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Bulletin 59

THE DEVELOPMENT OF AN
AUTOMATED BIOLOGICAL
MONITORING SYSTEM
FOR WATER QUALITY

John Cairns, Jr., et al.

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ABSTRACT

A rapid biological monitoring system has been developed which measures changes in the swimming movements and breathing rates of fish. The monitoring system eventually may provide an early warning of developing toxicity in wastes or streams. In a series of laboratory challenges to the monitoring system, a harmless diurnal temperature change was detected as well as harmful additions of chlorine and zinc sulfate, emphasizing the necessity of follow-up physical-chemical measurements to determine the cause of a biological detection and to decide whether control measures are necessary. The monitoring system correctly differentiated an upstream spill of chlorine from a simulated in-plant spill of zinc sulfate. The sensitivity of bluegills to a simulated zinc sulfate spill (introduction of a sublethal concentration of 3 mg/l, as zinc), did not decrease after 37 weeks of exposure to a low zinc concentration (0.075 mg/l), indicating that rapid desensitization of fish exposed to industrial waste may not be a problem, at least with wastes that act similarly to zinc. The monitoring system correctly indicated a reduction in toxicity when 3 mg/l zinc was antagonized by an addition of calcium chloride, but did not detect a mixture of copper sulfate and zinc sulfate predicted to be at the threshold of detection. A harmless increase in calcium chloride caused no breathing detections. The further development and eventual use of the monitoring system in industrial plants and river basins to maintain water quality for multiple use are discussed.

INTRODUCTION

Viable systems for continuously monitoring water quality are of critical importance for the future management and use of our watersheds. There are two reasons why it will only be through such continuous feedback information systems that sensible and efficient controls can be designed: first, a watershed's ability to receive waste fluctuates on a daily or even hourly basis; secondly, without constant monitoring, industrial spills do much damage to aquatic ecosystems before anyone is aware of the problem. It is significant that Section 308 of the Federal Water Pollution Control Act Amendments of 1972 states that the Administrator of the Environmental Protection Agency shall require the owner or operator of any point source to "install, use, and maintain such monitoring equipment or methods (including where appropriate, biological monitoring methods)" as the Administrator may require in fulfilling the objectives and requirements of the Act. Continuous monitoring systems using aquatic organisms as sensors may come to supplement the stream surveys and standard bioassays that are routinely used at present for biological monitoring. Contrary to commonly held opinions, such systems are not "pipe dreams". By comparison with the accomplishments of the space program, for example, research in water quality monitoring looks like the development of doorknobs.

The value of a regional monitoring program using chemical and physical measurements has already been well established by the Ohio River Valley Water Sanitation Commission's (ORSANCO) successful operation of automated monitoring devices for nearly 10 years on the Ohio River (Klein, et al., 1968). These devices record such things as dissolved oxygen and pH and have become indispensable in the management of the watershed and are stimulating similar developments in a number of countries. The major difficulty with this type of monitoring system, however, arises in the analysis of the data and in making evaluations of a complex ecosystem from the measurements of a few physical parameters. A possible solution to these problems involves the use of biological monitoring techniques.

The biological monitoring system is intended to complement physical-chemical monitoring techniques. It is difficult, if not impossible, to predict the biological effects of a complex, continuously changing industrial waste from chemical analyses alone. Damage may be caused by unsuspected contaminants; a janitor may dump a cleaning solvent down a drain, for example, or a change in upstream conditions in combination with a certain effluent may produce toxic conditions. The biological monitor is not

intended to determine the exact nature of toxic conditions. It can only indicate when these toxic conditions are developing. An early warning, however, can allow time for wastes to be temporarily stored until more exhaustive tests can be performed to determine the cause of the problem, or when used in combination with chemical and physical sensors can indicate which data should be looked at to determine the source of the toxicity.

A biological monitoring system using fish should: (1) provide continuous information on the effects of industrial waste on the health of fish; (2) prevent industrial spills from damaging fish populations in streams; and (3) help prevent expensive over-treatment of waste when a lesser degree of treatment would protect fish.

The monitoring system described in this paper continuously measures the breathing and swimming activity of fish exposed to an effluent in specially designed chambers, and compares their responses to normal activity and breathing rates obtained before any effluent was introduced. Previous research has shown that abnormal responses appear in the breathing and activity of fish exposed to sublethal concentrations of zinc, and also that abnormal responses appear well in advance of irreversible damage when fish are exposed to lethal concentrations of zinc (Waller and Cairns, 1971; Cairns and Sparks, 1971; Cairns, *et al.*, 1970). In other words, symptoms of ill-health occur in the test fish early enough so that corrective action can be taken to prevent immediate damage to fish in the monitoring system and presumably, to fish populations in the stream. The intent of this research has been to challenge the biological monitoring system in the laboratory, to determine whether it would fail under some complex or special conditions. Emphasis was on the following questions: (1) Can the monitoring system distinguish zinc spills from harmless fluctuations in temperature and water hardness? (2) Will fish exposed to low concentrations of zinc from an industrial waste for periods up to a year continue to exhibit warning responses to higher concentrations? (3) Can fish in the monitoring system respond to zinc and calcium (an antagonistic combination) and to zinc and copper (a synergistic combination)? We hypothesized that the fish would only respond abnormally to potentially harmful conditions.

We wish to emphasize that these tests were by no means exhaustive and that the monitoring system is not ready to be taken "off the shelf" and used by industry. Our intent is to stimulate comment on, and interest in, the development of biological monitoring systems. The system is flexible enough so that alternative test organisms, sensors, and statistical analyses can be developed to fit the requirements of different situations. Field tests, not

laboratory tests, must ultimately show whether these types of biological monitoring systems are feasible.

METHODS AND MATERIALS

Figure 1 is a diagram of a hypothetical in-plant monitoring unit, the operation of which was simulated in the laboratory. In the hypothetical unit, control fish are exposed to upstream water alone, while test fish are exposed to plant waste alone or plant waste diluted with upstream water. In the laboratory, the upstream water was municipal water dechlorinated by means of thiosulfate (Table 1 lists the chemical analysis).

I. Apparatus and General Procedure

The monitoring apparatus consisted of six experimental chambers in an 8 x 8 x 8 ft isolation room. The chambers were connected by cables to recording instruments outside the room (Figures 2, 3, and 4). Because activity and breathing of fish can be affected by disturbances such as noise or the presence of an observer, the isolation room was not opened after an experiment had started.

The chambers were 20.8-liter aquaria with Plexiglas* covers (Figures 2 and 5). The sides of the aquaria were painted black to keep the fish from seeing and being influenced by each other. Breathing movements of the fish were picked up by stainless steel electrodes located at the ends of the aquaria. Although there are various hypotheses in the literature as to the cause of the millivolt signal that is produced at an electrode immersed near a fish (Spoor, et al., 1971; Heath, 1972), there is agreement that a one-to-one correspondence exists between the peaks in the recorded signal and the breathing rate (opercular movements/minute). Thus, the breathing rate of the fish was determined by counting the number of peaks during a one-minute interval near the beginning of each recording period. The activity of the fish was monitored by light beams and photocells (Shirer, Cairns, and Waller, 1968). Three light beams, the thickness of a pencil lead, were used in each chamber: at the top, middle, and bottom levels. When a fish interrupted a light beam, a counter for that level of the test chamber incremented. At the end of an hour of this counting, an automatic camera took a picture of the counters, and the counters were then reset to zero. Thus fish activity rates; i.e., number of light beam interruptions for each level of each chamber per hour, were recorded on 35 mm film. The wavelength of the light beams was approximately 650 millimicrons.

*Plexiglas is a trade name of the Rohm and Haas Company

FIGURE 1

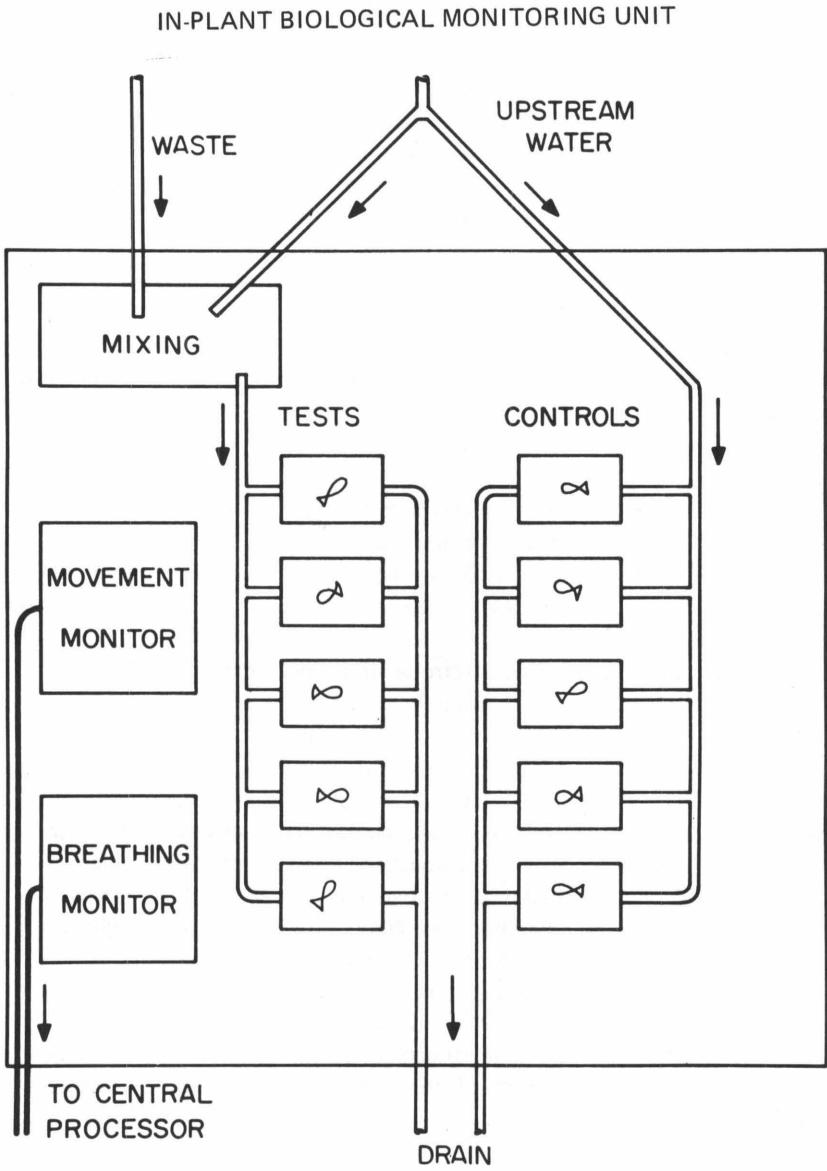


TABLE 1

CHEMICAL AND PHYSICAL CHARACTERISTICS
OF THE DILUTION OF WATER

<u>Water Characteristic</u>	<u>Mean</u>	<u>S.E.</u>	<u>Range</u>
Total hardness (mg/l as CaCO ₃)	55.1(284)*	7.2	51.0 – 68.0
Phenolphthalein alkalinity (mg/l as CaCO ₃)	0.0(284)	0.0	
Total alkalinity (mg/l as CaCO ₃)	37.8(284)	3.4	30.0 – 52.0
pH	7.9(281)	0.3	7.2 – 8.5
Temperature (°C)	22.8(223)	1.0	19.3 – 24.2
Dissolved oxygen (mg/l)	Maintained above 7.5		
Chlorine	None, except as noted in text		
Zinc, copper	Zinc and copper were not detected in random analyses of the dilution water by atomic absorption photospectrometry.		

*Number of analyses

FIGURE 2

FISH CHAMBERS

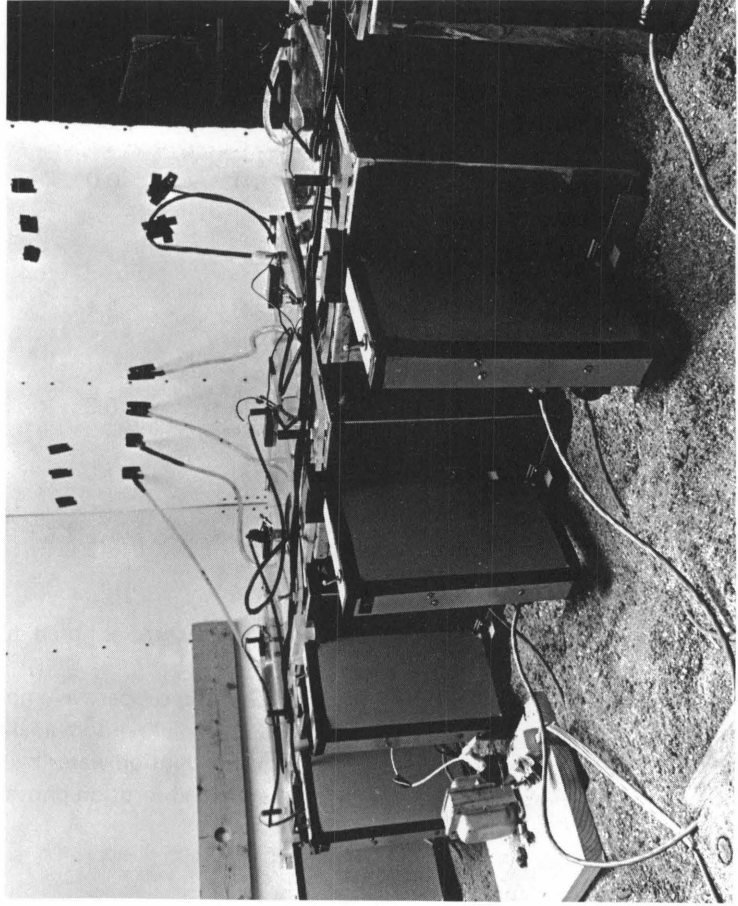


FIGURE 3

EQUIPMENT SET-UP

Far Left
Left Center
Right Center
Large Arrow
Small Arrow

Acclimatization Room
Polygraph and Tape Units
Isolation Room
Temperature Monitors
Water Sample Drains

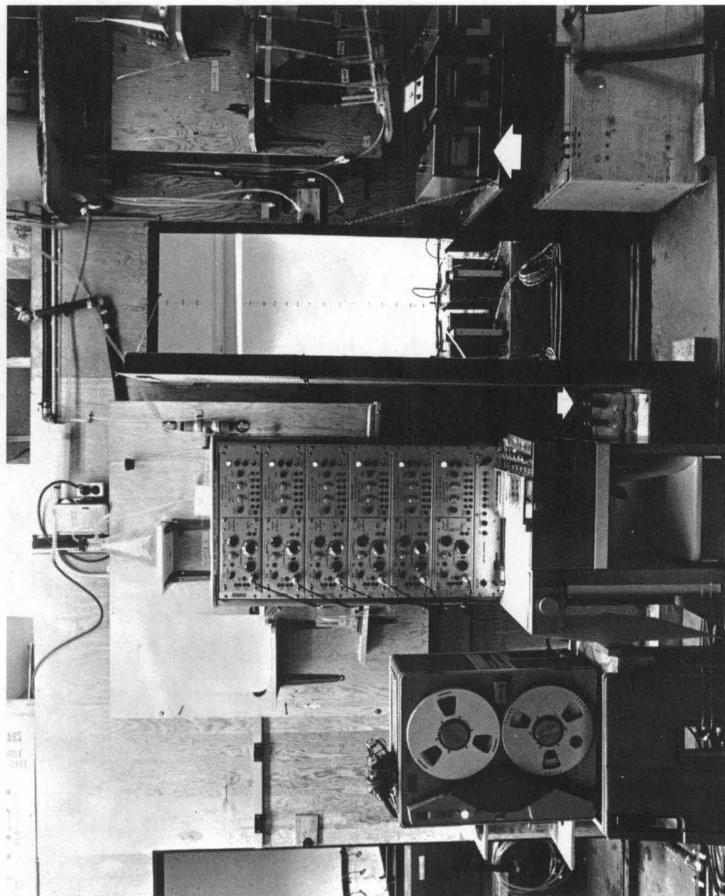


FIGURE 4

FISH SWIMMING ACTIVITY COUNTERS

Timer-Controlled Camera in Foreground

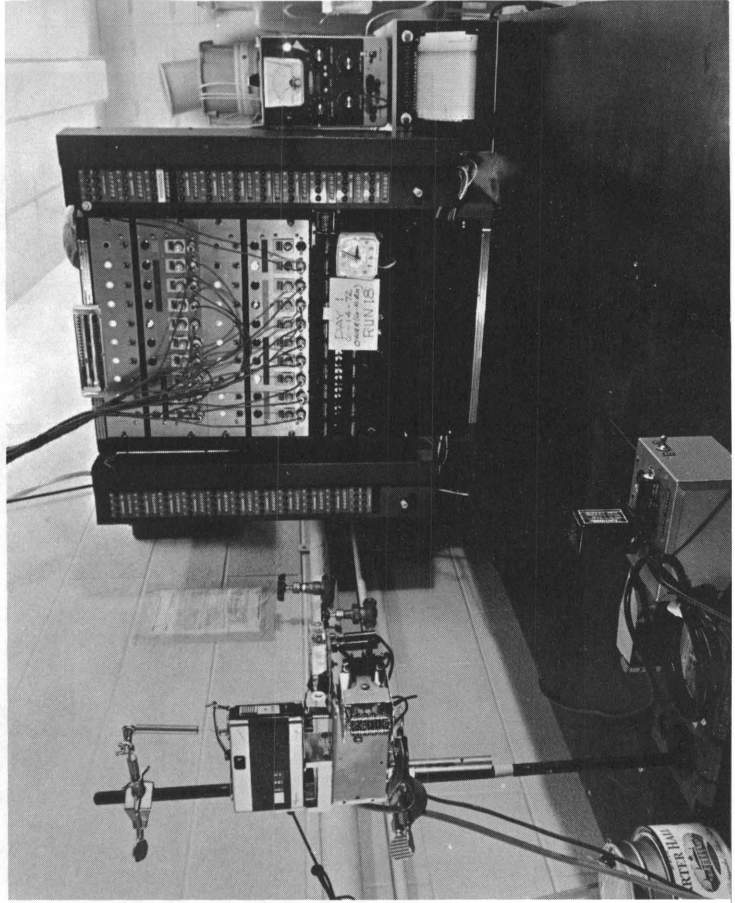
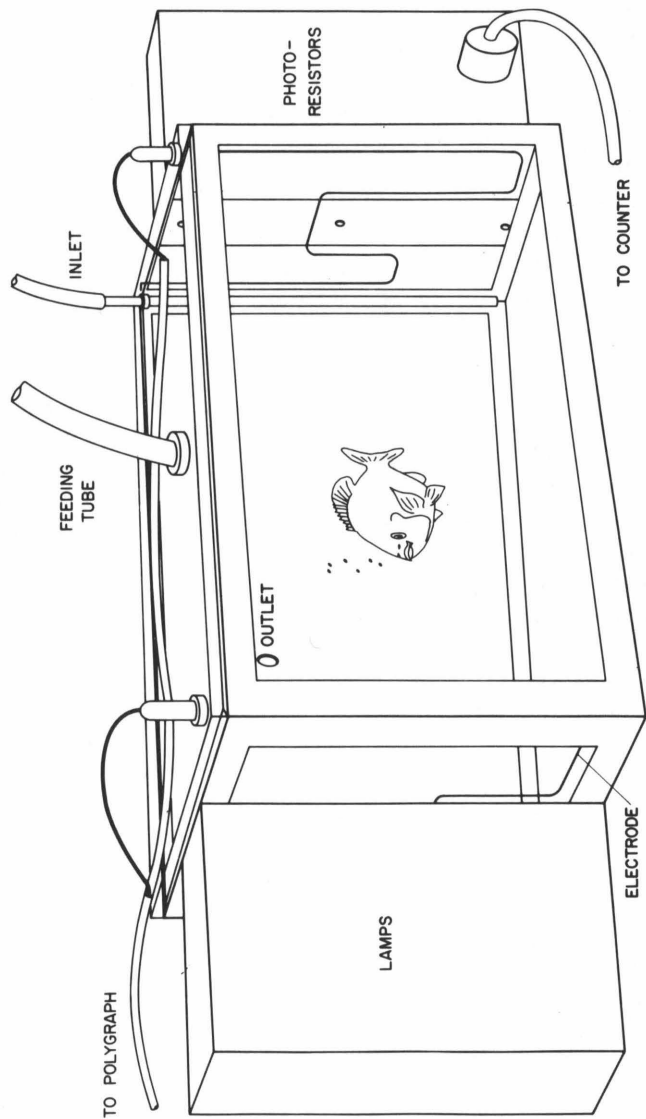


FIGURE 5

DIAGRAM OF CHAMBER



Timer-controlled fluorescent lights provided a photoperiod of 12 hours in the isolation room. In order to keep the apparatus as simple as possible, with the potential industrial application in mind, the room lights were turned on or off abruptly, whereas in earlier experiments the lights came on and off gradually to simulate dawn and dusk. Metering pumps were used in three experiments to deliver dechlorinated water or treatment solutions to the test chambers, but these were replaced by gravity-fed diluters of the type described by Mount and Brungs (1967) and by Brungs and Mount (1970). One diluter supplied two control chambers which were not treated during an experiment, and another diluter supplied four test chambers. The connections from the diluters were assigned randomly to the chambers at the beginning of each experiment. The diluters transfused replicate 300 ml doses into each chamber once every two minutes. These diluters were found to be more reliable than pumps and were not subject to electric power failures.

In the planned spills and treatments, it took nine hours for the toxicant concentration or level of treatment in the effluent from the chambers to reach the level of the in-coming water, after the treatment was initiated. The delay was due to the retention time of the chambers.

The fish used in these experiments were obtained in one batch by seining a pond in Salem, Virginia. The fish were acclimated to the 12-hour photoperiod and dechlorinated municipal water for at least two weeks prior to being used in an experiment. The average standard length of the 102 fish used in these experiments was 10.3 cm (S.D. $\pm$ 1.0), and the average weight was 36.9 gm (S.D. $\pm$ 11.0). At the beginning of every experiment, one fish was placed in each of the six chambers by 4 p.m. The first day of the experiment began at 7 a.m. the following day, when the first recordings began.

II. Data Analysis

a. Analysis of Respiration Data

Two normal sources of variation in breathing rates in bluegills had to be taken into account: individual variation and diurnal variation. To allow for individual variation, each fish served as its own control. During the first five days of the experiments (standardization period), the fish were exposed to dechlorinated tapwater alone, or in one series of experiments, to 0.075 mg/l zinc. Critical breathing rates were determined for each fish before the experimental treatment or simulated spill began on day 6. Treatment generally continued for four days, after which the experiment terminated and all fish were killed, weighed, and measured.

Critical breathing rates were the maximum rates obtained on day 1 of each experiment. The breathing rates were elevated due to incomplete recovery of the fish from the stress of being netted and transferred to chambers the previous afternoon. If no toxicants were introduced after day 1, the breathing rates returned to normal and rarely exceeded the maximum rates. In short, the handling procedure was purposely used to measure the individual response of the fish to a mild stress. Only a stress greater than that previously recorded on day 1 would result in a greater breathing rate.

Breathing and activity rates of bluegills are normally different at different times of day. For example, a rate that might be considered high during the dark interval of the photoperiod could be perfectly normal during the light interval. Therefore, critical values had to be determined at different times of the day to compensate for diurnal variation. For analysis of fish breathing, each day was divided into four intervals: Dawn (6 a.m. to 8 a.m.), Light (9 a.m. to 5 p.m.), Dusk (6 p.m. to 8 p.m.), and Dark (9 p.m. to 5 a.m.). The breathing rates of the fish were generally high during the light interval, low during the dark interval, and changed most rapidly when the lights went on or off during the dawn and dusk intervals. Recordings were made every half hour during the dawn and dusk intervals to delineate the rapid change in breathing rates, and every hour during the light and dark intervals. Critical breathing rates were determined for each of the four intervals of the first day.

A breathing rate that was greater than the critical rate was considered a response. For example, if the critical rate for fish 1 during the light interval was 35 breaths/minute, and the fish's breathing rate at 10 a.m. on day 7 was 40, then the fish was showing breathing response at 10 a.m. on day 7.

Based on previous experiments with toxicants, a detection of in-plant toxicity was defined as simultaneous responses by three or four of the four test fish that were exposed to the simulated plant effluent, and at the same time the absence of responses from two control fish. The function of the control fish was to indicate the presence of adverse conditions in the dilution (upstream) water, or the presence of an extraneous factor such as noise that disturbed all the fish. If it were positively known that no noise or floor vibrations had occurred, two simultaneous responses by the control fish were considered a detection of upstream toxicity. Since only the first day of the standardization period was required to establish critical breathing rates, the remaining four days of the standardization period were used to determine how many false breathing detections would occur. A false detection was one occurring when the fish were not being treated, there was no chlorine in the water, and no known extraneous disturbances to the fish.

b. Analysis of Activity Data

Critical activity rates were calculated from days three to six of the standardization period by employing the computer program and method of analysis described by Hall (1972). This technique does not lend itself to a short description and the reader should refer to Hall (1972) for a more complete description. Briefly, the method assumed that the observation (i.e., the hourly counts for: (a) the upper photocell and (b) the sum from the lower and middle photocells) followed a negative binomial distribution. Observations obtained during the initial standardization period were used to estimate the parameters of the negative binomial distribution. From these the upper critical limit could be calculated for the 95% level. Individual differences were accounted for by considering each fish separately, and diurnal fluctuations were accounted for by calculating a critical limit for each hour of the day. Any individual fish was said to show a response when the activity rates observed in the test period exceeded the corresponding critical limits for two consecutive hours. In other words, the fish was considered to be behaving "abnormally" when its rate of activity significantly exceeded the limits determined for the same fish at the same time of the day during the standardization period. A detection was said to occur in the system if two or more of the test fish and not more than one of the control fish showed a response during the same hour. The calculated probability of a detection occurring by chance (false detection) was 0.00048.

EXPERIMENTAL PROCEDURES

In three experiments, the chlorine concentration in the municipal water supply was so high that the thiosulfate dechlorinator was overloaded, and measurable quantities of chlorine occurred in the constant head tank which supplied the diluters. The first warning was always obtained when the control fish, as well as the test fish, began to show responses. The cause was confirmed with a Hach chlorine tester. The length of exposure was estimated, and the chlorine concentrations in both the head tank and the effluents from the chambers were checked frequently until they were completely free of chlorine. The water was tested for chlorine at least once a day during all the experiments, even when there was no warning of upstream toxicity by the control fish. The introduction of chlorine was an unplanned challenge to the monitoring system; the results of which are discussed in the next section. When the chlorine entered the water during the experiments, we were in precisely the same position as a hypothetical operator of a biological monitoring system, who is faced with an unknown substance in upstream water. We ran chemical analyses of the water, just as the operator would, and then took corrective measures. The chlorine spills, although unplanned, indicated the efficacy of the monitoring system.

Three other types of challenges were presented to the monitor: nontoxic changes in calcium and temperature, combinations of more than one factor, and chronic exposure to a low level of zinc followed by an increase in zinc concentration.

I. Nontoxic Factors

The response of fish to two nontoxic factors, calcium and temperature, was determined. It was originally planned to hold the water temperature constant in all experiments except one, in which the temperature was to be constant during the standardization period and fluctuate during the treatment period. However, it was found that the temperature of the effluent from the chambers increased steadily from 24.8 to 26.0°C when the room lights were on, even though the temperature of the water entering the chambers varied only 0.2°C per day. Apparently heat from the fluorescent light ballasts gradually warmed the well-insulated, unventilated isolation room which housed the chambers. The retention time of the chamber was long enough to allow the water temperature to increase 1.2°C. Thus, in all experiments, fish were exposed to a small diurnal temperature cycle (24.8 to 26.0°C), which may have contributed to the observed diurnal pattern in the breathing and

activity rates. In one experiment, the four test fish were exposed to a greater treatment cycle (24.8 to 29.2°C), which approximated the temperature regime reported for the Potomac River in June 1961, at a location free of extraneous thermal effluents (River Survey Report for the Potomac Electric Power Company, 1962). The effect of using the first treatment day (day 6) to establish critical breathing rates and of using four treatment days when establishing critical activity rates (days 6-9), was also determined. Since the 4.4°C cycle occurred during days 6-9, this method simulated what would happen if a diurnal temperature cycle occurred at an industrial site during the period for establishment of the critical values.

One experiment was used to test the effect of both a nontoxic treatment and treatment with an antagonistic combination of metals (described below). In the nontoxic phase of the experiment, the fish were exposed to dilution water alone for three days, then the water hardness was increased by increasing the calcium concentration from approximately 10 mg/l to 107 mg/l, using calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$). The high calcium concentration was maintained for three days. Only breathing responses to the high calcium concentration were monitored, because the entire six-day period (three days of exposure to dilution water alone plus three days of exposure to dilution water with added calcium) had to be used to establish the critical activity values.

II. Combined Factors

In the second phase of the experiment described above, the calcium concentration was maintained at 112 mg/l for four additional days, while the zinc concentration was maintained at 3.74 mg/l during the same time. Zinc chloride and calcium chloride were added to distilled, deionized water to make the stock solution for the combined treatment.

The detection threshold of the monitoring system for zinc is approximately 3 mg/l, which is 0.4 of the 96-hour LC50 (7.5 mg/l zinc) for adult bluegills in our dilution water. To use the terminology of Sprague and Ramsay (1965), the 96-hour LC50 represents one toxic unit, and the threshold for zinc detection is 0.4 toxic unit. In order to test whether the effects of copper and zinc on fish breathing and activity rates are more than additive, the fish were exposed to a mixture of copper and zinc which was calculated to be equivalent to 0.4 toxic unit, with zinc and copper each contributing 0.2 toxic unit. Two such experiments were conducted, one in which only the breathing rates were monitored, and a second in which only activity rates were monitored.

Time was not available to run a conventional bioassay with copper to determine the 96-hour LC50 for our stock of bluegills in dechlorinated municipal water, so the LC50 was estimated as follows: Since the 96-hour LC50 for bluegills exposed to zinc was 7.5 mg/l in our laboratory and 4.2 mg/l in another laboratory using water of similar hardness, but bluegills of different sizes (Cairns and Scheier, 1968), we assumed that the 96-hour LC50's for copper in the two laboratories would be in the same proportion. We therefore multiplied the 96-hour LC50 for copper (1.2 mg/l) obtained by Cairns and Scheier, by 7.5/4.2 to obtain an estimated LC50 of 2.2 mg/l copper under our laboratory conditions. Two-tenths of 2.2 mg/l copper is 0.44 mg/l copper, and 0.2 of 7.5 mg/l zinc is 1.50 mg/l zinc. If all the assumptions made above are correct, and if zinc and copper are merely additive in their effects, some breathing detections and very few activity detections would be expected when fish were exposed to the mixture of 0.44 mg/l copper and 1.50 mg/l zinc, since the activity monitor is slightly less sensitive than the breathing monitor. If zinc and copper act synergistically, many breathing and activity detections would be expected to occur. There is a deflection in the plot of the zinc concentration in Figure 12 (day 9), because the zinc component of the stock solution was increased when it became apparent that the measured zinc concentration in the test chambers was not going to reach the desired level of 1.5 mg/l. Since zinc has an affinity for surfaces, some may have been removed from solution in the stock bottle and dilution apparatus. The mean zinc and copper concentrations were measured by an atomic absorption spectrophotometer during the two experiments and are shown in Table 4—both were slightly below the desired concentrations.

III. Chronic Zinc Exposure Followed by a Zinc Spill

Eleven experiments, conducted at one-month intervals, were designed to determine how often the fish in the monitoring system would have to be replaced if they were continuously exposed to a presumed biologically "safe" concentration of 0.075 mg/l zinc (1/100 the 96-hour LC50). This concentration is substantially above the background concentration of zinc in the dilution water, which is usually less than 0.010 mg/l. Approximately 100 fish were kept in a continuous flow of water containing an average zinc concentration of 0.075 mg/l (S.D. = 0.011, range 0.053 - 0.096), starting August 4, 1971. After five weeks of exposure, and at four-week intervals thereafter, six fish were taken from the stock tanks and exposed for six days in the monitoring system to the same zinc concentration (0.075 mg/l), then

four fish were exposed to approximately 3 mg/l zinc for four days while two fish continued to be exposed to 0.075 mg/l zinc, as controls. Three mg/l zinc was chosen because it is at the detection threshold of the monitoring system, and if the fish became resistant to zinc or desensitized in some way, they would not show responses. An additional test was conducted with fish that had been exposed to 0.075 mg/l zinc for ten months. In this experiment, after being exposed to 3 mg/l zinc for four days, the fish were exposed to the low concentration (0.075 mg/l) again for four additional days to determine whether their breathing and activity rates would return to normal. Zinc analyses for the experiments in this phase are shown in Table 3.

TABLE 3

NUMBER OF DETECTIONS* OBTAINED FROM BLUEGILLS BEFORE AND DURING 4-DAY EXPOSURE TO CALCIUM, CALCIUM AND ZINC, COPPER AND ZINC, OR A DIURNAL TEMPERATURE FLUCTUATION OF 4.4°C

Treatment	<u>Number of Detections</u>		<u>Activity</u>
	<u>Breathing</u>		
	Before Exposure	After Exposure	During Exposure
Temperature**	3	31	2
Temperature†		0	0
109.7 mg/l Ca plus 3.7 mg/l Zn	0	1	0
0.3 mg/l Cu plus 1.4 mg/l Zn	0	0	
0.4 mg/l Cu plus 1.2 mg/l Zn			0

*Defined in text.

**Critical breathing and activity rates determined before exposure to 4.4°C cycle.

†Same experiment as ** above, but with critical breathing and activity rates determined by including exposure to 4.4°C cycle (see text).

TABLE 4

NUMBER OF DETECTIONS* OBTAINED FROM BLUEGILLS BEFORE AND DURING A 4-DAY EXPOSURE TO A SIMULATED ZINC SPILL (INTRODUCTION OF 3 mg/l ZINC) FOLLOWING CHRONIC EXPOSURE TO 0.075 mg/l ZINC

Duration of exposure to 0.075 mg/l Zn (weeks)	Zn (mg/l) during spill **	Number of Detections		Activity During Treatment
		Before Spill	Breathing During Spill	
5	3.515	0	0	—
13	3.004	0	0	0
21	2.640†	2	6	—
25	2.979	0	1	1
29	2.846	1	1	2
33	3.192	0	11	17
37	3.028	0	1	0
41	3.076	0††	16	0
	Total	3	36	20

*Defined in text.

**Means of measurements by atomic absorption spectrophotometry.

†Detections occurred, but were attributable to failure of water supply to test chambers.

††Delivery of toxicant stopped for 6 hours, and zinc concentration reached a low of 1 mg/l on day 9.

RESULTS

The effect of sublethal concentrations of toxicants on the breathing and activity of fish sometimes was obvious to an observer of the recordings, even without further analysis of the data. For example, polygraph records obtained before and after four fish were exposed to 3 mg/l zinc are shown in Figure 6 (all six fish had been exposed to 0.075 mg/l zinc for 21 weeks). The breathing rate of fish 5 and 6 increased noticeably after zinc addition.

Generally, more responses were obtained in all the experiments during the dark interval of the photoperiod than during the light interval. The breathing and swimming activity rates were generally lower and less variable during the dark interval than the light, so the critical values were also lower at night. A slight increase in breathing rate or activity variance caused by the experimental treatment was more apt to produce responses at night than during the day. Although other response criteria were tested, in an effort to make the breathing and activity responses at all intervals of the day equally sensitive to toxicants, the alternative criteria either resulted in over-sensitivity and too many false responses when no treatment was being administered, or decreased sensitivity and too few responses when the fish were being treated.

In all thirteen experiments, there were 1,671 occasions (28 recording sessions per day) when breathing detections could have occurred. Six false detections actually occurred so the probability of a false breathing detection occurring during one recording session can be estimated at 0.0036. The probability of a false activity detection was calculated to be 0.00048.

I. Upstream Toxicity Caused by Chlorine

It is noteworthy that no false in-plant breathing detections occurred in experiments (Table 2 and Figure 7) where chlorine accidentally entered during the standardization period. The breathing rate of both control and test fish increased, indicating an upstream, rather than an in-plant source of toxicity.

In experiments 3 and 18 (Table 2 and Figure 8), an upstream source of toxicity was detected, while the test fish were being exposed to a spill of 3 mg/l zinc. Chemical tests on water in the constant head box showed that the toxic agent was chlorine. Mean measured concentrations in effluent from the chambers ranged from undetectable to 0.05 mg/l. Although the control fish recovered quickly when the chlorine was removed with sodium thiosulfate,

FIGURE 6

BREATHING SIGNALS

Recorded before and after fish 1,3,5, and 6 were exposed to 3 mg/l zinc (a sublethal concentration). Fish 2 and 4 were controls and were not exposed to 3 mg/l zinc. All six fish had been exposed to 0.075 mg/l zinc for 33 weeks.

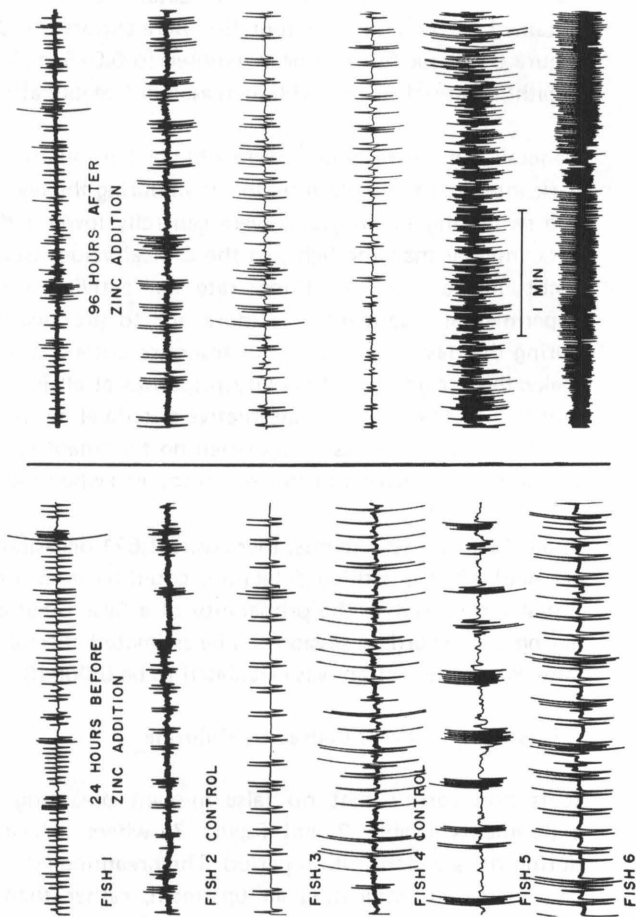


FIGURE 7

BREATHING AND ACTIVITY RESPONSES TO SEQUENTIAL ADDITION OF CHLORINE AND ZINC

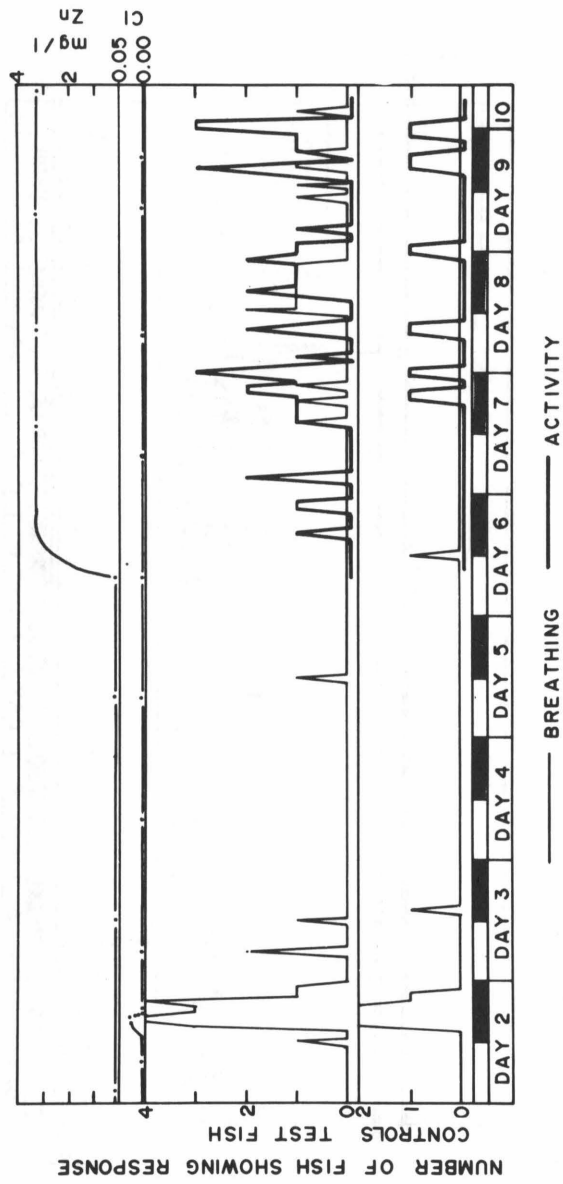


FIGURE 8

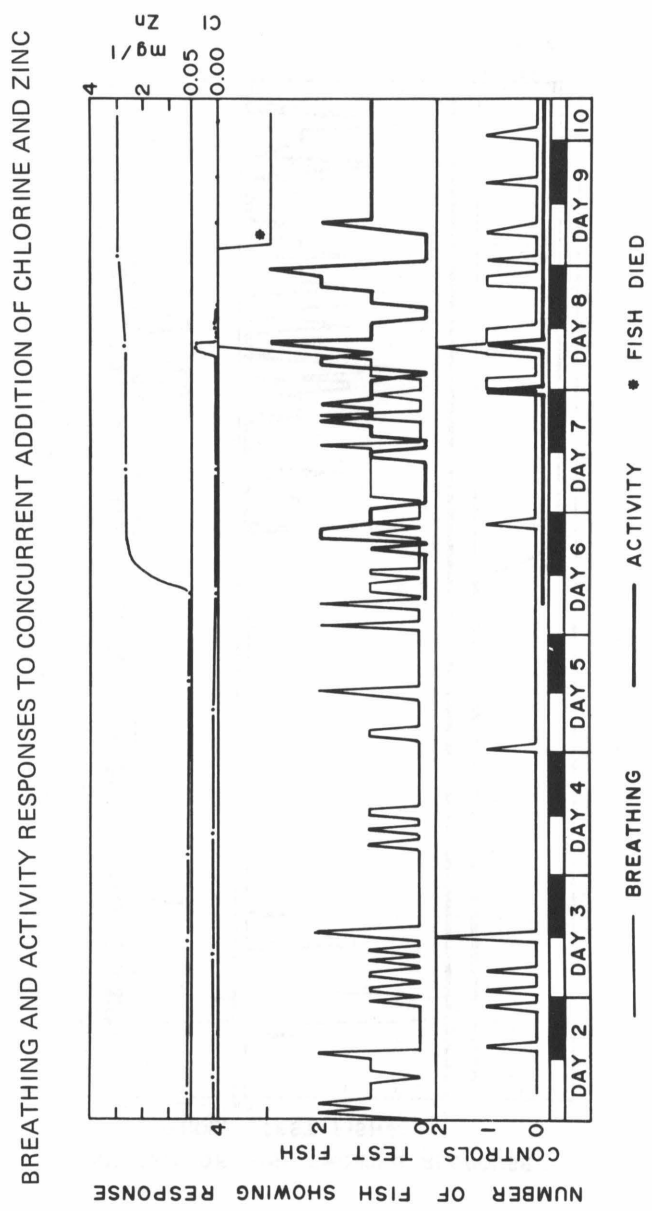


TABLE 2

NUMBER OF DETECTIONS* OBTAINED FROM BLUEGILLS IN EXPERIMENTS
WHERE CHLORINE ACCIDENTALLY ENTERED THE DILUTION WATER

In each experiment four experimental fish (EX) were exposed to 3 mg/l zinc during a 4-day treatment period and two control fish (CON) were exposed to 0.075 mg/l zinc. All fish had been previously exposed to 0.075 mg/l zinc.

Experiment No.	Duration of exposure to 0.075 mg/l Zn (weeks)	Fish	Chlorine Concentration Range (mg/l)	Zn (mg/l) during spill**	Chlorine entered before/after Zn treatment began	Estimated duration of exposure (hrs)	Number of Detections Breathing		Activity During treatment	No. of fish killed
							Before treatment	During treatment		
3	9	EX	.04 - .05	3.083	After	3	—	—	—	1
		CON	.04 - .05	0.060	—	—	0	12	12	—
6	17	EX	.01 - .03	3.327	Before	3	—	—	—	0
		CON	.01 - .03	3.327	Before	1.5	—	—	—	0
18	45	CON	n.d.t	0.086	—	—	0	0	3	—
		EX	.08	2.831	After	1.5	—	—	—	0
		EX	n.d.t	2.831	After	3	—	—	—	1
		CON	n.d. - .05	0.070	—	—	0	44	1	—
						3	—	—	—	0

*Defined in text **Means of measurements by atomic absorption spectrophotometry n.d. = not detectable

the test fish showed an increased number of breathing detections, in comparison to other experiments, and one out of four test fish died in each experiment. Although simultaneous exposure to zinc and chlorine was lethal, detection of both in-plant toxicity (due to zinc) and upstream toxicity (chlorine) occurred well before any fish had died (Table 2 and Figure 8).

II. Nontoxic Factors

When critical breathing rates were determined on day 1 of the temperature experiment, three breathing detections occurred during the standardization period when the fish were exposed to a 1.2°C cycle, and 28 detections occurred when the fish were exposed to a 4.4°C cycle. The control fish were exposed to a 1.2°C cycle throughout the experiment. The maximum number of breathing responses was obtained between 7 and 11 p.m., when the temperature peaked and began to decrease (Figure 9). Detections started when the temperature of the effluent from the test chambers reached 29°C and continued until the temperature dropped below 27°C.

When critical activity rates were determined during the standardization period, two detections occurred during the treatment period, both on the last day of treatment (Figure 10). On all treatment days, most of the activity responses occurred between 1 and 7 a.m. when the temperature was lowest. As stated earlier, the nighttime activity rate of bluegills is less variable than the daytime rate, and a smaller increase in variance is required to produce a response at night than during the day.

When critical breathing rates were determined on the first day of treatment, day 6, no detections were obtained on days 7-10. Similarly, when critical activity rates were established from the records of days 6-9, no detections occurred during the remaining treatment period.

Figure 11 shows that the number of breathing responses by the fish did not appreciably increase in response to an increase in calcium concentration from 10 to 107 mg/l (the calculated hardness due to Ca, Mg, Zn, and Fe increased from 33 to 301 mg/l as CaCO₃).

III. Combined Factors

When bluegills were exposed for four days to 3.7 mg/l zinc and 112 mg/l calcium added together (total calculated hardness, 318 mg/l), only one

FIGURE 9

BREATHING RESPONSE TO DIURNAL CYCLES OF 1.2°C (days 2-5)
AND 4.4°C (days 6-9)

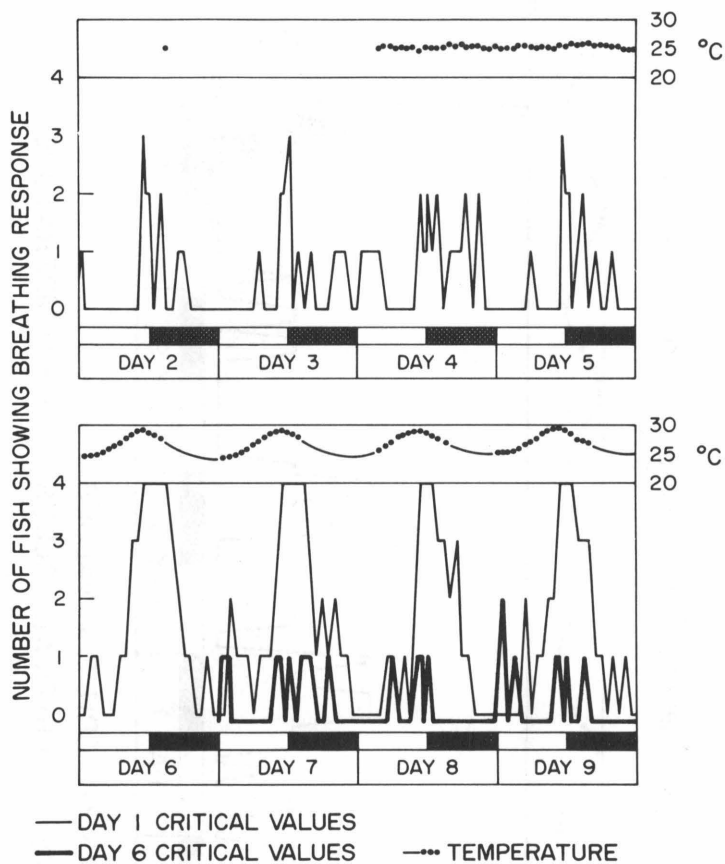


FIGURE 10

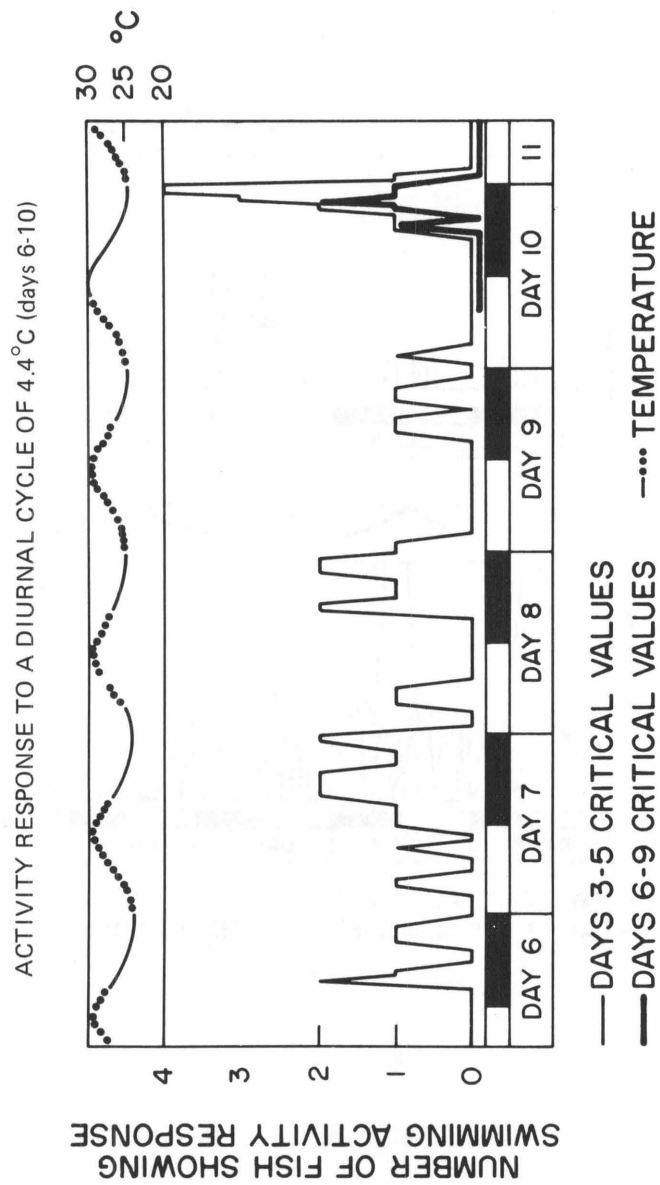
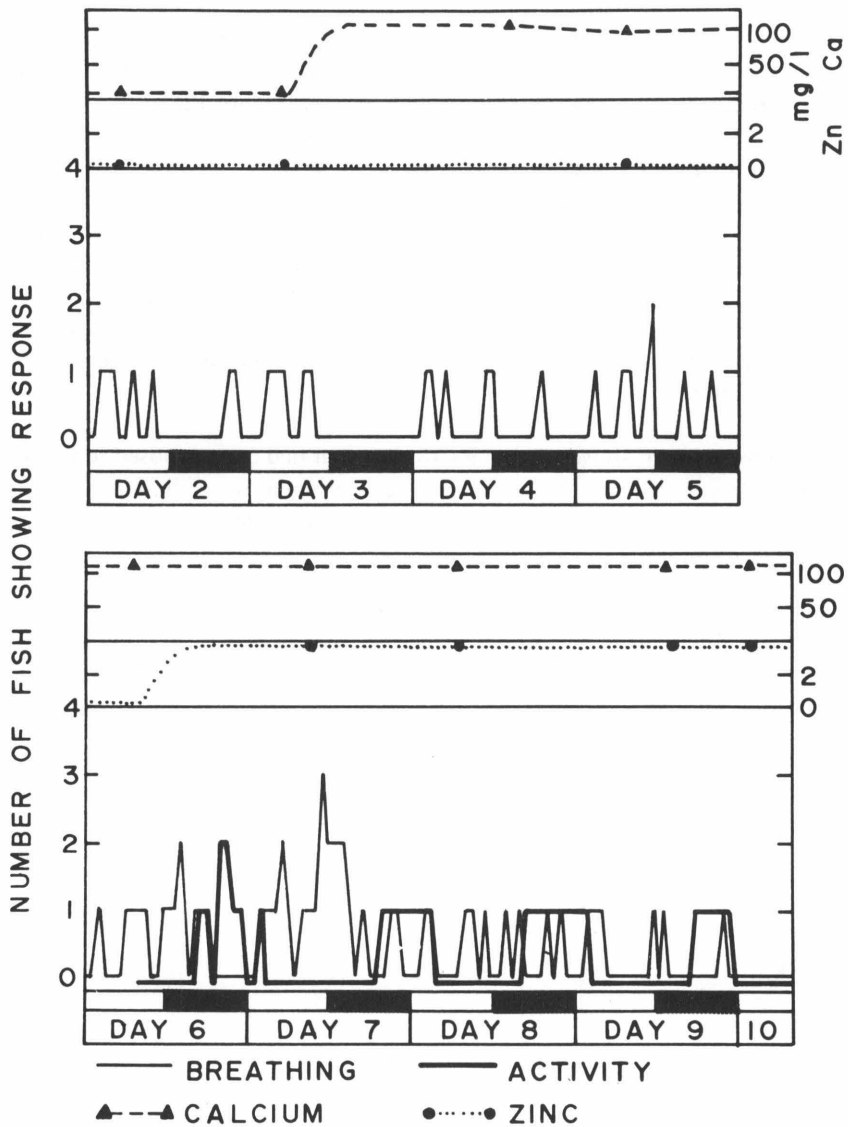


FIGURE 11

BREATHING AND ACTIVITY RESPONSES BY FOUR TEST FISH
TO ZINC AND CALCIUM



breathing detection and one activity detection occurred (Table 3). Figure 11 shows that the number of activity responses was very low throughout the experiment. The largest number of breathing responses, and the single detection, occurred during the first two days after zinc flow started. The number of responses on the last two days of the experiment was comparable to that on days before zinc was added.

When fish were exposed to a mixture of copper and zinc, no breathing or activity detections occurred (Table 3) although more breathing responses resulted during treatment (days 9-12) than before (Figure 12).

IV. Chronic Zinc Exposure Followed by a Zinc Spill

Table 4 shows that chronic exposure to a presumed biologically "safe" concentration of 0.075 mg/l zinc for periods up to 41 weeks did not decrease the sensitivity of bluegills, as measured by breathing and activity responses, to a simulated zinc spill (introduction of 3 mg/l zinc). In fact, the most breathing detections occurred after the longest exposure to the low zinc concentration.

In the experiment shown in Figure 13, fish that had been exposed to the low zinc concentration for 29 weeks retained their ability to show activity and breathing detections to a sublethal zinc spill, and they recovered when zinc stress was removed.

FIGURE 12

BREATHING AND ACTIVITY RESPONSES BY FOUR TEST FISH
TO A MIXTURE OF ZINC AND COPPER

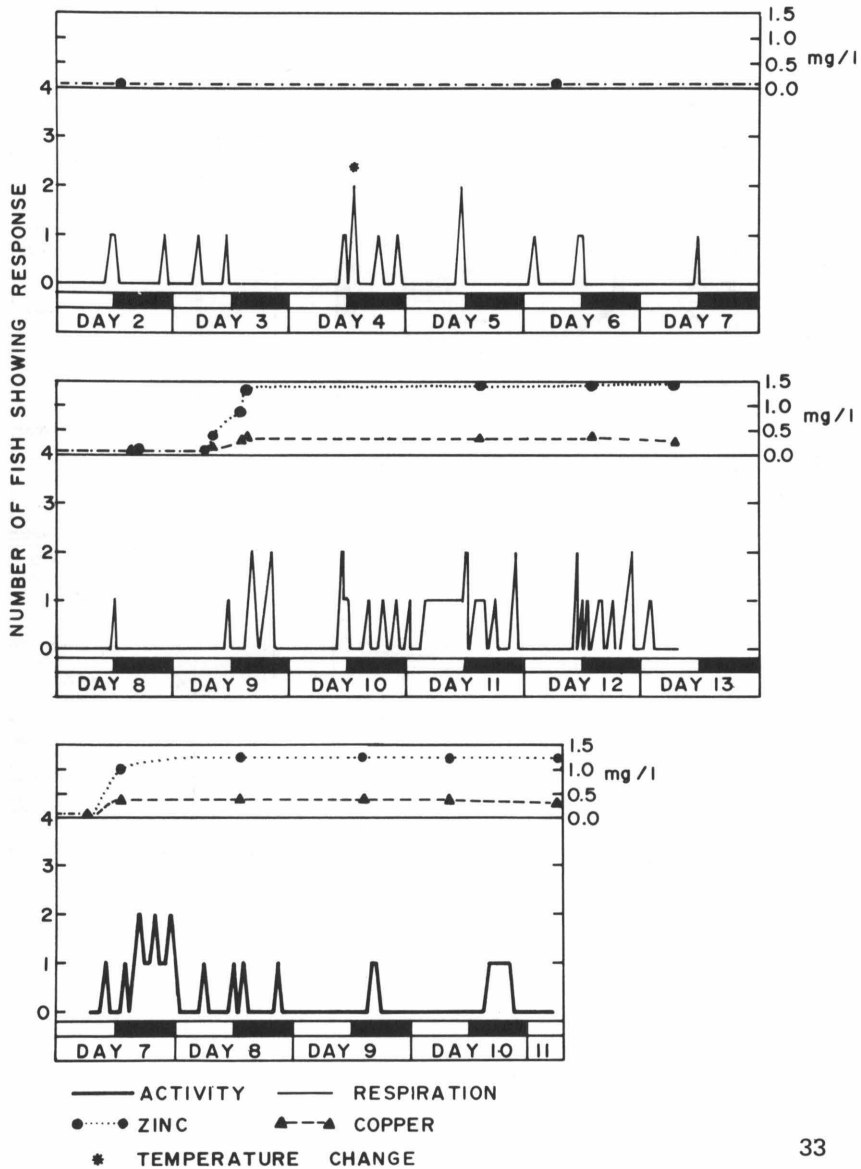
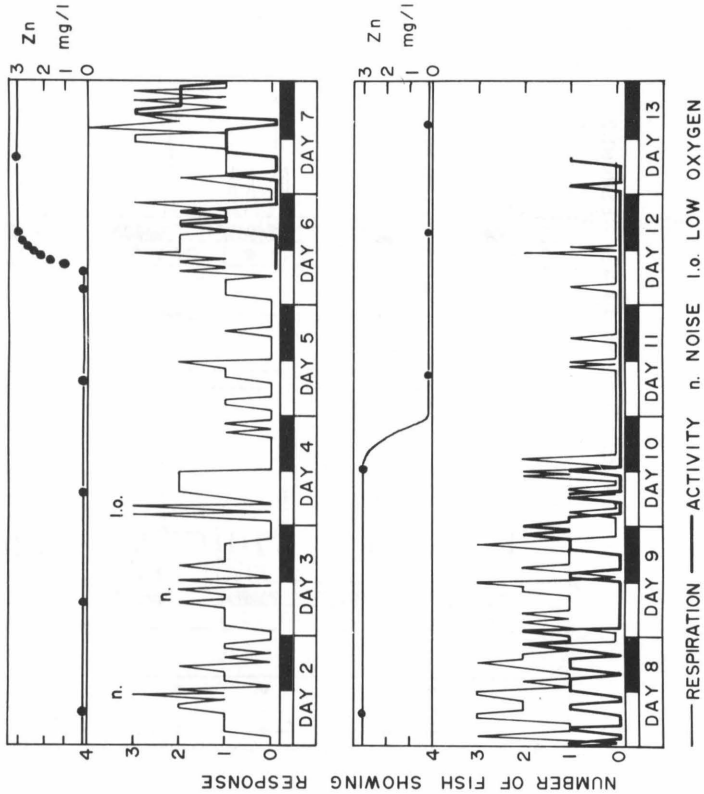


FIGURE 13

BREATHING AND ACTIVITY RESPONSES BY FOUR TEST FISH TO A SIMULATED ZINC SPILL
ON DAY 6, FOLLOWING EXPOSURE TO 0.075 mg/l ZINC FOR 41 WEEKS



DISCUSSION

The biological monitor has been found to be sensitive to several kinds of environmental changes. These include more than just the water quality. For example, it was discovered that the fish respond to mild seismic shock (Sparks and Cairns, 1972). By controlling temperature and photoperiod, and by sound proofing and vibration damping, however, it was possible to concentrate on selected water quality parameters.

The problem of the increased sensitivity of the system during the night is most likely a statistical one since the respiration rates are lower and activity is less variable at this time. However, it is also possible that the sensitivity of the sunfish to toxicants and other factors such as temperature has a circadian periodicity in view of the cycles of susceptibility to drugs, toxicants and temperature that have been found in fish and other animals (Ertel, Ungar, and Halberg, 1963; Haus and Halberg, 1959; Cole and Adkisson, 1964; Starky, et al., 1972).

The responses resulting from accidental introduction of chlorine into both the control tanks and the test tanks on three occasions indicate that the monitor is capable of distinguishing between upstream and in-plant sources of toxicity since both control and test fish had increased responses. The lack of breathing detections and the small number of activity detections during the zinc spill (Table 2 and Figure 7) after an unplanned chlorine spill in the low-zinc period may indicate a desensitization of the fish by the chlorine.

Since detections occurred in response to an increased temperature cycle, it is evident that this variable would have to be carefully controlled or accounted for in the statistical method of any industrial installation. It is not likely that a diurnal cycle of 4°C was a stress to the fish since cycles of this magnitude are common in nature. When the critical limits were calculated for both activity and respiration so as to include the first of the treatment period, however, there were no detections. This suggests that, while the monitor is sensitive to abrupt changes in nontoxic as well as toxic conditions, these conditions can be taken into account when the critical limits are determined. It also indicates the need for periodically up-dating the critical limits during monitoring over long periods. Physiological periodicities are known to be influenced by temperature cycles as well as photoperiod, and any change in the nature of the cycles would show up as detections by the method of analysis that was used. It is surprising that the increase in respiration occurred just after the warmest part of the cycle, while the increase in activity

occurred after the coolest part of the cycle. Since one would expect a positive correlation between these two variables, this aspect of the results should have further investigation.

It is likely that the system is not sensitive to moderate fluctuations in calcium since no breathing responses occurred when the calcium concentration was increased from 10 to 107 mg/l. The effect of combining an increase in calcium with an increase in zinc is another matter, however. One breathing and one activity detection, and a number of breathing and activity responses occurred during the first two days after the combined zinc-calcium flow started (Figure 11). On the last two days of the experiment, the number of responses was comparable to that on days before the zinc and calcium were added, showing that the tolerance of the fish to zinc increased. An increase in tolerance did not occur in other experiments where zinc was added alone (see Figure 13, for example). This may be due to a lag in the effect of the change in hardness. It has been shown by Lloyd (1965) that it takes five days for fish to change their acclimation to water hardness. Until then, they responded to metal toxicity as if they were still in water of the hardness to which they had previously been acclimated.

The lack of responses to a combination of zinc and copper may have been because the concentrations were below the detection threshold of the system. The mixture was calculated to be close to the threshold concentration that would increase fish activity and above the threshold that would increase fish breathing, with each metal contributing approximately one-half the effect. In a discussion of joint toxicity, Lloyd (1961) concluded that effects of copper and zinc are generally additive, rather than synergistic, except in high concentrations. The hypothesis that copper and zinc synergistically affect fish breathing and activity cannot be rejected until the lower detection limit of the monitor for copper is measured rather than estimated from acute toxicity data reported by other researchers. The monitoring system should then be tested with several mixtures, all calculated to be at the threshold of detection by the monitor, but with copper and zinc contributing various fractions of the threshold effect.

The sensitivity of the breathing responses to a zinc spill did not appear to be decreased even after 41 weeks of continuous exposure to a low level of zinc. The sensitivity of the activity response, however, may decrease with time. Activity detections occurred after 25 and 29 weeks of chronic exposure when the mean treatment concentrations were 2.98 and 2.85 mg/l respectively, but not after 37 and 41 weeks, with mean treatment concentrations of

3.03 - 3.08 mg/l. An alternative interpretation of the activity data is that the absence of detections in some experiments was a chance occurrence, attributable to the fact that the zinc concentration of 3 mg/l was close to the lower detection limits of the monitoring system. In any event, the development of resistance by fish kept in monitoring systems in industrial effluents which act similarly to zinc may not be a problem for periods up to 37 weeks. Nevertheless, it would be advisable to replace fish in a monitoring system at an industrial site on a regular schedule (such as replacing two fish a month) until it could be demonstrated that decreased sensitivity to the effluent was not a problem.

If one species of test organism is relatively insensitive to a waste, another species (including species other than fish) could be substituted or added with appropriate modifications to the monitoring system. It would also be possible to make the waste delivered to the test organisms more concentrated than the waste in the stream, to increase the response.

The Future of Biological Monitoring

Now that several of the important aspects of the biological monitoring system have been evaluated, it is appropriate to discuss the role that biological monitoring has in future developments of water quality surveillance techniques.

Generally, physical-chemical automatic sensing devices fall into two categories. The first type includes automatic sampling techniques which require reagents and usually photometric determinations (Malz, *et al.*, 1968). Although sensors of this type can tell a great deal about the chemical nature of the water, they are not likely to be used for surveillance systems in the near future mainly for practical and economic reasons since they require a great amount of maintenance. The second type, like the ones used in the ORSANCO project, includes probes (any transducer that can directly measure a parameter in terms of an electrical output) that measure such things as pH, O_2 , and temperature. The data collection from these devices can easily be automated and a number of such recording instruments are in use today. In order to employ these techniques for water quality management, however, it is essential that the data recording be coordinated on a watershed basis in the form of "on-line" systems. The problem then boils down to evaluating the health of a complex ecosystem from a few physical parameters. This evaluation requires round-the-clock services of highly trained ecologists, and even at that the evaluation is difficult. Some sensors should be placed directly at major pollution sources rather than just in water treatment plants, as in the case with the Ohio river system.

A possible solution to the problem is to use biological monitoring devices in combination with physical and chemical sensors. A biological monitor can (1) give a warning of potentially hazardous conditions in time for specialists to be called in to evaluate the problem; (2) detect toxic conditions that pass physical sensors undetected; and (3) give continuous, immediate information on the toxicity of the effluent to aquatic organisms.

Figure 14 illustrates how a system of such monitors might be organized on a watershed. There would have to be two different types: in-plant and in-stream monitors (the nature of these would depend on the conditions at each location). The in-plant monitors would be placed at all major industries and sewage treatment facilities. The in-stream devices would be placed at strategic locations in the watershed itself. The data recorded at all of these would be transmitted continuously or at regular intervals to a central location for analysis.

The time-consuming hand procedures of keypunching, counting, and film developing presently keep the biological monitoring system described in this paper from being installed at an industrial site. With readily available electronic technology, these steps in the analysis can be completely automated. Figure 15 illustrates the nature of such a system presently under development. Essentially it consists of a minicomputer which can, by specific program instructions, either collect data or clear one of a number of registers. The data in each of these registers are either the output of a counter recording respiratory movements or activity or output from analog-to-digital converters recording such factors as temperature or oxygen (Cairns and Westlake, unpublished). Not only can the observations from both biological and physical-chemical monitors be printed out as they are collected, but also statistical analyses on the data and warning messages can be printed at the same time.

The statistical techniques described previously for the activity data have already been applied to the respiration data. Lower critical levels for breathing and activity rates, as well as the upper critical levels are also determined. Figure 16 is part of the output from this program. From left to right each line contains the time in minutes of the year, the activity count for an hour for all three photocells from tank 1, the respiration rate for the same hour for tank 1, the activity for tank 2, etc. A number of messages are also indicated. For example, "CODE 1H" indicated that the activity count was abnormally high but that the respiration rate was in the expected range. If a sufficient number of breathing and activity responses occur in the same hour, the message "WARNING" is printed out suggesting that corrective measures

FIGURE 14

PROJECTED USE OF IN-PLANT AND IN-STREAM BIOLOGICAL MONITORING UNITS

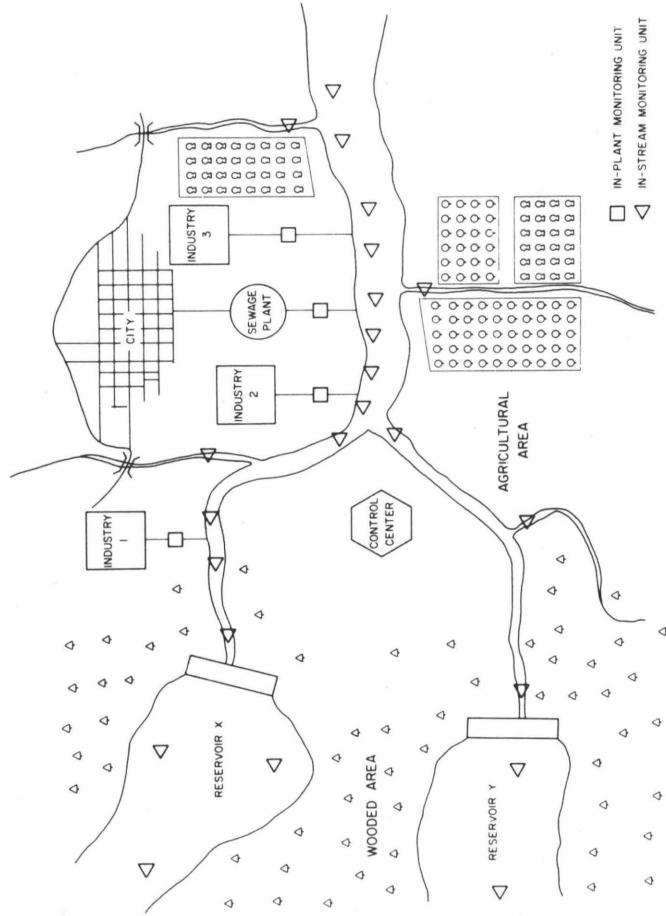


FIGURE 15

BLOCK DIAGRAM OF A COMPUTERIZED SYSTEM FOR MONITORING FISH MOVEMENT AND BREATHING

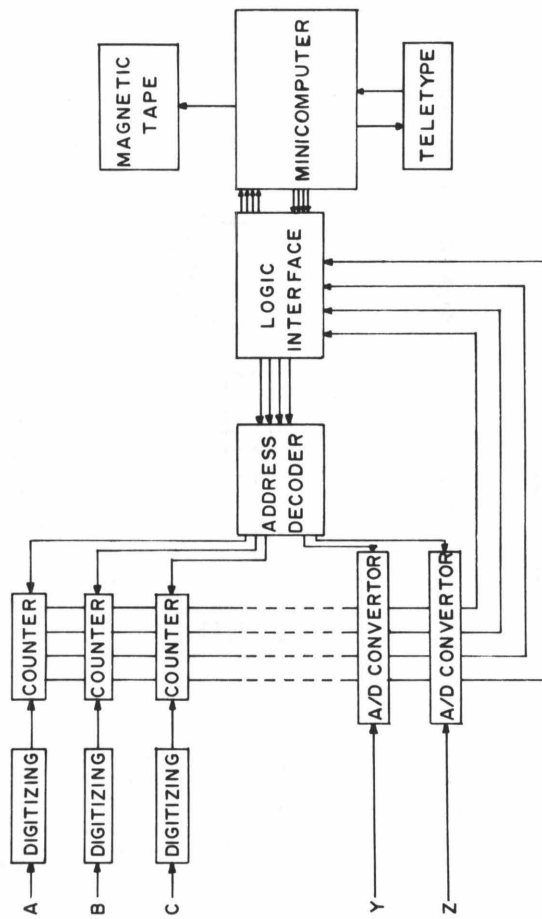


FIGURE 16

COMPUTER OUTPUT FROM A MONITORING EXPERIMENT
SHOWING WARNING STATEMENTS

209700	72	35	121	22	184	17	379	28	36	19	192	42
									CODE 1L			
209760	174	36	124	14	45	17	527	22	38	20	178	38
							CODE 1H					
209820	74	38	157	18	100	17	338	26	41	26	167	40
209880	59	36	169	18	46	19	339	22	46	16	187	40
	CODE LH											
209940	33	26	170	17	72	18	316	32	122	27	160	48
	CODE 1L				CODE 1L							
210000	10	43	224	16	70	26	409	39	221	18	362	38
	CODE 1L										CODE 2H	
210060	190	40	192	17	219	19	375	45	164	19	243	40
	CODE 2H						CODE 2H				CODE 2H	
WARNING												
210120	7	39	76	16	103	20	407	64	63	17	162	58
	CODE 2H											
210180	23	22	57	13	70	21	565	47	70	15	204	40
WARNING												
210240	35	25	15	12	43	17	361	26	79	17	180	40
							CODE 3H		CODE 1H		CODE 3H	
WARNING												
210300	11	28	44	16	2	15	388	28	75	12	153	58
			CODE 1L						CODE 1H		CODE 3H	
210360	102	25	0	14	53	16	524	23	39	13	122	40
			CODE 1L				CODE 1H				CODE 3H	
WARNING												
210420	45	21	52	11	27	15	259	24	24	15	89	51
							CODE 1H				CODE 3H	
WARNING												
210480	56	34	37	11	1	15	418	37	20	11	54	41
					CODE 1L						CODE 3H	
210540	72	21	40	10	27	15	351	22	38	14	38	47
					CODE 1L							
210600	39	21	13	14	16	19	165	33	29	15	29	35
									CODE 2L		CODE 2H	
210660	89	17	15	16	17	16	168	29	21	18	23	35
	CODE 2L		CODE 1L								CODE 2H	

be taken. The program is designed in such a way that it could be easily adapted to an on-line operation.

Once this critical step of automation is accomplished it is important that the system gain industrial experience. As well as continuing to challenge the monitor with a variety of conditions, a similar device might be installed at an industrial site, or better still, in a trailer which could be moved to a number of industrial sites. In any event, only the sensors and a small part of the interface need be moved out of the lab. The minicomputer and the rest of the system could remain in the lab and receive data from the monitor through telephone lines.

It should be pointed out that the monitoring of respiration and activity of fish in the manner described in this paper is not the only possible type of biological monitor nor is it necessarily the best. It is difficult to imagine a type of data, however, that could not be described by either counters or analog-to-digital converters. The design is therefore flexible enough to accept future modifications in the sensors and these will no doubt be developed as actual field experience is gained. In addition, it is not likely that the same type of monitoring system will work efficiently in all situations, so the logistics would have to be tailored to fit the circumstances. For example, several species of aquatic organisms could be used simultaneously as test organisms, if some species were sensitive to some components of the effluents and other species were sensitive to other components. Since the data analysis is done entirely according to the programming instructions, it is also possible to make necessary modifications in the statistical techniques with experience.

There are other biological monitoring techniques that are under development at present which show a great deal of promise. Besch and Juhnke (1971) describe an apparatus which determines the ability of fish to maintain their position in a current under various toxic conditions. The detection of movements of fish by means of heated thermistors is another possibility (Heusner and Enright, 1966). The disturbances caused by fish swimming in an ultrasonic field can also be used as a measure of activity (Byrne, 1971). O'Hara (1971a,b), Shaumburg, *et al.* (1967), and Livingston (1968, 1970) described techniques for measuring oxygen consumption of fish exposed to toxicants. It is important that developments of this nature occur along several lines since the monitoring devices need to be tailored to different conditions at different sites. Gradient tanks could make it possible to use the extraordinary olfactory capabilities of fish to detect materials at concentrations well below toxic levels (Sprague, 1968; Ishio, 1960; Kleerekoper, *et al.*, 1972; Westlake, *et al.*, unpublished; Jones, 1956; Spoor

and Drummond, 1972). There is a great need for developing monitoring techniques not only using fish but also other aquatic organisms (e.g. Butler, 1969).

In summary, it appears likely that a family of biological monitoring methods will be developed, and that techniques for automatically analyzing the data (and perhaps even for decision making) will make it feasible to use such systems to help control water quality in effluents and river basins.

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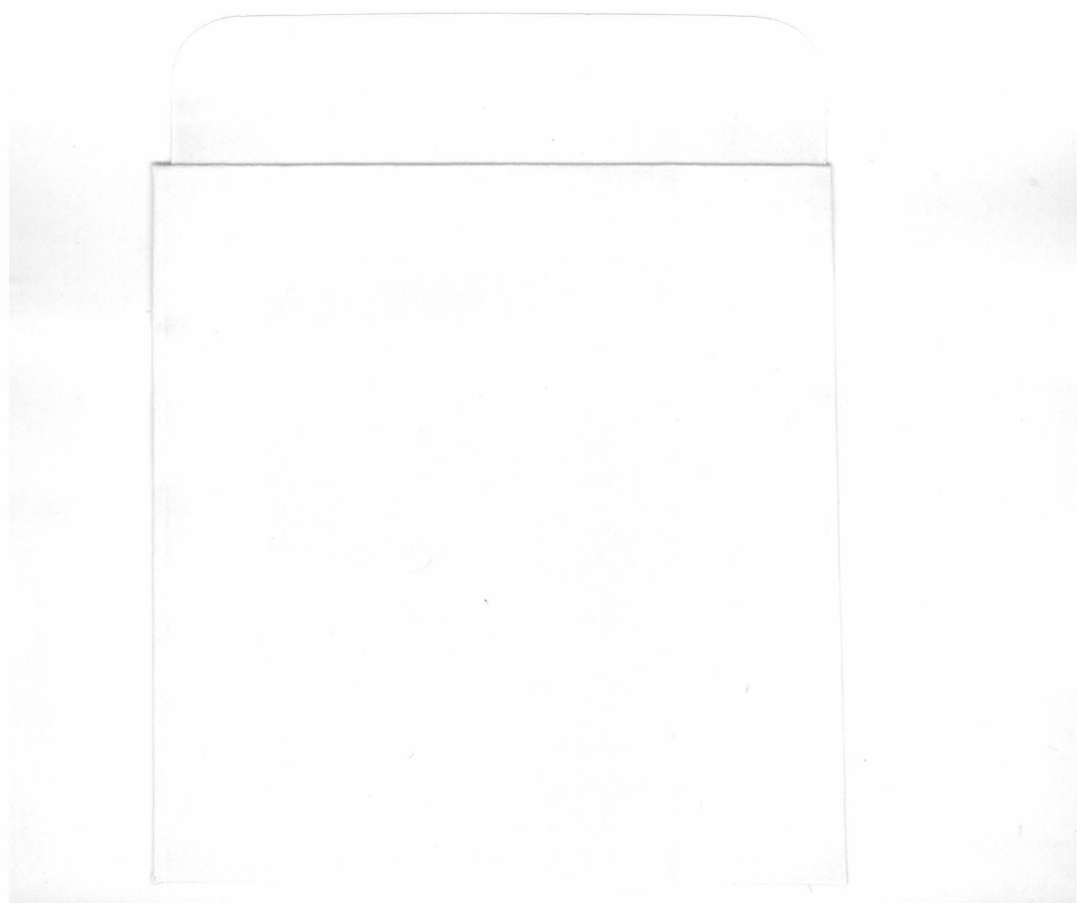
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