T H E U N I V E R S I T Y O F M I C H I G A N

COLLEGE OF ENGINEERING
Department of Mechanical Engineering

Supplement to Final Report

THE EFFECT OF LONG-TERM EXPOSURE TO OUTBOARD ENGINE EXHAUST EMISSION ON THE FATHEAD MINNOW

Norman K. Schenck
Walter J. Weber, Jr.
David E. Cole

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INTRODUCTION

Concern about the pollutional effects of outboard motors has grown as a result of the increasing use of power boats in lake and river recreational activities. In some lakes this may be the primary source of man-caused contamination. The question is: to what extent does this affect the aquatic environment? The study being perfomed at The University of Michigan is an attempt to assess these effects qualitatively and quantitatively by employing a laboratory bioassay, i.e., by subjecting aquatic animals to different levels of exhaust contamination and measuring their response in a systematic way. A short-term bioassay has previously shown that outboard motor exhaust products are lethal to fish only at extremely high concentrations which are not likely to be found in realistic situations.[1] The present study is a long-term bioassay to determine if there are more subtle, sublethal effects on a particular species of fish which is representative of temperate waters.

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1. English, et al., Water Pollution Control Federation Journal, July 1963, p. 923.

TEST METHOD AND EQUIPMENT

TEST FISH

Fathead minnows (Pimephales promelas) were chosen as the test fish. There are several reasons for selecting this species over the many possible choices. Its small size permits a large number to be used in the limited space available. Its spawning behavior allows control of "nesting" sites which permits reliable data observation. It serves as a food fish for larger temperate water sport fishes such as bass, pike, crappie, and perch. It is neither particularly tolerant nor intolerant to environmental stresses, it is readily available, and it has been often used in laboratory bioassays.

TEST CONCENTRATIONS

The test fish are being exposed to a wide range of concentrations of "exhaust water" (water subjected to the submerged exhaust emissions from outboard engines). This range was selected to bracket conditions resulting from moderate to extremely heavy motor boat usage. Table I shows the nominal exhaust water concentrations in terms of volume of fuel consumed per volume of dilution water.

TABLE I

TEST CONCENTRATIONS

1.	(Highest)	1/10,000
2.		1/40,000
3.		1/160,000
4.		1/640,000
5.	(Lowest)	1/2,560,000
6.	(Control)	0

TEST FUELS

Exhaust waters from leaded and non-leaded gasolines are being tested simultaneously in a side-by-side arrangement. (Lead is a known toxic substance which is not required for two-stroke engines but is used because non-leaded gasoline has not been generally available.) The test fuels are Indolene Clear (non-leaded) and Indolene 30 (containing 3 grams per gallon of tetraethyl lead). These are fuels of high quality control from Standard Oil and are widely used in

exhaust emission studies. Johnson lubricating oil is mixed with the gasolines at the recommended ratio for these engines of 1:50. This is the common oil-fuel ratio for modern outboard engines. Older engines require larger proportions of oil.

TEST WATER PREPARATION AND DISTRIBUTION

Duplicate test chambers for each concentration (including controls) are set up for each gasoline. Similer 1-1/2 hp Johnson motors are used to produce the exhaust water from the two gasolines. A high-strength "stock solution" of each exhaust water is produced by operating these engines in a 50-gallon tank. This stock solution (which is renewed three times per week) is diluted and delivered to the test tanks by a serial dilution system which mixes the prepared exhaust water with freshwater in the proper proportions through a series of constant-head orifice tube devices shown pictorially in Figure 1 and schematically in Figure 2. The dilution water is Ann Arbor tap water which has the typical characteristics shown in Table II. This water must be dechlorinated

TABLE II

ANN ARBOR TAP WATER ANALYSIS

Methyl orange alkalinity	34 ppm as $CaCO_3$
Phenolphthaline alkalinity	37 ppm as $CaCO_3$
Hydroxide alkalinity	3 ppm as $CaCO_3$
Bicarbonate alkalinity	0 ppm as $CaCO_3$
Total hardness	80 ppm as $CaCO_3$
Non-carbonate hardness	46 ppm as $CaCO_3$
pH	10.0
Chlorine	.5 - .7 ppm
Calcium	21.6 ppm
Magnesium	5.3 ppm
Chromium	< .002 ppm
SiO_3	8 ppm

and its pH must be lowered to render it suitable for fish life. Dechlorination is effected by passing the water through activated carbon which reduces the active compounds of chlorine to harmless chloride. Adjustment of pH is accomplished by bubbling carbon dioxide gas through the water as it enters the distribution tank. The CO_2 forms carbonic acid, H_2CO_3, in water which ionizes and thus increases the hydrogen ion concentration in the water. The CO_2 input is regulated by a Fisher titrimeter which senses the pH and opens and closes the CO_2 valve accordingly. A schematic diagram of the experimental apparatus is shown in Figure 3. Figure 4 is a photograph of the system.

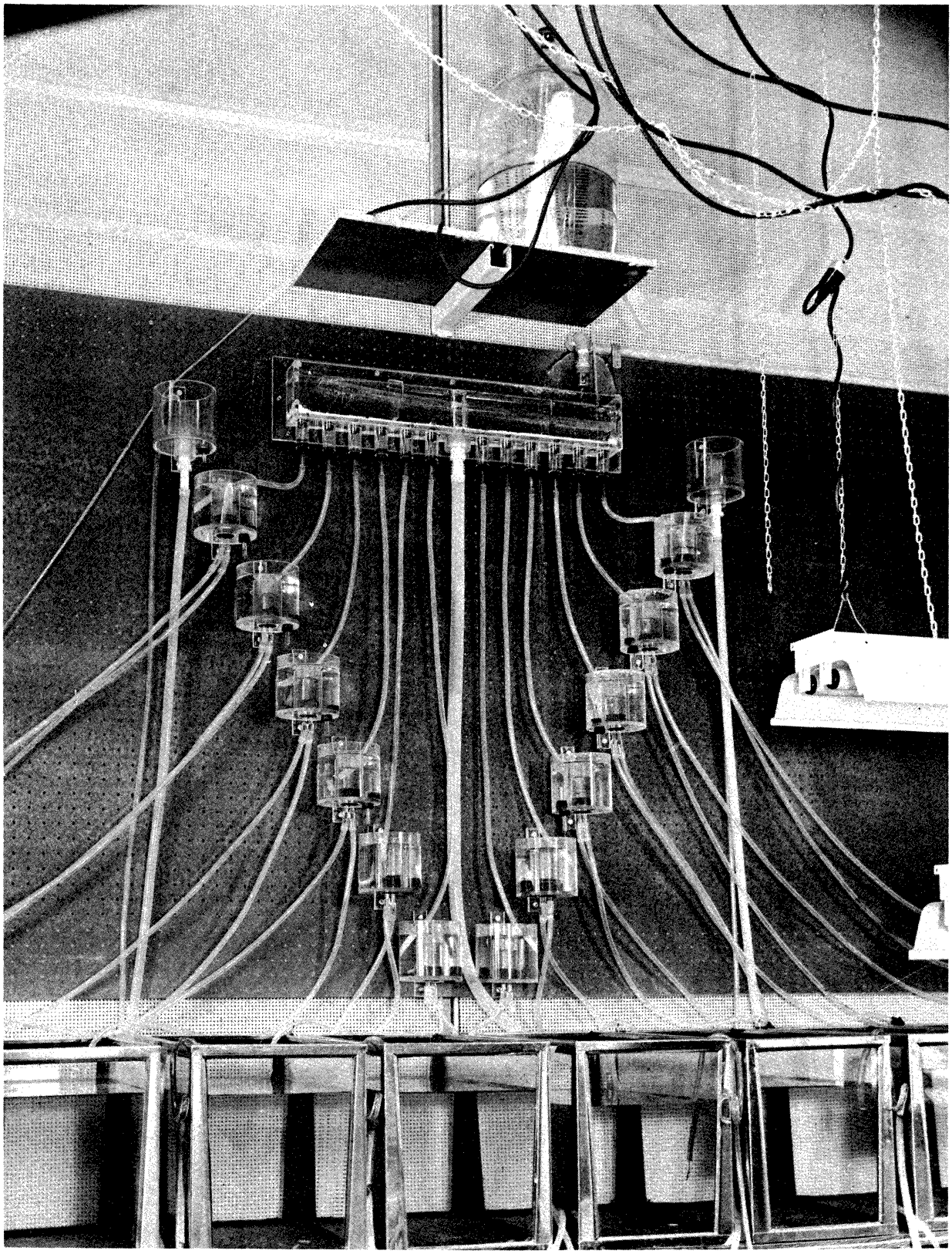

Figure 1. Photograph of serial dilution system.

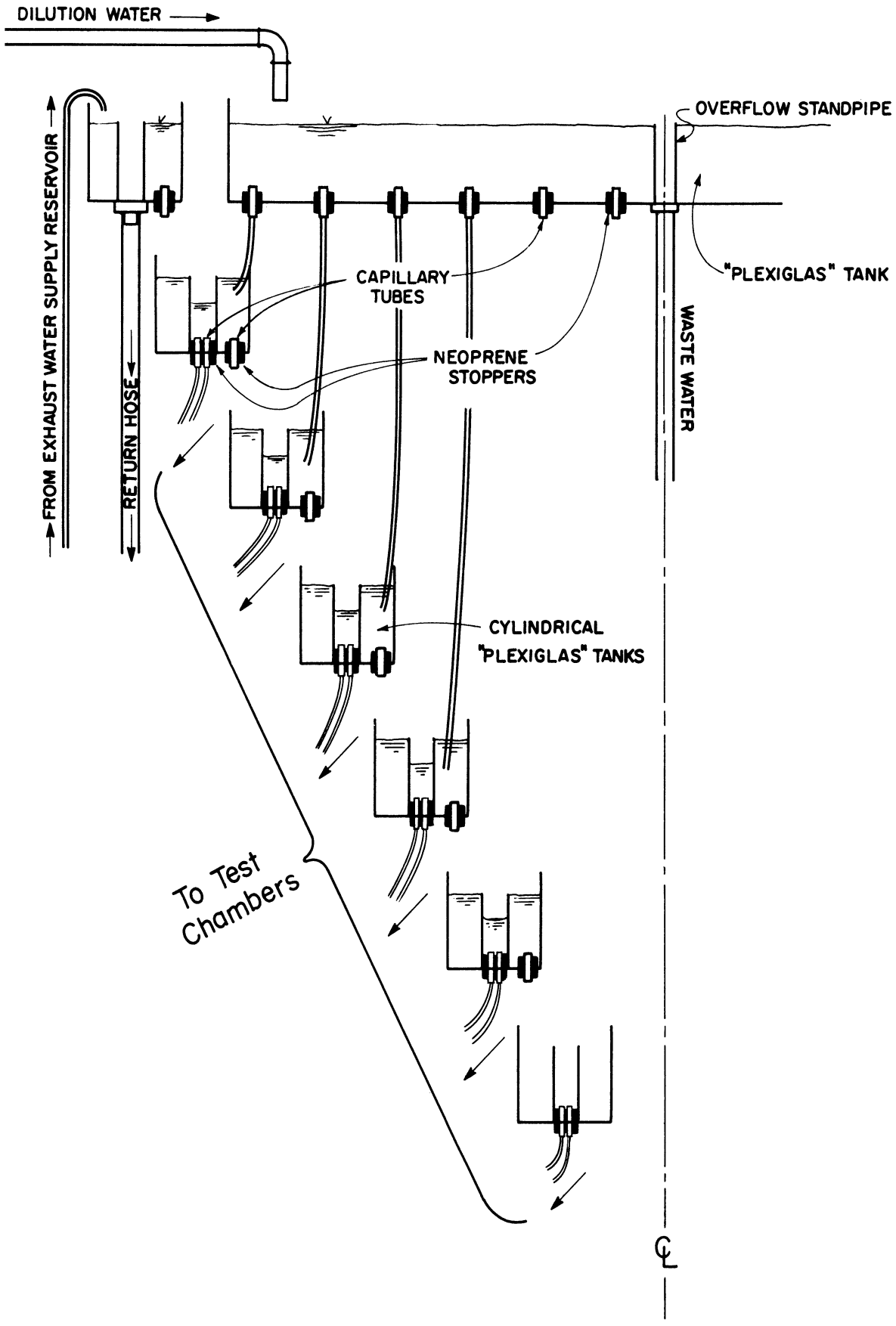

Figure 2. Schematic diagram of serial dilution system (symmetrical about centerline).

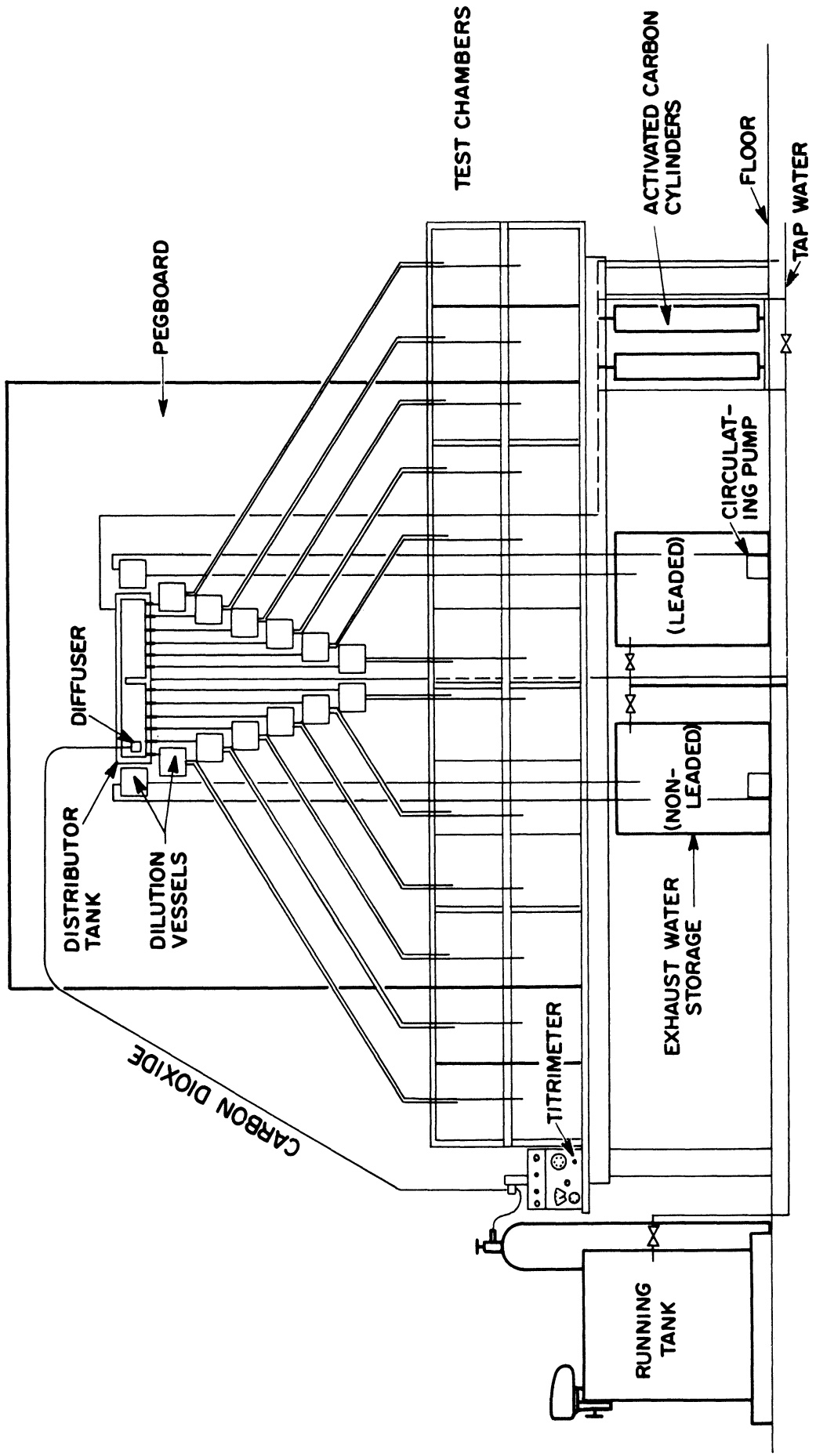

Figure 3. Schematic diagram of the experimental apparatus.

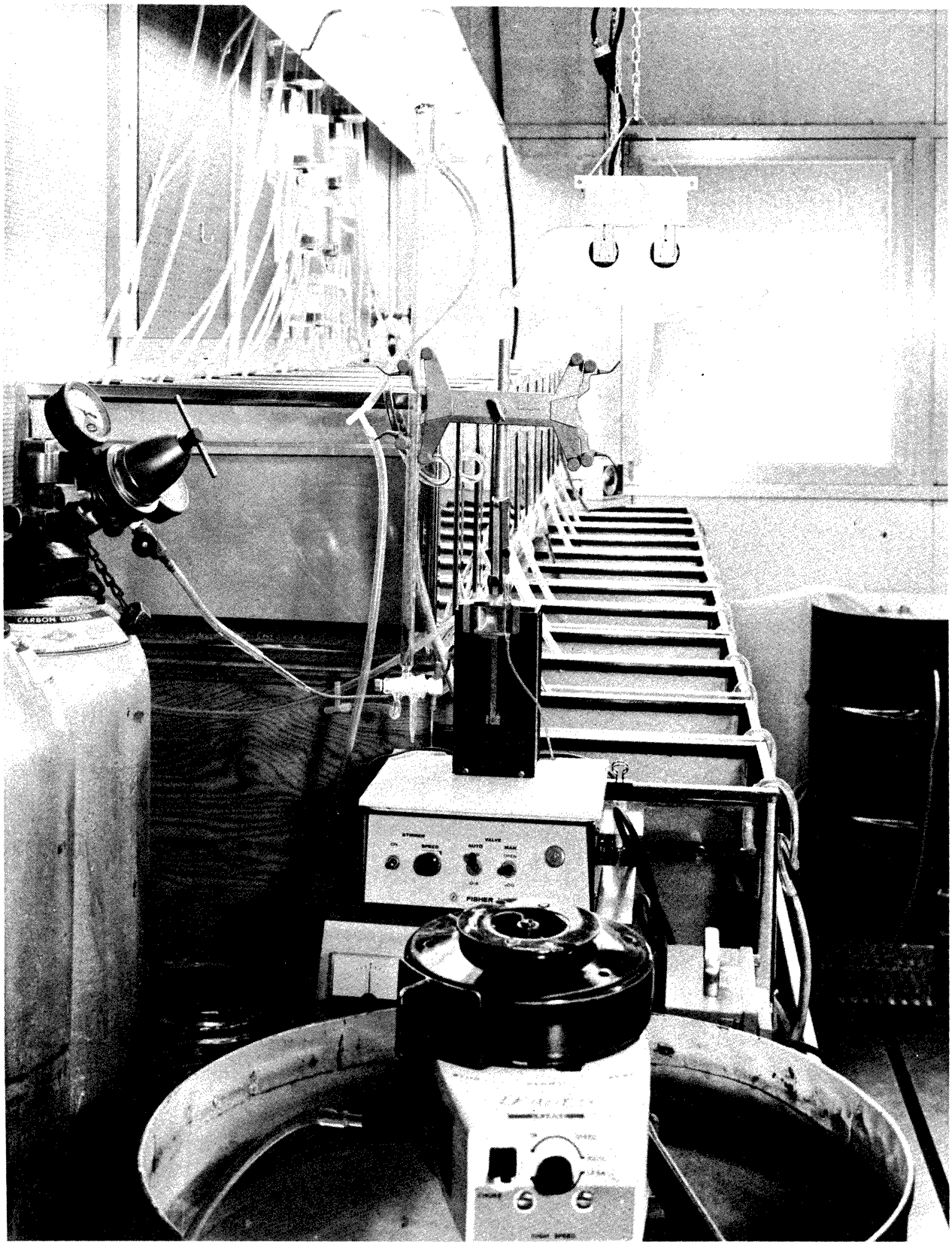

Figure 4. Photograph of the experimental apparatus.

ENVIRONMENTAL CONTROL

Light and temperature are important environmental factors which affect spawning. Artificial lighting is provided by a double row of 40-watt fluorescent lamps 24 inches above the water surface. These lamps are controlled on a 24-hour cycle by a timing device which is adjusted to approximate the natural photoperiod. Seasonal variations in ambient temperature have resulted in a suitable variation of temperature in the test chambers, so no special means for temperature control have been necessary. Temperatures in the tanks have varied from a low of 10°C in the winter to 27°C so far during the summer months.

DIET FOR TEST FISH

An adequate diet is ensured for the test fish by feeding them a variety of foods, including "Tetramin" (a commercial staple food), brineshrimp, bloodworms, tubifex worms, and Daphnia (small aquatic animals). These are supplemented by periphyton (algae) which grows on the walls of the tanks.

TEST CHAMBERS AND ASSAY CRITERIA

The test chambers (24 in all) are 10-gallon aquariums with stainless steel frames. Each is aerated continuously, the air being supplied by four vibrator pumps. Each test chamber contains a complex of nesting sites shown in Figure 5 where spawning can take place, and an incubating cup illustrated in Figure 6 to which eggs can be transplanted and permitted to develop and hatch guarded from the adult fish. When a spawning occurs the eggs are counted and recorded. About 50 of these eggs are placed in the incubating basket. The percent of these eggs which hatch and the percent of fry which survive a specified period (14 days) are recorded. These observations comprise the data which will be analyzed. The basic method, though not the experimental apparatus, is modeled after the well established technique of Donald Mount at the National Water Quality Laboratory in Duluth.[2]

Originally, ten immature fish were placed in each test chamber. It is almost impossible to identify the sex of young fathead minnows so the number of males and females in each tank was not initially known. During the spawning season, however, the males develop facial spines, a blue-gray pad between the head and dorsal fin and broad vertical stripes, permitting easy sex identification. The number of spawnings which occur in the different tanks are then compared on a per-female basis.

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2. Mount and Stephan, "A Method for Establishing Acceptable Toxicant Limits of Fish," Trans. Am. Soc., 96, 185 (1967).

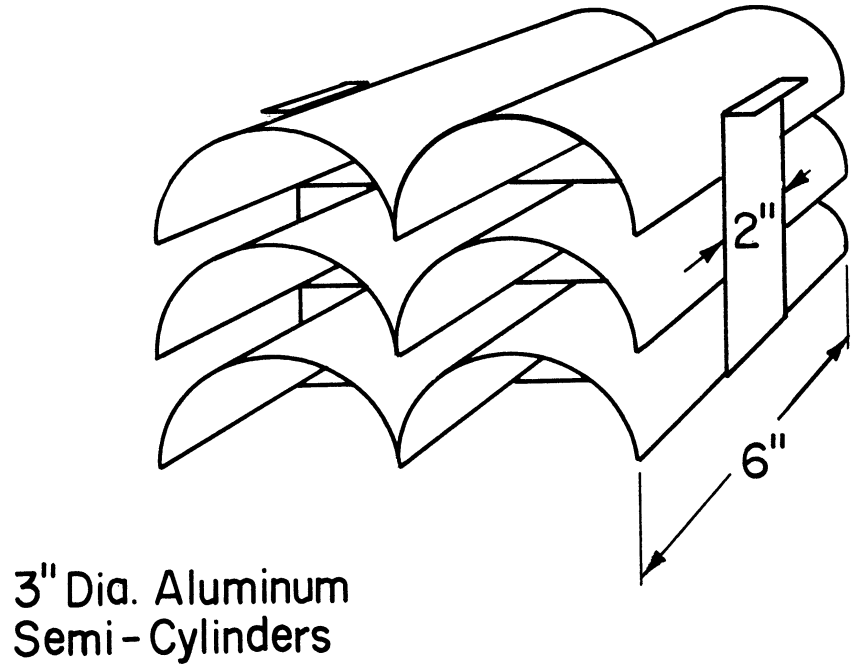

Figure 5. Nesting site complex.

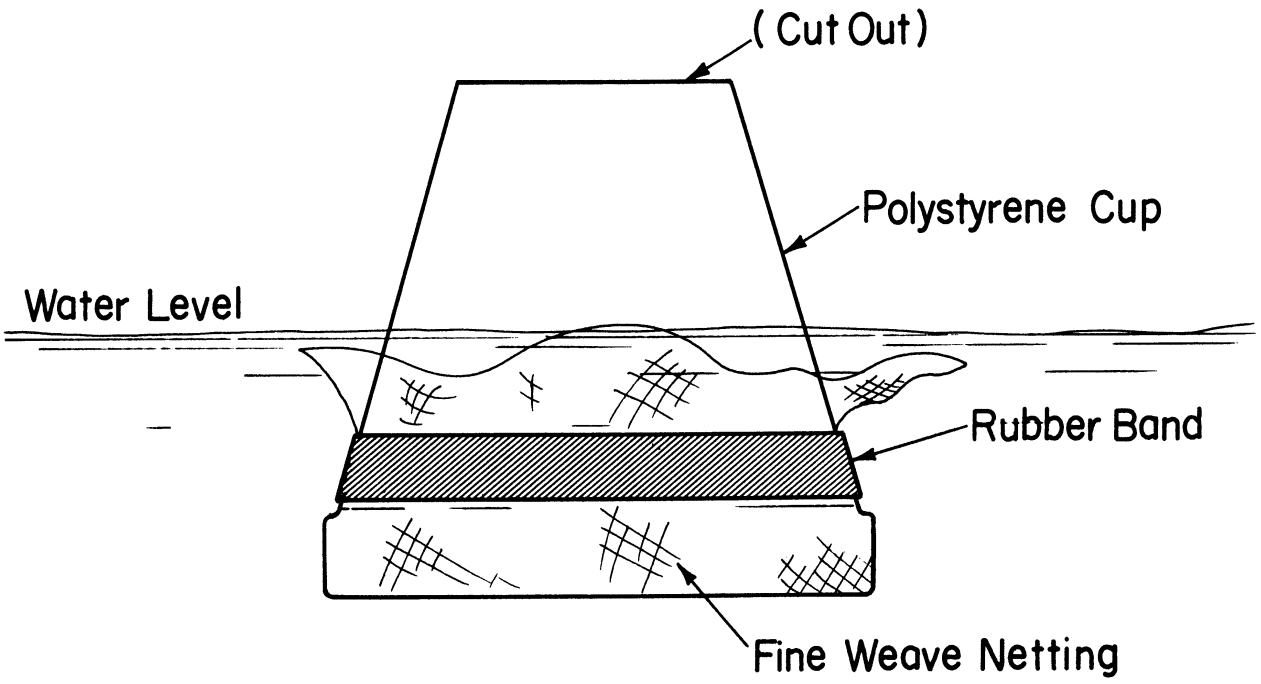

Figure 6. Incubating cup.

PROJECT HISTORY TO DATE

The test fish were obtained from a state-owned hatchery pond near Saline, Michigan in October 1969. They were acclimated to the laboratory water for about four weeks while the experimental apparatus was being completed and tested. They were then transferred to the 10-gallon test chambers where they were acclimated for two weeks more and treated with formalin and antibiotics. Late in November injection of exhaust water was begun and gradually brought up to the test concentrations.

During the early part of the test, 5 to 10% of the fish were lost before the population stabilized. A serious setback occurred early in January when the fish showed signs of sickness and began dying rapidly. In a short time half the original number were lost. The deaths were apparently not related to the engine exhaust emissions as they occurred randomly in all tanks, including the controls. A concerted effort was made to determine the cause of the mortalities. Fish biologists at the University were consulted and some of the dead and dying fish were sent to the State of Michigan fish pathologist at Grayling. No one was able to diagnose the cause however. It is difficult to establish a healthy fish culture in an artificial environment such as this and this experience, though unfortunate, is not at all unusual in bioassay work. Much has yet to be learned about fish diseases and stresses and how they can be minimized in the laboratory. Bioassayists concede that to acquire and maintain a healthy population is the most difficult part of the bioassay.

Because of the possibility that the deaths were due to environmental stresses, rather than to infectious disease, several changes were made in the system and its operation. For example, the 24-hour per day continuous flow was ceased. Instead the system is now operated about six hours per day. This was done to allow a longer "conditioning" period for the water and perhaps make it more "livable." It was at this point that the aeration system was installed. This was done actually to deaerate the supersaturated incoming water rather than to increase dissolved oxygen concentration.

The survivors of each pair of duplicate tanks were combined to one of these tanks and new fish were placed in the other. Since that time very few deaths have occurred among the survivors of the original test fish, and they have remained in a healthy condition. A gradual loss has been experienced among the newer fish however and their population is now less than half the original 120. There have been no deaths in these tanks for more than a month but the total sample has been reduced significantly and some individual tanks have been drastically affected.

RESULTS TO DATE

The plans for obtaining growth rate data were disrupted by the loss of so many of the original fish. Since the fish were weighed in aggregate for each tank and since they varied somewhat individually in size, the death of some made the initial weight data meaningless. Because of fear that the weighing process might be harmful to the fish and cause further problems, this part of the experiment was abandoned.

Data on reproduction had to wait, of course, on the spawning season which was expected to begin in May. The first spawning occurred June 2. It took place in Tank 1B with the highest concentration of non-leaded gasoline exhaust water and the original test fish. 251 eggs were counted, 50 were incubated and apparently all 50 hatched. Four more spawnings have occurred since, three in the same tank and one in 2B, the next most concentrated tank, also containing original test fish. The average number of eggs per spawning in 1B is about 250. The single spawning in 2B produced 205 eggs.

DISCUSSION

It should be noted that the study as it now stands deviates from the original plan at least in four ways. (1) Instead of duplicate tanks at one period of exposure, two exposure times are now involved, but only one tank for each concentration. (2) For both exposure times, the fish remaining have survived stresses which have killed half their original numbers. They therefore may be suspected to have a tolerance level for the exhaust water which is higher than average for the species. (3) Renewal of the test concentrations of exhaust water is not continuous but rather limited to six hours per day. This allows more time for degradation of the exhaust products but at the same time it is actually more representative of the diurnal input to lakes from typical motorboat operation. (4) The continuous aeration may accelerate degradation and detoxification of the exhaust products. This effect will be tested in the acute toxicity studies to be performed later.

Though the data is sparse to date the results so far indicate there may be little if any adverse effect on spawning at even the highest test concentration. There are no reliable data so far on hatching success and survival.

FUTURE PLANS

Acute toxicity tests will be performed in accordance with the Routine Bioassay Method as described in Standard Methods[3] to determine the 24- and 96-hour median tolerance limits (TL_m) of immature fathead minnows to the exhaust water. This will be done both in batch-style and later on, after termination of the long-term test, with the continuous flow system.

A culture of Daphnia has been started with the possibility in mind of testing the effect of exhaust water on this aquatic species. Daphnia is a major source of food for fishes and therefore plays an important role in the ecology of freshwater streams and lakes. A common snail, Limnia stagnalis, may also be used as a test organism.

An experiment will be performed to determine the kinetics of degradation of exhaust products in both natural water and sterile distilled water. Another planned experiment will be designed to determine the possible accumulation of lead compounds in bottom sediments. Benthal deposits from a natural lake where motorboats are prohibited will be used in this experiment.

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3. Standard Methods for the Examination of Water and Wastewater, 12th Edition, 1965, American Public Health Association, New York.