insidious pollution could have a serious effect on an important fishery without being detected by conventional methodology. Only through long-range integrated laboratory-field studies can the impact of various long-lasting pollutants such as DDT and petrochemicals be assessed. Although behavioral experimentation cannot be directly interpreted as a field index, it nevertheless can allow an expansion of ecological perspective when reviewing the usefulness or possible detrimental effects of a particular contaminant on an estuarine species or community. The study of behavior can thus be used to indicate field interactions and the potential impact of pollution.

SEAWATER DELIVERY SYSTEM

Seawater systems for controlled laboratory experiments have been described for various environmental situations (Atz, 1964; Cargo, 1964; Wisby, 1964). It is clear that such systems must be carefully designed and constructed to allow maximal control of environmental conditions without impairment of water quality. Such factors as temperature, salinity, dissolved oxygen, turbidity, toxic substances, and growth of fouling organisms are some of the more important parameters that should be considered. Each system must be adapted to the immediate environment for this to be accomplished. Seawater at the Florida State Marine Laboratory (Turkey Point, Florida) is pumped from the shallow grass flats of the nearby Gulf of Mexico. There are seasonally high levels of turbidity and suspended sediments in this area. This situation has been approached by the development of a series of 1,000 gallon settling tanks where much of the suspended matter is allowed to settle out (Figures 1 and 2). Water is then pumped to a master headbox through a temperature control system composed of a thermoregulator heater-cooler unit similar to that described by Livingston (1970). This system is designed for a rough adjustment of water temperature and has been altered in certain ways to allow the processing of relatively large flows of water. The water passes through approximately 24 feet of 1 inch tubing that has 4 indentations per inch to increase the surface area. The tubing sits inside a water-filled chamber containing several heating elements with a combined capacity of 1×10^4 watts. From the heat-exchange coils the water travels into a reservoir headbox containing a thermoregulator which is wired to the heater-cooler apparatus. The output from this apparatus controls heavy duty relays carrying 208 VAC to the heating elements; this has the capacity to raise the temperature of 3 gallons of water per minute 10° centigrade. The master headbox complex remains above the laboratory headboxes so that water is gravity fed to the various experimental devices. All of the water in the reservoir box which is not used in the labs is returned to one of the settling tanks to maintain temperature stability.

Seawater enters each laboratory headbox (water conditioning unit) and is passed through 2 inches of oyster shell for initial filtration and pH control. The water is then run over a partition which facilitates the exposure of the water to ultraviolet light from a 15 watt germicidal light bulb. The seawater then seeps through 5 inches of activated charcoal for further purification and passes down a series of troughs with heaters for precise temperature control. Standpipes at both ends of the headbox control the water level, and the water is continuously aerated as it procedes through the system. Incoming seawater can be monitored for important

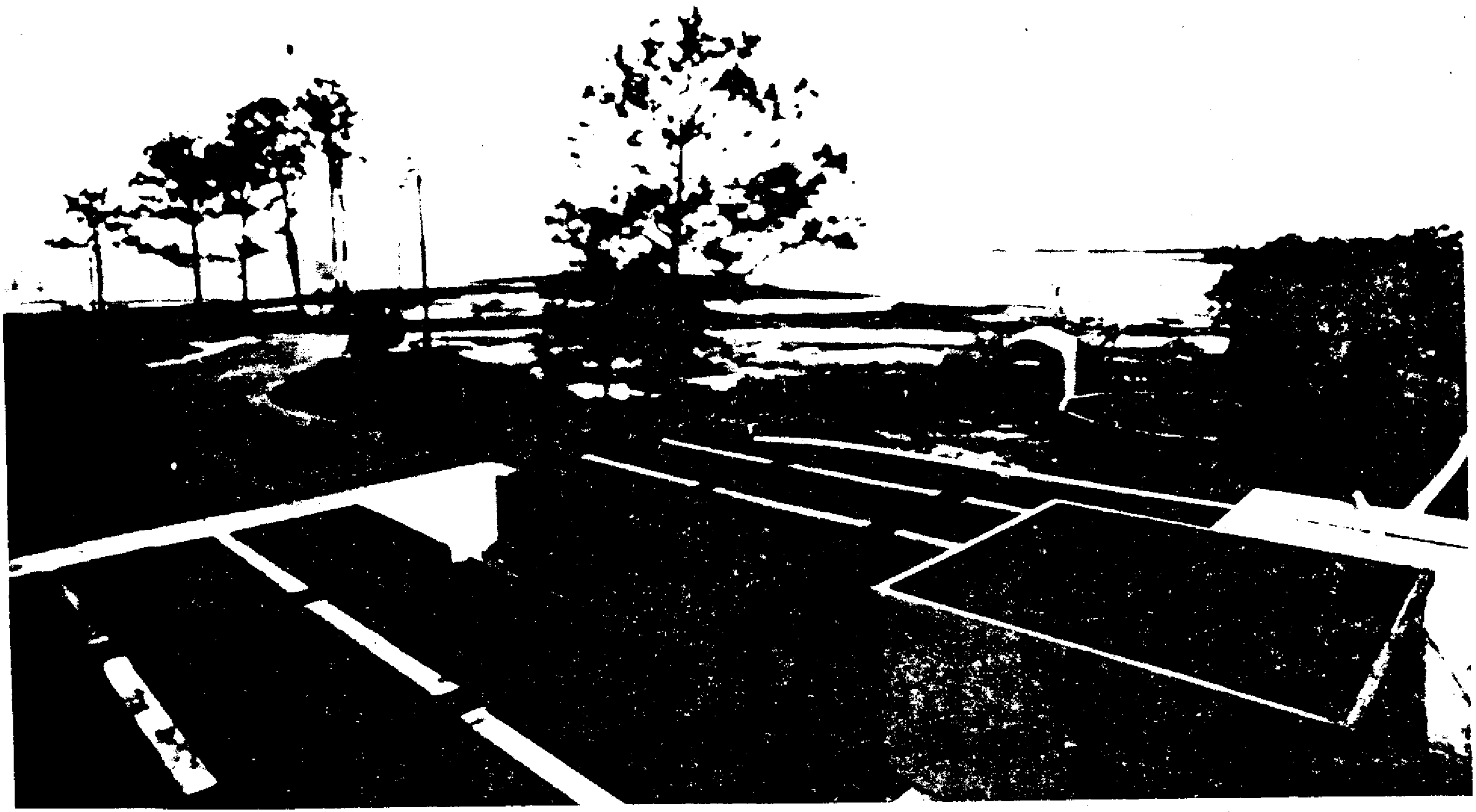
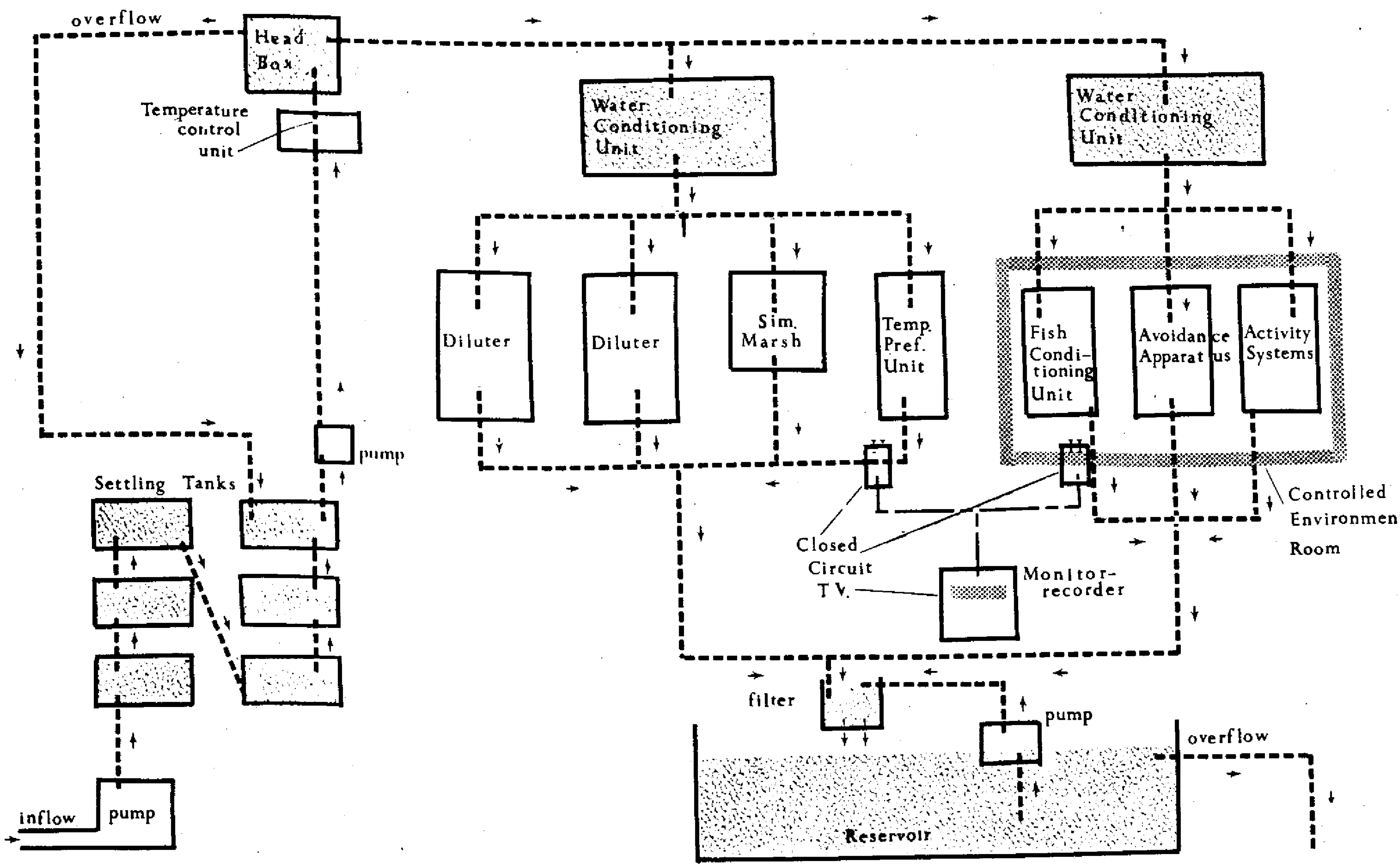


Figure 1: Settling tanks overlooking the northern Gulf of Mexico at the Edward Ball Marine Laboratory (Turkey Point, Florida).



SEA WATER DELIVERY SYSTEM

Figure 2: Flow diagram of sea water delivery system showing relationships of the water

parameters such as temperature, salinity, dissolved oxygen, and turbidity. In this way, seawater is processed and made available to the various experimental laboratory systems.

DILUTER SYSTEMS

Various types of continuous flow diluters have been developed to maintain low concentrations of pesticides in experimental aquaria for prolonged periods. Several diluters have already been described (Lane and Livingston, 1970). One diluter was designed to deliver fixed concentrations of contaminants in seawater continuously to experimental chambers (Figure 3). The water is delivered from the constant head reservoir through a series of U-shaped siphon tubes which may be adjusted up or down to change the rate of flow. The water then passes through several slant tubes which receive measured injections of pesticide. The solution is mixed in the lower reservoirs and passed directly to the test tanks. The flow rate to the test tanks may be adjusted by varying the constriction of the ends of the delivery tubes and also by changing the height of the mixing reservoirs. The pesticide solution is delivered from a modified Mariotte bottle to a series of syringe needle injectors. The injectors are inserted through split tubes covering the longitudinal slits along the upper faces of the slant tubes. Injection rate may be varied by raising or lowering the injectors along the length of the slant tubes and by changing the bore diameter of the needles. A solenoid shutoff controlled by a pressure switch in the main constant head reservoir serves as a fail-safe system to shut off the pesticide flow into the test tanks in case of a water source failure.

Since photoperiod can be a critical factor in any behavioral experiment, lighting systems have been developed for all experimental setups. Care has been taken to simulate as closely as possible the natural environment. Each system consists of a combination of dimmers and timers to provide a variable photoperiod and a simulated dusk/dawn transition. A modified version of a time-dependent lamp dimmer is used with a timer that consists of a set of rotating cams that open and close a series of microswitches. At sunrise, one cam causes the dimmer to increase the voltage continuously to 5 (100 watt) incandescent bulbs for a period of about 25 minutes. Another cam then switches the (160 watt) fluorescent bulbs on and a third one turns the incandescent bulbs off, thus reducing the amount of heat generated. This cycle is reversed for the dusk transition. One can simulate the photoperiod of any time of the year by appropriately adjusting the cams.

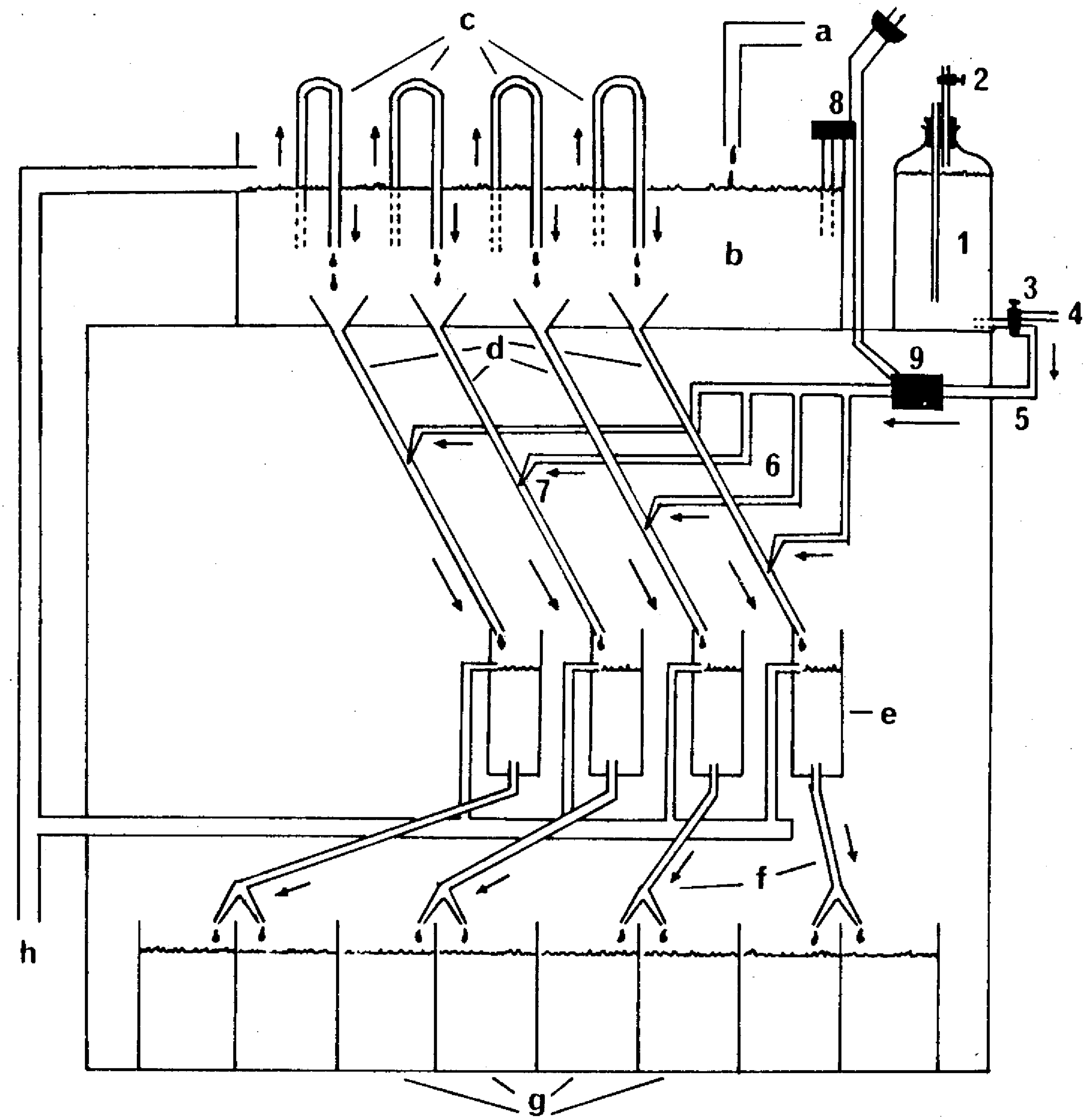


Figure 3
Schematic diagram of continuous flow pesticide dilutor system.

Water delivery system: a. temperature and salinity controlled water source, b. constant-head reservoir, c. adjustable siphon tubes, d. slanting glass tubes with a plastic covered longitudinal slit on upper slide, e. constant-head mixing reservoirs, f. delivery tubes constricted and split distally, g. test tanks, h. drain.

Pesticide delivery system: 1. modified Mariotte bottle containing pesticide solution, 2. pressure release valve (open only during refilling), 3. three-way valve, 4. refilling and vacuum starting outlet, 5. main line to injectors, 6. injector lines, 7. syringe needle injectors, 8. water level fail-safe switch controlling, 9. solenoid shut-off.

AVOIDANCE APPARATUS

To test the avoidance reactions of marine fishes to toxic substances, a differential avoidance trough has been constructed similar to that used by Sprague (1968) and Sprague and Drury (1969). This experimental apparatus consists of a rectangular plywood trough (42 inches long by 6 inches wide by 6 inches deep), covered with white polyester resin on the inside and clear resin outside. Seawater flows into each end of the trough and drains at the center by means of 4 constant level siphons that maintain the water level at a depth of 4 inches. Water flows through the trough at a rate of 1 gallon per minute in each arm. Dye studies have shown a distinct boundary between the two sides of the trough with little or no mixing evident.

A Sony video camera and television monitor is used for remote viewing of the trough which is located in a sound proof controlled environment room. In this way, fish behavior in the trough can be taped and analyzed with minimal disturbance to the experimental subjects. Fishes are placed in the trough and allowed to acclimate for an adequate period of time before testing. The toxicant is metered into one side of the trough from a constant pressure head (Mariotte bottle) while the other end of the trough remains uncontaminated. The position of the fish in the trough is then recorded every 30 seconds for 60 minutes and avoidance is determined by the percent of time spent in uncontaminated water. The following formula (Sprague, 1968; and Sprague and Drury, 1969) has been used to compute the avoidance index (AI).

$$AI = \frac{X - Y}{X + Y}$$

X = number of counts in clean water
Y = number of counts in test concentration

Another avoidance system is presently under construction that utilizes an electronic system of monitoring activity behavior. The trough is made of transparent plexiglass, and has 8 sets of infrared light-emitting diodes (LED) and phototransitors on each end of the trough. Beams of infrared light are directed across the trough. If a moving fish breaks one of the light beams, one of the two counters will be activated thus allowing the quantification of fish movements in the trough. A closed circuit television can be used in conjuction with a tape recorder to monitor the counters at specific time periods. A comparison of the numbers on the counters should then give an indication of any possible avoidance of or attraction to the contaminated water. This can also be used as a device to measure activity (see following section).

ACTIVITY MONITORING SYSTEM

Activity Patterns of fishes can be quantitatively determined in monitoring tanks that are equipped with sets of infrared lights and phototransistors as described above. Each 5 1/2 gallon aquarium is fitted with 8 sets of LED (Figure 4), and the entire set up is connected to one channel of an Esterline-Angus event recorder. Each time a light beam is broken by the movement of a fish, notation is made; these are counted in 30 minute intervals and graphed accordingly. In addition to the measurement of the activity of the experimental subjects, observations of the actual behavior of the fish can be made and recorded with the closed circuit television apparatus. These experiments are run in a controlled environment room so that the subjects remain in relative isolation from extraneous sources of distraction. With careful control of light, temperature, salinity, dissolved oxygen, etc., experiments can thus be run on a whole range of activity related modes of behavior. The chronic effects of such compounds on activity patterns can be analyzed by exposing fishes to low concentrations of various types of pollutants.

TEMPERATURE PREFERENCE APPARATUS

The experimental appartus consists of a plywood (4.5 inches by 2.7 inches by 5 inches) trough (Figures 5 and 6). The trough (painted with clear polyester resin on the sides and white polyester resin on the bottom) is subdivided into 10 regions with narrow black polyethylene strips. Addition of heated and cooled water to the trough at specific locations creates the desired temperature gradient. Hot water is added at the bottom of the trough and cold water at the surface. The addition of about 1/4 gallon per minute (of hot and cold water combined) through 10 small tubes provides sufficient mixing to establish a continuous gradient. Water to be cooled flows through heat exchange coils in a water/methanol bath located in the freezer section of a refrigerator. Water is heated with two 615 watt Corning PC351 hot plate stirrers, and flows are adjusted with Teflon stopcocks or screw clamps. The gradient has a range of 10°C as determined by 6 glass-enclosed thermistors placed along the side of the trough. The resistance of the thermistors is read on an ohmmeter and converted to °C. The temperature of each region is thus determined by interpolation from the temperature of the thermistors. The trough is located in a light-proof area and illuminated with two 8 foot 40 watt fluorescent bulbs mounted 6 feet above the trough and attached to an automatic timer. Observation of the trough is facilitated by a Sony video camera and Sony television monitor. Fishes are allowed to acclimate to the trough and gradient for appropriate periods, and the location of each fish is

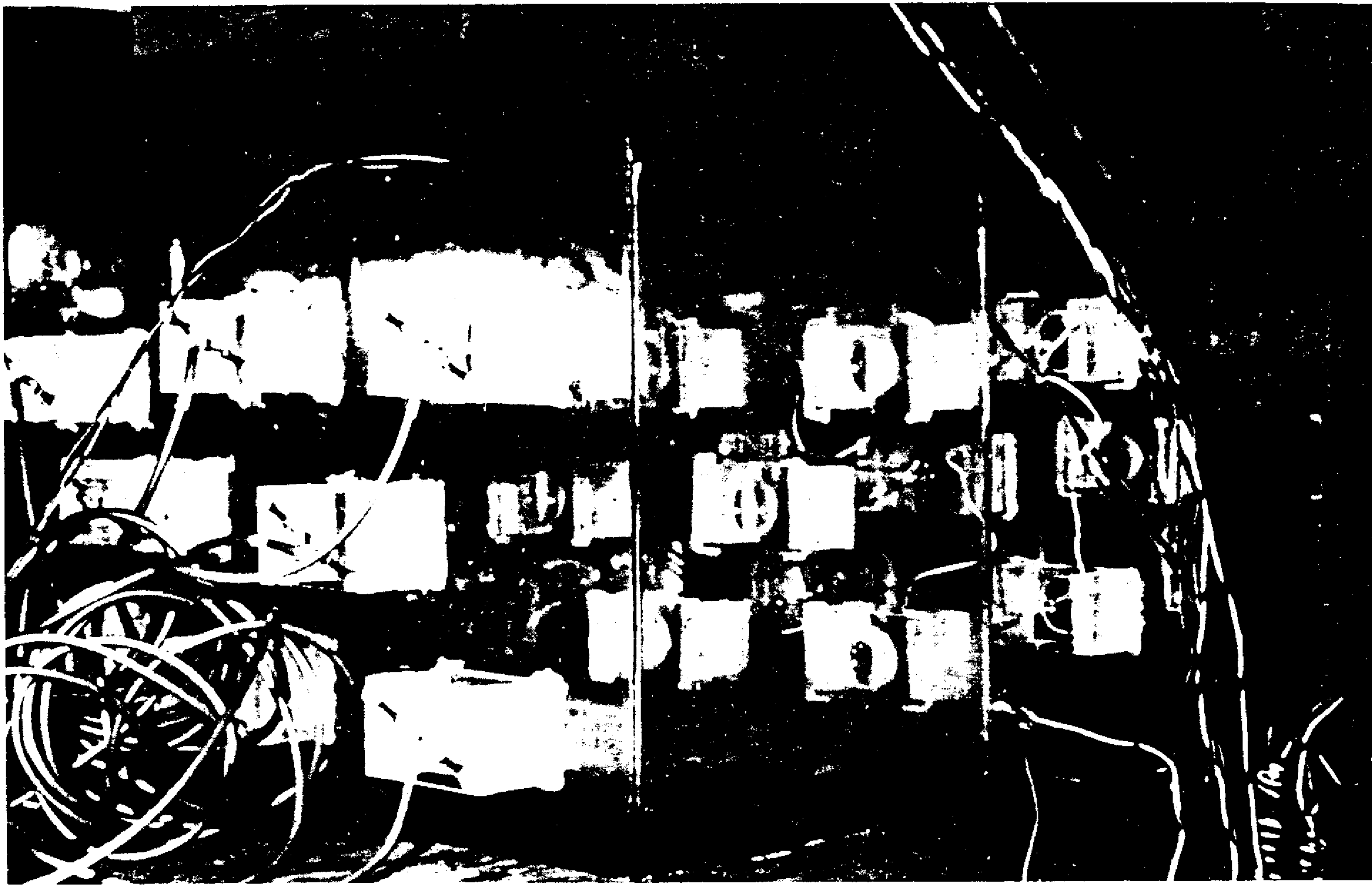


Figure 4: Activity monitoring apparatus with infrared light emitting electrodes that send a series of beams across an aquarium.

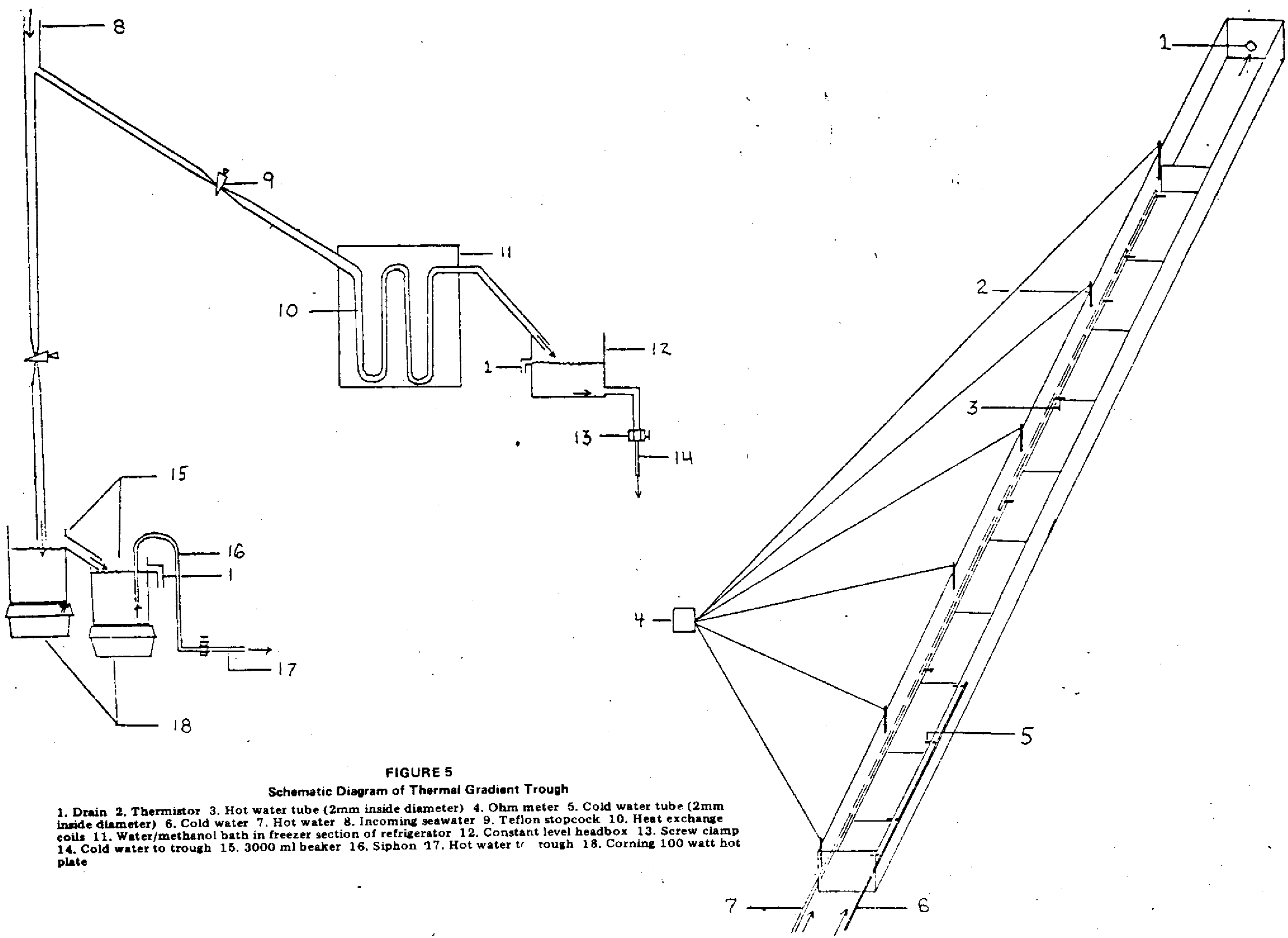
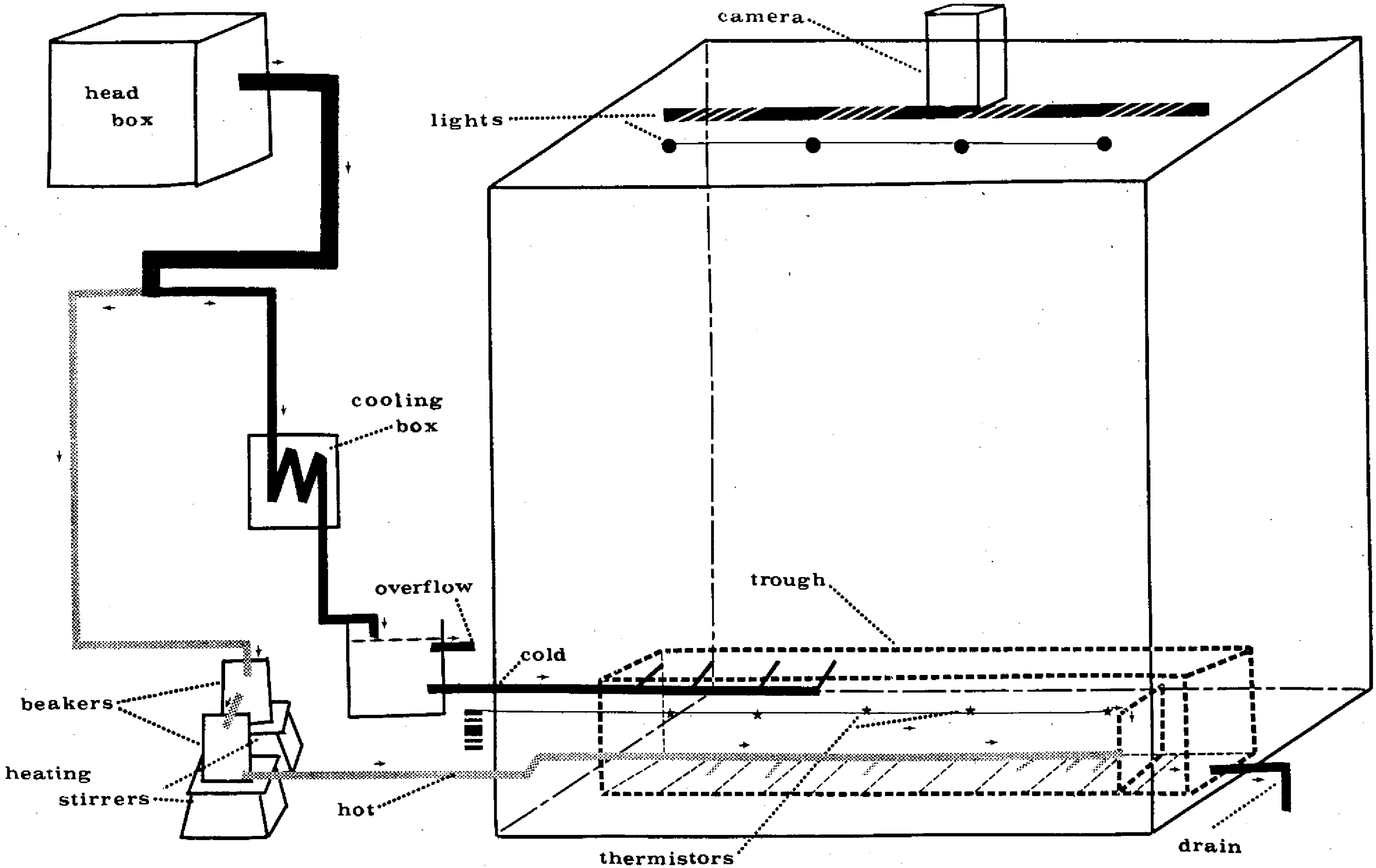


FIGURE 5

Schematic Diagram of Thermal Gradient Trough

1. Drain 2. Thermistor 3. Hot water tube (2mm inside diameter) 4. Ohm meter 5. Cold water tube (2mm inside diameter) 6. Cold water 7. Hot water 8. Incoming seawater 9. Teflon stopcock 10. Heat exchange coils 11. Water/methanol bath in freezer section of refrigerator 12. Constant level headbox 13. Screw clamp 14. Cold water to trough 15. 3000 ml beaker 16. Siphon 17. Hot water to trough 18. Corning 100 watt hot plate



TEMPERATURE PREFERENCE APPARATUS

Figure 6: Temperature preference apparatus, showing relationship of T.V. monitor and trough.

recorded once each minute for 60 minutes. One set of such observations is considered one trial. The location of the anterior end of the head is arbitrarily designated as the location of the entire fish. This apparatus thus allows flexibility of experimental and monitoring conditions as well as a rapid quantification of the experimental data.

SIMULATED MARSH HABITATS

Three simulated marsh habitats (Figure 7) have been established in circular wading pools (10 feet diameter). Each pool is supplied with a constant flow of 3/4 gallon of sea water per minute. Mud, plants, and animals were combined in the pools as balanced systems that could serve in the determination of the dynamics and effects of pesticide flow. A tidal flow is created by electronically raising and lowering a constant level siphon in each pool. The height of the siphon is changed with a motor-gear system to produce one complete tidal cycle once every 12 hours and 48 minutes. High tide comes to within a few inches of the mud plateau and forms an intertidal zone of 5 to 6 inches. In addition to a zone of marsh grass (*Spartina alterniflora*), various organisms such as fiddler crabs, juvenile blue crabs, oysters, and certain species of top minnows have been used successfully in this habitat. The use of the simulated marsh systems under strictly controlled conditions; it is thus an attempt to utilize the advantages of laboratory experimentation without subjecting the organisms to the narrow restrictions that usually accompany such operations.

SUMMARY

An experimental system has been set up to facilitate the use of high quality sea water in experiments concerned with the chronic effects of pollutants on the behavior and physiology of selected marine organisms. This system can be used to determine quantitative changes in behavior under closely controlled conditions. By utilizing a sound proof controlled environment room, there is a minimal amount of outside distraction for the experimental organisms. The closed circuit television allows continuous monitoring in such undisturbed surroundings, and is used effectively for rapid quantification of behavioral data. With this system, it is hoped that more can be learned about the long-term sublethal effects of pollutants on the behavior of key estuarine and coastal organisms so that another dimension can be added to our understanding of the significance of pesticide residues found in natural populations.

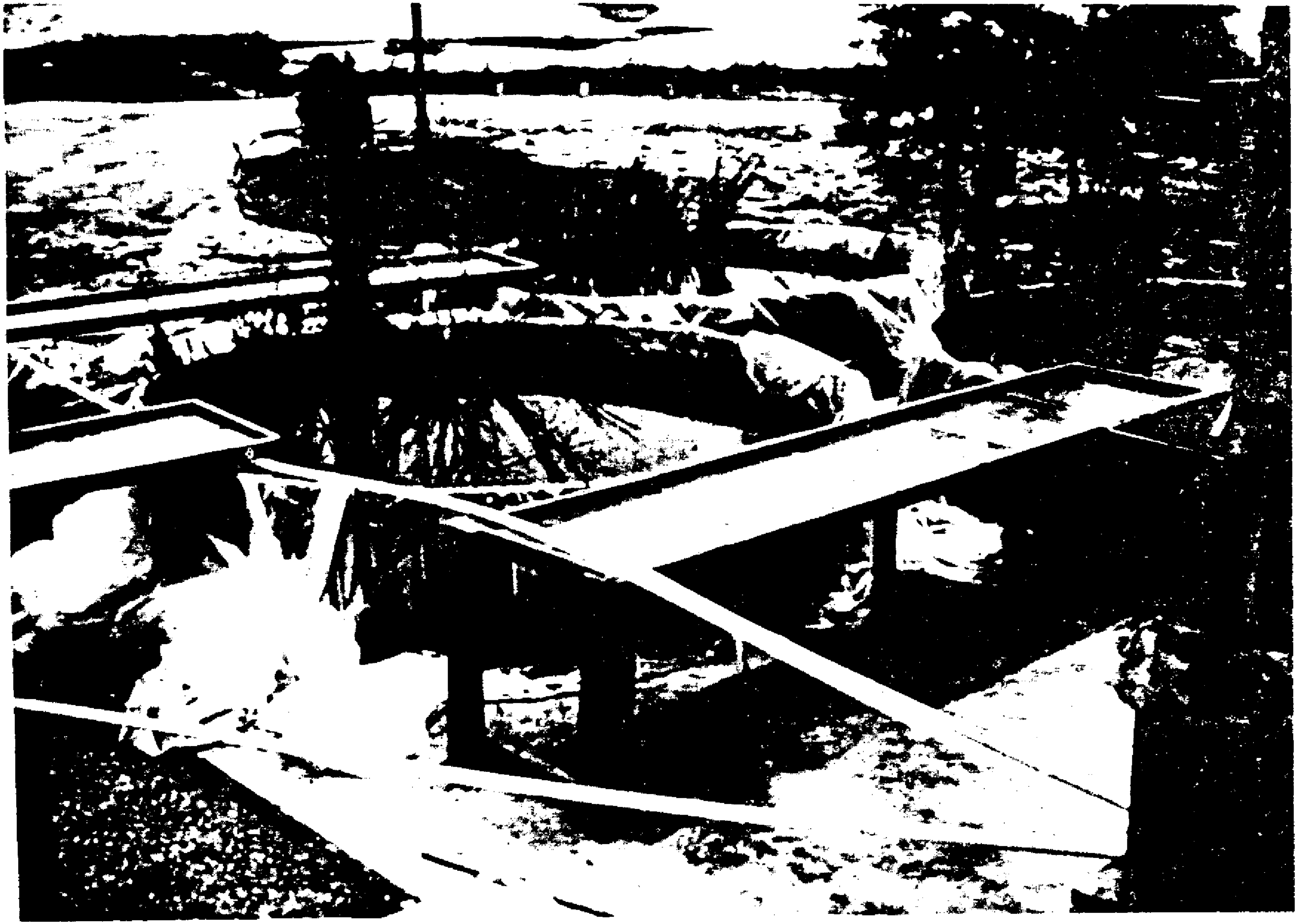


Figure 7: Simulated marsh habitants with troughs for deposition of corn cob grits carrying pesticides such as Mirex.

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