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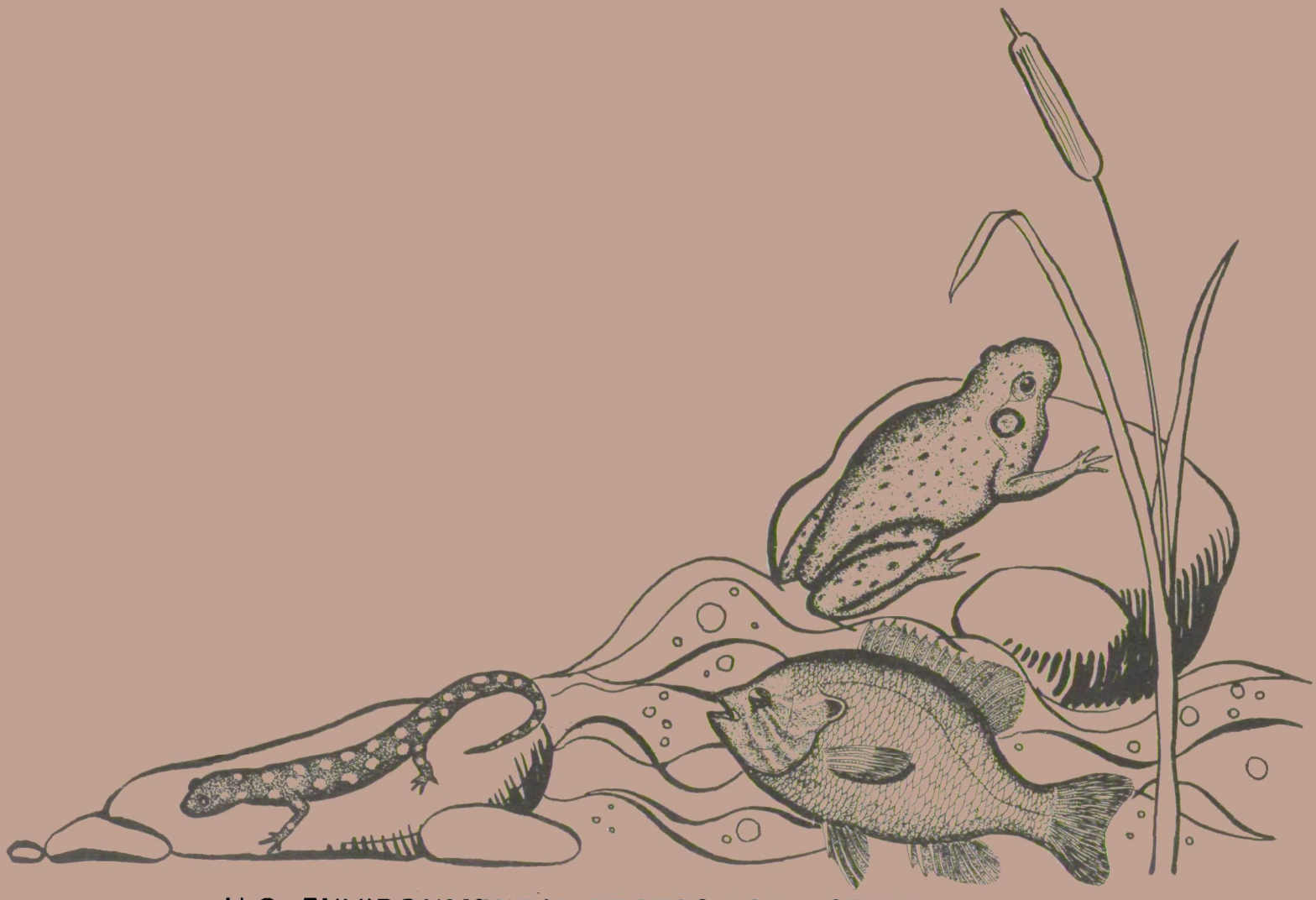
The Use of Fish Movement Patterns to Monitor Zinc



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THE USE OF FISH MOVEMENT PATTERNS TO MONITOR ZINC

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ABSTRACT

The feasibility of using fish movement patterns measured by light beam interruption as a technique for continuous monitoring of the response of fish to zinc was investigated. In conjunction with the monitoring studies the growth and reproductive success of the B luegill sunfish (Lepomis macrochirus) exposed to various fractions of the lowest concentration of zinc detected by the monitoring apparatus were studied.

The monitoring apparatus does not in any way interfere with fish movement within the test chamber and allows for the maintenance of fish for long time periods. Under the conditions described the system detects premortal aberrations in fish movement caused by zinc. The detection of stress occurs in sufficient time to permit survival of the test fish if stress conditions are reversed at the time of detection. The lowest concentration of zinc detected by the system during a 96-hour exposure was between 3.64 and 2.94 mg/1 Zn^{++}. The system's range of effective measurement as related to turbidity is discussed. This method should detect other toxicity equally well.

The growth and reproductive success of the bluegill was tested in concentrations approximately equal to 1/10 and 1/100 the lowest concentration of zinc detected by the monitoring system and 1/100 of the 96 hour TL50 (median tolerance limit) determined under continuous flow conditions. The growth and reproductive success in 1/100 the lowest detected zinc concentration and 1/100 the 96 hour TL50 value did not differ appreciably from the controls while a concentration of approximately 1/10 the lowest detected zinc concentration in effect eliminated reproduction in the bluegill.

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FIGURE

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SECTION I

CONCLUSIONS

1. The monitoring system used in this study detects aberrations in movement patterns of fish exposed to lethal and sublethal concentrations of zinc.

2. The monitoring system does not detect aberrations in movement patterns of bluegills exposed to concentrations of zinc which effectively eliminate reproduction.

3. The system does not require direct contact with the fish being monitored and therefore allows for the maintenance of the fish for extended periods of time.

4. The monitoring system as described is limited in its applicability to continuously monitor effluents unless turbidity is controlled.

5. The reproduction of bluegill sunfish under the conditions described is effectively eliminated at a concentration of approximately .255 mg/l Zn^{++}.

SECTION II

RECOMMENDATIONS

This laboratory study was designed to develop the equipment and precedures necessary to test the hypothesis that fish movement patterns could be used as a means of detecting premortal aberrations. This goal was achieved, and it is recommended that further laboratory studies using different toxicants and or combinations of stressors be carried out to refine the techniques developed.

The relationship between the removal of suspended solids and toxicity should be studied to determine if extending the effective range of measurement of the instrument by removing the solids in turn alters the predictability of the system.

In depth statistical evaluations should be undertaken to determine if the sensitivity and speed of response through analysis can be facilitated.

It is recommended that when growth and reproductive studies are undertaken utilizing fish of the size range used in these experiments that larger containers be employed to house the spawning fish. The container should probably be at least twice the length of the tanks employed in these studies.

In growth and reproductive studies several duplicates of each concentration should be simultaneously analyzed to insure that the apparent inherent biological differences in behavior and reproductive potential are not interpreted as absolute differences due to the toxicant being studied.

In these studies, contrary to that reported elsewhere, spawning was not generally confined to a certain time interval within the photoperiod. Therefore checking the nests twice a day once in the morning, and once in the evening is recommended as a means of eliminating the loss of some spawnings due to hatching occurring prior to the time of nest checking.

SECTION III

INTRODUCTION

As the pollution problems associated with an expanding industrial base and increasing population size become more acute, the need for an overall program of ecosystem management to minimize the adverse effects of man's activities on the environment must be developed.

The report of the Council on Environmental Quality (1970) repeatedly stresses the need for the development of predictive, simulative and management capabilities to combat air and water pollution. These techniques must be developed if the concept of multiple use set forth in the Water Quality Act of 1967 (Public Law 89-234) is to succeed and if the present practice of alteration without comprehension is to be eliminated.

A great deal of progress has been made in determining biologically safe concentrations for fish (Mount and Stephan, 1967; Mount, 1968; Water Quality Criteria, 1968; Sprague, 1969; Brungs, 1969; and Eaton, 1970). There remains, however, at least one critical area of effluent quality control which must be effectively monitored before the standards for chronic exposure of fish to toxicants will prove effective. This protection should include safeguards against the development of acutely toxic conditions resulting from either industrial or municipal accidents or from changes in the total environmental variation and industrial processes.

If an industry conforms to the predetermined standards for chronic exposure 363 days out of the year and over a period of two days due to human error or a combination of human error and changes in the physical and chemical characteristics of the receiving stream acutely toxic conditions develop which are not detected in time to prevent deleterious conditions from developing--then the standards for chronic exposure alone can not protect the receiving stream. Also an industrial or municipal effluent could foreseeably conform to the standards for chronic exposure all the time and still not insure against the development of acutely toxic conditions. For example, if an upstream industry changed processes resulting in the release of a compound, at a predetermined biologically safe concentration, which interacted synergistically with an effluent from a downstream industry, also released at a predetermined biologically safe concentration for fish, the result could be not only chronic stresses but the development of acutely toxic conditions. The standards for chronic exposure without sufficient protection against the development of acutely toxic conditions will not maintain a vigorously functioning aquatic ecosystem.

Continuous physical and chemical monitoring systems provide a partial answer to this problem in that they may be used to detect almost instantaneously a single environmental variable which has reached a

lethal level. However, aquatic organisms respond to the collective effect of the environmental factors and these effects can not be predicted from the chemical-physical analyses alone without direct feedback from the organisms.

Cairns (1970) and Cairns (in press) presents a plan for a systems approach to aquatic ecosystem management on a regional basis. In order to implement this plan both instream and inplant systems for continuous biological monitoring must be developed (Shirer, et al., 1968; Cairns, et al., 1970).

The objective of this study was to determine the feasibility of using fish movement patterns monitored by light beam interruption as a technique to detect premortal aberrations.

SECTION IV

EFFECTS OF ZINC ON FISH MOVEMENTS

Three species of fish were tested under varied environmental conditions in a total of twenty experiments to determine the feasibility of using fish movement patterns as a means of detecting acute and chronic exposure to pollutants.

Monitoring Apparatus: The design philosophy of the monitoring apparatus was based on technical simplicity and reliability. Common five-gallon tropical fish aquaria of about 41 cm length, 21.5 cm width, and 26 cm height were used as the test tanks. Basically the monitoring units consisted of light beams which were arranged to traverse the length of the tanks near the bottom, middle, and just below the surface (Figure 1). Photoresistors at the opposite end of the tanks were illuminated solely by the lamp sources with the aid of collimating baffles. Interruption of the light falling on the photoresistors operated relays, via two-transistor amplifiers, which in turn advanced counters and deflected pens of an event recorder. Six tanks, each with three light beam sensors, were used in each experiment. For a detailed description of the circuitry see Shirer, Carins, and Waller (1968).

Static Tests: Ten of the twenty experiments analyzed were carried out under static test conditions. In these experiments the test tanks were housed in a light-tight plywood chamber 2 x 1 x 2.5 meters. Ambient illumination within the chamber was provided by a pair of 40 Watt fluorescent tubes cycled daily for 12 hours darkness and 12 hours light with a time switch.

Each tank, filled to a calibrated 17 liter mark, was equipped with an air diffusion stone housed in a perforated plastic cylinder. The plastic cylinder was located midway along the length of each tank and contained the rising air bubbles. The cylinder served to eliminate any erroneous signals which might have resulted from bubbles breaking the light beam.

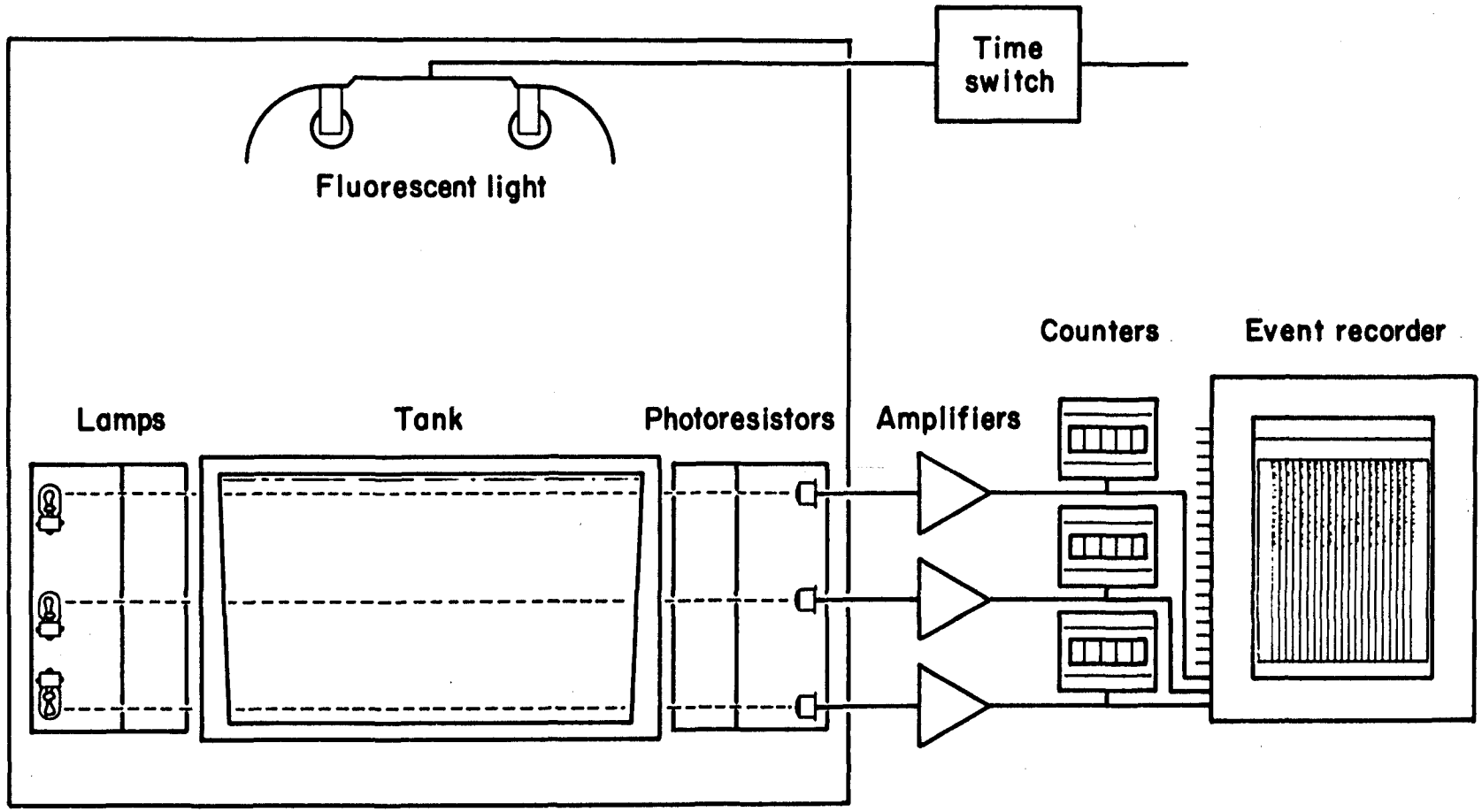
Synthetic dilution water was used for all static tests (Scheier and Cairns, 1966). The chemical characteristics of the synthetic dilution water are based on determinations made at the beginning and end of each experiment. Dissolved oxygen was 7.5 mg/1 for all determinations. The pH was always highest at the beginning of each test ranging from 7.0 - 7.8. For any given tank pH did not decrease by more than 0.5 during any experiment. Temperature always increased during an experiment. Temperature ranged from 20 C - 25 C. Initial and final hardness and methyl orange alkalinity values were constant for both factors and were 51 mg/1 as $CaCO_3$ and 68 mg/1 as $CaCO_3$ respectively.

Test Organisms: Two species of fish, the golden shiner Notemigonus

Figure 1

Block diagram of

monitoring apparatus


Time switch
Fluorescent light
Counters
Event recorder
Lamps
Tank
Photoresistors
Amplifiers

crysoleucas (Mitchell) and the goldfish Carassius auratus (Linnaeus), were tested under static conditions. Because of the difficulty in maintaining stocks of golden shiners under static test conditions only a single experiment using this species was completed. Test fish were acclimated to experimental dilution water, temperature, and photoperiod for a period of at least two weeks prior to their use in an experiment. During this period they were fed a commercial food preparation (Tetra-Min) ad libitum twice daily. The test fish were not fed during an experiment.

Two days prior to the beginning of an experiment a single test fish was placed in each of the six test chambers and allowed to readjust after handling. After the two-day readjustment period recording was started at 6:30 a.m. and continued for eight days. At 6:30 a.m. on the fifth day the calculated concentration of toxicant was added in solution to each of the experimental tanks; the control received the same volume of dilution water minus the toxicant. The gradual addition of the toxicant was accomplished by adding the solution via plastic tubing to each tank. Mixing was quite rapid due to the air driven circulation of tank water.

At the beginning of the recording period the counts registered on the electric counters for each of the three photocells in a single tank were recorded in a notebook. Subsequent records were made every three hours throughout the test period, except for a daily interval from 12:30 a.m. to 6:30 a.m. during the dark cycle of the photoperiod. Cummulative movement for the desired interval was obtained by subtracting two successive readings. Upon completion of an experiment, total length and wet weight was recorded for each fish (Table I). All tanks and equipment were washed in EDTA between experiments.

Continuous Flow Experiments: Initial analyses of the movement patterns recorded in the static tests revealed a high level of variance. Because of the high level of variance and because continuous flow testing more realistically approximated the conditions under which this apparatus would be used, a second series of ten experiments using the bluegill Lepomis macrochirus (Rafinesque) as the test species were performed. All procedural changes incorporated in the continuous flow tests were done in an attempt to increase reliability and reduce variance.

Monitoring Apparatus: The only change made in the monitoring apparatus was the addition of red 650 mu filters to each of the light paths. Although one can not say that visual detection of the beams would be impossible at this level, the lighting conditions would provide reasonable "darkness" i.e., at a level which would not exceed moonlight nights (J. R. Brett, personal communication).

All movement experiments were carried out in an isolation room. The isolation room was designed to reduce the effects of noise produced by normal laboratory traffic. The experimental and stock tanks housed in the isolation room were placed on a 35cm deep sand bed.

TABLE 1. SUMMARY OF GENERAL FISH DATA

Expt. Number	Weight (grams)		Standard Length (cm)		Total Length (cm)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
1	3.4	0.258	---	---	7.9	0.568
2	5.4	0.854	---	---	7.9	0.531
3	5.0	1.162	---	---	8.2	0.564
4	3.2	0.337	---	---	7.2	0.334
5	4.5	1.001	---	---	7.7	0.606
6	4.6	0.656	---	---	7.9	0.206
7	5.3	0.579	5.5	0.213	8.2	0.459
8	4.1	0.544	4.9	0.233	8.2	0.729
9	3.5	0.571	4.8	0.242	7.3	0.278
10	3.9	0.520	4.9	0.147	7.6	0.524
11	14.2	3.023	7.7	0.432	10.2	0.567
12	15.5	1.778	8.0	0.273	10.6	0.350
13	19.6	2.377	8.7	0.297	11.3	0.312
14	12.3	2.053	7.4	0.322	9.7	0.436
15	13.0	3.172	7.4	0.561	9.5	0.739
16	12.3	2.851	7.6	0.450	9.9	0.588
17	11.5	2.317	7.4	0.404	9.7	0.470
18	12.4	2.180	7.5	0.460	9.6	0.563
19	14.3	1.989	7.9	0.281	10.2	0.337
20	13.9	2.234	8.1	0.368	10.3	0.444

Two pieces of lucite, painted black, were cemented to the side and bottom of each 17-liter stock and experimental tank to form the top and outside wall of a 14 cm long by 6 cm open chamber. It was hoped that the chamber would provide a refuge for the fish when stressed, thereby magnifying the changes in normal movement patterns.

Both isolation rooms and the stock and growth and reproduction room were maintained on the same simulated dawn-dusk photoperiod. A motor driven dimming unit was used to increase light intensity at "sunrise" from zero intensity to a maximum intensity of 100 foot candles, measured at the water's surface of the experimental tanks. The increase in light intensity from zero to full intensity occurred over a thirty-minute period. The reverse sequence occurred during the simulated sunset. Forty-Watt vita-lite fluorescent tubes provided the ambient illumination.

Dechlorinated municipal tap water flowed by gravity from a 500 gallon reservoir to two smaller 189-liter reservoir. The smaller constant head reservoir served as the immediate source of water for the movement experiments, and final temperature adjustments were accomplished at this point. Routine chemical analyses were performed daily on the water in the 189 liter reservoir (Table 2). Two modified Harvard 1210 variable speed peristaltic pumps were used to provide flow to the experimental tanks. Three tanks were serviced by each pump. A single hose for each tank was anchored in the 189-liter reservoir five cm beneath the water surface. The water was pumped up through a glass stopcock and flowmeter after which the line split and passed through the head of the peristaltic pump. After passing through the pump the line was rejoined and continued to a small mixing vessel before continuing to the appropriate experimental tank. The continuous flow line to each tank entered its respective tank in one corner, the flow being released approximately one centimeter from the bottom of the tank. A 0.64 cm effluent port was drilled through the glass wall of each tank at the opposite end and side of influent water. The effluent port was drilled at the 17-liter mark in each tank. The effluent water for each tank was returned to the outside of the isolation room for sample collection and if not used for that purpose was discharged.

Flow rates were determined twice daily by collecting the effluent from each tank five times for one-minute intervals. The mean flow rate for this period was calculated and the flow to each tank adjusted accordingly. An attempt was made to maintain all flow rates at 100 mls/min.

Toxicant introduction was accomplished by switching from the 189-liter constant head reservoir to two 95-liter constant head containers in which a calculated concentration of reagent grade $ZnSO_4 \cdot 7H_2O$ had been mixed. Care was taken to avoid temperature differences between the regular dilution water and the batch mixed zinc solutions. The zinc analyses were determined using atomic absorption spectrophotometry made at least twice daily. The initial measurement was made after nine hours to insure that 95 - 99% particle replacement had occurred within the test chambers. At the conclusion of each experiment the tanks and glass-

TABLE 2. CHEMICAL CHARACTERISTICS OF DILUTION WATER.

Water Characteristics Continuous Flow Experiments	Number of Analyses	Mean	S.D.
Temperature (C)	396	19.7	1.79
pH	397	7.8	0.26
Total Hardness (mg/l as $CaCO_3$)	394	51	10
M.O. Alkalinity (mg/l as $CaCO_3$)	393	41.3	8.8
Dissolved Oxygen	7.5 in all cases		
Chlorine	not detectable unless otherwise noted		

ware were washed with EDTA and all tubing was replaced.

Water from the 189 liter reservoir also flowed into the stock and growth and reproduction room where it was pumped to the elevated constant head reservoir. Temperature adjustments were made at this point before the water flowed by gravity to the five 189-liter stock tanks. Water also flowed from the elevated constnat head box back into the isolation room where it was distributed to the six 17-liter stock tanks.

Fish: The bluegill stock used in these experiments were obtained by seining a pond located on the property of the Veterans Administration Hospital in Salem, Virginia. In the laboratory the fish were maintained under continuous flow conditions for a period of at least two weeks before being moved to isolation room two. During acclimation fish were fed Gordon's formula (Axelrod, 1952) once daily between the hours of 8:00 a.m. and 5:00 p.m. The exact hour of feeding was determined from a random number table. This was done in an attempt to eliminate the effect of anticipated feeding which may have developed had the fish been fed at the same time each day (Davis and Bardach, 1964). Experiments 19 and 20 represent the only tests in which fish were fed. The methods for bioassay fish (A.P.H.A., 1965) were adhered to as closely as possible.

Six test fish were moved from the community stock tanks and placed one fish per tank in the 17-liter stock tanks located in isolation room two. The fish were acclimated to conditions in these tanks for a period of at least two weeks before being used in an experiment. Four days prior to the beginning of an experiment the six fish located in the 17-liter stock tanks were transferred to the experimental tanks. After a four-day readjustment period recording was started and continued for a period of eight days. At the beginning of the fifth day of recording the flow was switched from the 189-liter constant head reservoir to the constant head batch mixed zinc reservoirs.

Data recording was facilitated by the use of a 35 mm Ricoh Auto Shot spring advance camera. The camera was tripped by a solenoid activated by a time switch. Pictures of the electric counters (Figure 1) were taken at hourly intervals throughout each 24-hour period except for the hours divided by the simulated sunrise and sunset. During this period pictures were taken on the half hour. At the end of an experiment the fish were killed and wet weight, standard length, and total length measurements were taken (Table 1).

Statistical Analyses: The statistical test used to analyze the fish movement patterns was a two-sample test for homogeneity of variance. A computer program written by Sokal and Rohlf (1969) was used to facilitate handling the large volume of data. In all analyses each fish served as its own control. For the experiments carried out under the static test conditions day to day comparisons were made by using the six time intervals for which records were made to obtain an estimate of the variance in movement patterns for a given day. The variance for the data recorded during day 1 was then compared to the variance recorded for day 2, the

the largest variance value being divided by the smaller value. If the test for homogeneity of variance was not significant the fish was not considered to be showing abnormal movement patterns and the variance estimate for day 2 was then tested for homogeneity against the variance for day 3. If at any time the test for significance indicated heterogeneity the data for the last recorded day was dropped and the preceding day was compared to the variance estimate for the next day. For example, if the test for homogeneous variance between day 2 and day 3 indicated the variances were heterogeneous, the variance estimate for day 3 was dropped from the analysis and day 2 was compared to day 4. An analysis showing heterogeneity was considered to indicate abnormal changes in the movement patterns.

The data from the continuous flow tests were treated in the same manner except the time interval for data collection was reduced from the day to day comparisons in the static stest to comparisons made four times during a 24-hour period. The level of significance used for all tests was = .002.

Results and Discussion: The results presented here represent a progression from relatively crude static tests to more sophisticated continuous flow tests. The results do not include fifteen tests which actually formed the basis for the methods employed in the tests reported. The preliminary studies included experiments in which more than one fish per test chamber was used, more than three photocells per test chamber were used, and various periods of acclimation were investigated as well as different photoperiod regimes.

The criterion used to determine abnormal movement patterns was a positive test for heterogeneity. The definition of "stress detection" is arbitrary and is based on the results obtained, rather than some pre-conceived idea as to what might constitute "stress" in terms of fish movement patterns as monitored by light beam interruption. For example, the results presented in Tables 3-15 represent a series of experiments in which the total movement of the fish (the sum of the light beam interruptions for the three photocells in a given tank) was used to obtain an estimate of the variance used in the statistical test for heterogeneity. Based on the results obtained from these experiments the definition of "stress detection" is the occurrence of two or more positive tests for heterogeneity (abnormal movement) during the same time interval.

Since light beam interruptions are recorded for three different photocell levels within each tank there exists seven possible combinations of photocell levels on which the variance estimates used to determine abnormal movement can be based. The results presented here are based entirely on the sum of the light beam interruptions for all three photocells in a given tank. The advantages or disadvantages obtained from analyzing the light beam interruptions from the three levels in all possible combinations has not as yet been established but because there is an observable tendency for bluegills under stress to increase activity at the tops of the test chambers, the continuous flow data have been

analyzed using light beam interruptions recorded for the top photocells in each tank. The analyses in which the light beam interruptions recorded for the top photocells alone were used agree in general with those obtained using the total light beam interruptions from all three photocells with one major distinction. The definition for stress detection given for analyses based on the total light beam interruptions from all three photocells does not give consistent or reliable results when the light beam interruptions for the top photocells in each tank are analyzed alone. It may be that analyses based on the light beam interruptions from the six possible combinations of photocell levels will each have a different set of criteria on which stress detection is based. The fact that the arbitrarily defined stress detection is dependent on which combination of photocells are being analyzed is not important. What is important is that once the criteria have been established for stress detection using a certain combination of photocell levels, the results based on this combination must give a consistent and reliable index of premortal aberrations.

It should be noted that the system does not directly monitor the total activity of the fish but rather reflects changes in the variances of movement through the light beams as compared to the variance in light beam interruptions recorded for a previous time interval. The magnitude of change in the number of light beam interruptions does not necessarily mean that a positive test for heterogeneity will be recorded. What is necessary is an increase or decrease in the variance of one of the time intervals compared.

Tables 3, 4, and 5 give the results obtained from static experiments 1 through 10. These results show that during the first four days of any experiment the variance calculated from the number of light beam interruptions recorded for the test fish only periodically deviated from the preceding day giving rise to a positive test for abnormal movement. A positive test for abnormal movement is indicated by an asterisk in the tables. The most important aspect of these analyses is that during the first four days of these experiments, in only one special case was there ever more than a single positive test for abnormal movement recorded during a given time interval. For example, in Table 3, experiment 1, the comparison of variances in light beam interruption for day 1 vs day 2, fish number six, indicates a positive test for abnormal movement as shown by the asterisk. During this same time interval (day 1 vs day 2) this was the only analysis in which a positive test for abnormal movement was detected.

The results from the second four days of experiments 1, 2, and 3 (Table 3) show that stress detection (the occurrence of two positive tests for abnormal movement during the same time interval) occurred during the first day of exposure to 7.5 mg/l Zn^{++} for the golden shiner, and 15.5 mg/l Zn^{++} and 7.5 mg/l Zn^{++} for goldfish. For goldfish these were acutely toxic levels as indicated by the deaths recorded on the following days. The fact that stress detection occurred before the onset of death in both cases indicated that the use of light beam interruption as a technique

TABLE 3. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING STATIC EXPERIMENTS 1-4.

Fish	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4		Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
			Experiment No. 1 Golden Shiner 7.5 mg/l Zn^{++}					
1	0	0	0	--Zn^{++} Added--	0	0	0	0
2	Fish died during day 1				-	-	-	-
3	0	0	0		*	0(4vs6)	0	0
4	0	0	0		0	0	0	0
5	0	0	0		*	0(4vs6)	0	0
6-C	*	0(1vs3)	0		0	0	0	0
			Experiment No. 2 Goldfish 15 mg/l Zn^{++}					
1	0	0	0	--Zn^{++} Added--	0	Dead		
2	0	0	0		*	Dead	Experiment	
3	0	0	0		*	Dead	Terminated	
4	0	0	0		0	Dead		
5-C	0	0	0		0	0		
6	0	0	0		0	0		
			Experiment No. 3 Goldfish 7.5 mg/l Zn^{++}					
1	0	0	0	--Zn^{++} Added--	0	0	0	0
2	0	0	0		0	0	Dead	-
3	0	0	0		0	0	Dead	-
4	0	0	0		*	0(4vs6)	0	0
5-C	0	0	0		0	0	0	0
6	0	0	0		*	*(4vs6)	0(4vs7)	0
			Experiment No. 4 Goldfish 5.6 mg/l Zn^{++}					
1	0	0	0	--Zn^{++} Added--	0	0	0	*
2	0	0	0		0	0	*	0(6vs8)
3-C	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	0	0		0	0	0	0
6	Leak in Tank		-		-	-	-	-

for monitoring movement is sufficiently sensitive to detect premortal signs of stress.

In experiments 4 through 10 (Tables 3, 4, and 5) no stress detection occurred when goldfish were exposed to levels of zinc from 5.6 mg/l to 1.8 mg/l except in the special case recorded during experiment 6 (Table 4). Experiment 6 represents a weakness in the technique of analysis, the correction of which involves programming a subjective decision into the computer used for analysis. The statistical analysis of the light beam interruptions for fish five revealed a positive test for abnormal movement when day 1 was compared to day 2. This positive test continued when day 1 was compared to day 3 and eventually lead to stress detection by the system when day 1 was compared to day 4 for fish five and abnormal movement was also recorded for fish 1 during the same time interval. This was a false stress detection which must occur only rarely if the system is to function properly. This type of response for fish five was perpetuated after the comparison of day 1 and 2 by the rules governing the handling of data after a positive test for abnormal movement had been recorded. This rule states that when two intervals are compared and a positive test for abnormal movement is recorded the data for the most recently recorded time interval is dropped and the next time interval is compared to the last interval in which normal movement was recorded. Obviously in this case the variance calculated from the data recorded for day 1 was not in line with those recorded for the successive time intervals. A programmable instruction to the computer to eliminate this weakness would be based on the following logic. If a positive test for heterogeneous variances is recorded for a single fish for x number of comparisons in a row, during time periods when no other analyses are indicating positive responses, then re-evaluate the analysis in which the first heterogeneous variance was recorded. The re-evaluation, in this case, would mean instead of dropping the estimate of variance recorded for day 2 as was done during the original analysis, drop the estimate for variance for day 1 and proceed with the comparisons. This was done for these data and the results showed homogeneous variances (normal movement patterns) for all subsequent comparisons involving data from fish number five. As noted in this discussion, x number of comparisons was used as opposed to some specific number. Specifying a number of comparisons at this point would be premature because of the limited number of instances in which the perpetuation of heterogeneity has occurred.

Experiment 8 (Table 4) was carried out to determine the effects of maintaining the test organisms for eight days under static test conditions without feeding. In this experiment the fish were handled as in the zinc addition experiments except that at the beginning of the fifth day, instead of adding zinc in solution, the equivalent amount of dilution water minus zinc was added to each test chamber. As the results show there does not appear to be any tendency toward increased heterogeneity over the eight-day test period.

The results from the continuous flow tests are presented in Tables 6

TABLE 4. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING STATIC EXPERIMENTS 5-8.

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4		Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
		Experiment No. 5 Goldfish 3.2 mg/1 Zn^{++}						
Fish				--Zn^{++} Added--				
1	0	0	0		0	0		
2	0	0	0		0	0	Experiment	
3	0	0	0		0	0	Terminated	
4	0	0	0		*	0(4vs6)	Power Failure	
5-C	0	0	0		0	0		
6	0	0	0		0	0		
		Experiment No. 6 Goldfish 3.2 mg/1 Zn^{++}						
1	0	0	0	--Zn^{++} Added--	*	0(4vs6)	0	*
2-C	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	*	*(1vs3)	*(1vs4)		*(1vs5)	*(1vs6)	0(1vs7)	*
6	0	0	0		0	0	0	0
		Experiment No. 7 Goldfish 1.8 mg/1 Zn^{++}						
1	0	*	0(2vs4)	--Zn^{++} Added--	0	0	*	0(6vs8)
2	0	0	0		0	*	0(5vs7)	0
3	0	0	0		0	0	0	0
4-C	0	0	0		0	0	0	0
5	0	0	0		0	0	0	0
6	0	0	0		0	0	0	0
		Experiment No. 8 Goldfish All Tanks Control						
1	0	0	0	--Zn^{++} Added--	0	0	0	0
2	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	*	*(2vs4)		0(2vs5)	0	0	*
6	0	0	0		0	0	0	0

TABLE 5. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING STATIC EXPERIMENTS 9-10.

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4		Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
			Experiment No. 9 Goldfish 3.2 mg/l Zn^{++}					
Fish				--Zn^{++} Added--				
1	0	0	*		0(3vs5)	*	0(5vs7)	0
2-C	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	0	0		0	0	*	0(6vs8)
6	0	0	0		0	0	0	*
			Experiment No. 10 Goldfish 3.2 mg/l Zn^{++}					
1-C	0	0	0	--Zn^{++} Added--	0	0	0	0
2	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	0	0		0	0	0	0
6	0	0	0		0	0	0	0

through 15. These data are reported as first half day values, second half day values, first half night values, and second half night values. The segment referred to as first half day values was from 7:00 a.m. to 1:00 p.m., but because data were recorded at half hour intervals during sunrise this interval includes seven record units on which the variance estimate for this interval was based. Second half day values include the time from 1: p.m. to 7:30 p.m. during which seven record units were made. The first half night values include the time from 7:30 p.m. to 1:00 a.m. and six record units, while the second half night values include the time from 1:00 a.m. to 7:00 a.m. during which six record units were made.

The results presented in Table 6 (Experiment 11) indicate stress detection occurred during a time period when no zinc was being added to the system, and would initially appear to be a false detection. However, the reason for this detection was traced to a breakdown in the chlorine neutralizing system. This breakdown probably occurred sometime during the morning or early after noon of day 4 and by early evening the chlorine content in the effluent from the test chambers reached 0.10 mg/1. The chlorine neutralizing system was repaired and by 12:00 a.m. on day 4 the effluents from the test chambers was < 0.05 mg/1. Because the fish had received this short term stress prior to the normal time for zinc addition no additional stress was applied. The experiment was run to normal termination to determine the recovery patterns of the fish. Once the chlorine stress was removed the return to normal movement patterns was quite rapid as can be seen from the lack of abnormal movement recorded for the second half night comparisons between day 3 and day 4.

The results presented in Tables 7 through 12 represent a series of experiments designed to determine the lowest concentration of zinc detected by the system under the conditions described. Each table includes a value for the calculated concentration of introduced zinc and in parentheses a mean measured concentration (atomic absorption).

The results from this series of experiments indicate that the lowest detectable zinc concentration based on measured concentrations is between 3.64 (Table 11) and 2.94 mg/1 Zn^{++} for a 96-hour exposure (Table 12).

Some of the experiments in this series presented special problems and interpretation and need further qualification. Tables 9 and 13 show the results obtained from two experiments in which problems with the recording equipment were encountered. These experiments are not consecutively numbered but were carried out consecutively. The problem was due to the loss of bias control in the main recorder, resulting in erroneous counts in those cells for which no data are given. This equipment malfunction was due to insufficient cooling in the main recorder, and the addition of a fan to cool electrical components eliminated the problem.

The results from experiment 15 (Table 10) indicate that stress detection occurred during the first half night values when day 4 was compared to day 5. Comparing this response time to stress detection with that for

TABLE 6. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 11. BLUEGILL ALL TANKS CONTROLS.

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values			
Fish							
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				Second Half Day Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	*	*(4vs6)	0(4vs7)	0
				First Half Night Values			
1	0	0	*	0(3vs5)	0	0	0
2	0	0	0	0	0	0	0
3	0	0	*	0(3vs5)	0	0	0
4	0	0	*	*(3vs5)	0(3vs6)	0	0
5	0	0	0	0	0	0	0
6	*	0(1vs3)	*	0(3vs5)	0	0	*
				Second Half Night Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0

TABLE 7. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 12. BLUEGILL 7.5 mg/l Zn^{++} (5.93 mg/l Zn^{++}).

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values			
Fish							
1-C	0	0	0	0	0		
2	0	0	0	0	*		
3	0	0	0	0	*	Experiment	
4	0	0	0	0	0	Terminated due to	
5	0	0	0	0	0	Power Failure	
6-C	0	0	0	0	0		
				Second Half Day Values			
1-C	0	0	0	0	0		
2	0	0	0	0	0		
3	0	0	0	0	0		
4	0	0	0	0	0		
5	0	0	0	0	0		
6-C	0	0	0	0	0		
				First Half Night Values			
1-C	0	0	0	0	0		
2	0	0	0	0	0		
3	0	0	0	0	*		
4	0	0	0	0	*		
5	0	0	0	0	0		
6-C	*	0(1vs3)	0	0	0		
				Second Half Night Values			
1-C	0	0	0	0	0		
2	0	0	0	*	0(4vs6)		
3	0	0	0	*	0(4vs6)		
4	0	0	0	*	0(4vs6)		
5	0	0	0	*	0(4vs6)		
6-C	0	0	0	0	0		

Zn^{++} Added

TABLE 8. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 13. BLUEGILL 7.5 mg/1 Zn^{++} (6.33 mg/1 Zn^{++}).

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4		Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values				
Fish								
1-C	0	0	0		0	0	0	0
2	0	0	0		*	0(4vs6)	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	-
5	0	0	0		0	0	0	-
6	*	0(1vs3)	0		0	0	*	*(6vs8)
				Second Half Day Values				
1-C	0	0	0		0	0	0	0
2	0	0	0		0	0	0	0
3	0	0	0		0	*	*(5vs7)	*(5vs8)
4	0	0	0		0	0	*	-
5	0	0	0		0	0	*	-
6	0	0	0		0	0	0	0
				First Half Night Values				
1-C	0	0	0		0	0	0	0
2	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	Dead	-
5	0	0	0		0	*	Dead	-
6	0	0	0		0	0	0	0
				Second Half Night Values				
1-C	0	0	0		0	0	0	0
2	0	0	0		*	*(4vs6)	*(4vs7)	*(4vs8)
3	0	0	0		*	0(4vs6)	0	*
4	0	0	0		0	*	Dead	-
5	0	0	0		0	*	Dead	-
6	0	0	0		0	0	0	0

Zn^{++} Added

TABLE 9. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 14. BLUEGILL 5.6 mg/l Zn^{++} (4.33 mg/l Zn^{++}).

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values			
Fish							
1	-	-	-	-	-	-	-
2	0	0	0	0	0	*	0(6vs8)
3	0	0	0	*	0(4vs6)	*	0(6vs8)
4	0	0	0	0	0	0	*
5	-	-	-	-	-	-	-
6-C	0	0	0	0	0	0	0
				Second Half Day Values			
1	-	-	-	-	-	-	-
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	-	-	-	-	-	-	-
6-C	0	0	0	0	0	0	0
				First Half Night Values			
1	-	-	-	-	-	-	-
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	*
4	0	0	0	0	0	0	0
5	-	-	-	-	-	-	-
6-C	0	0	0	0	0	0	0
				Second Half Night Values			
1	-	-	-	-	-	-	-
2	0	0	0	0	*	*(5vs7)	0(5vs8)
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	-	-	-	-	-	-	-
6-C	0	0	0	0	0	0	0

Zn^{++} Added

TABLE 10. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 15. BLUEGILL 5.6 mg/l Zn^{++} (3.87 mg/l Zn^{++}).

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4		Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values				
Fish								
1-C	0	0	0		0	0	0	0
2	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	0	0		0	0	0	0
6	0	0	0		0	0	0	0
				Second Half Day Values				
1-C	0	0	0	--Zn^{++} Added--	0	0	0	0
2	0	0	0		0	0	0	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	0	0		0	0	0	0
6	0	0	0		0	0	0	0
				First Half Night Values				
1-C	0	0	0		0	0	0	0
2	0	0	0		*	0(4vs6)	0	0
3	*	0(1vs3)	0		0	0	0	0
4	0	0	0		*	0(4vs6)	0	0
5	0	0	0		0	0	0	0
6	0	0	0		0	0	0	0
				Second Half Night Values				
1-C	0	0	0		0	0	0	0
2	0	0	0		0	*	0(5vs7)	0
3	0	0	0		0	0	0	0
4	0	0	0		0	0	0	0
5	0	0	0		0	0	0	0
6	0	0	0		0	0	0	0

TABLE 11. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 16. BLUEGILL 4.2 mg/1 Zn^{++} (3.65 mg/1 Zn^{++}).

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values			
Fish							
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	*	0(5vs7)	*
4-C	0	0	0	0	0	0	0
5	0	0	*	0(3vs5)	0	0	0
6	0	0	0	0	0	0	0
				Second Half Day Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4-C	0	0	0	*	*(4vs6)	*4vs7)	*4vs8)
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				First Half Night Values			
1	0	0	0	0	0	0	0
2	0	0	*	0(3vs5)	*	*(5vs7)	*(5vs8)
3	0	0	0	0	0	0	0
4-C	0	0	0	0	0	0	0
5	0	0	0	0	0	*	0(6vs8)
6	0	0	0	0	0	0	0
				Second Half Night Values			
1	0	0	0	0	0	0	0
2	0	0	0	*	0 (4vs6)	0	0
3	0	0	0	0	0	0	0
4-C	0	0	0	0	0	0	0
5	0	0	0	0	*	0(5vs7)	0
5	0	0	0	0	*	0(5vs7)	0
6	0	0	0	0	*	*(5vs7)	*(5vs8)

--Zn^{++} Added--

TABLE 12. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 17. BLUEGILL 3.5 mg/l Zn^{++} (2.93 mg/l Zn^{++}).

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values			
Fish							
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3-C	0	0	0	0	0	0	0
4	0	0	0	0	*	0(5vs7)	0
5	0	0	0	0	0	0	0
6	0	0	*	0(3vs5)	0	0	0
				Second Half Day Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3-C	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				First Half Night Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3-C	*	0(1vs3)	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				Second Half Night Values			
1	0	0	0	0	0	0	0
2	0	0	0	*	0(4vs6)	0	0
3-C	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0

Zn^{++} Added

TABLE 13. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING CONTINUOUS FLOW EXPERIMENT 18. BLUEGILL ALL TANKS CONTROLS.

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
			First Half Day Values				
Fish							
1	-	-	-	-	-	-	-
2	0	0	0	0	0	-	-
3	0	0	0	0	0	0	0
4	0	*	*(2vs4)	0(2vs5)	0	0	0
5	0	0	0	0	0	0	-
6	0	0	0	0	0	0	0
			Second Half Day Values				
1	-	-	-	-	-	-	-
2	0	0	0	0	0	-	-
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	-
6	0	0	0	0	0	0	0
			First Half Night Values				
1	-	-	-	-	-	-	-
2	0	*	*(2vs4)	0(2vs5)	0	-	-
3	0	0	0	0	0	0	-
4	0	0	0	0	0	0	-
5	0	0	0	0	0	-	-
6	0	0	0	0	0	0	-
			Second Half Night Values				
1	-	-	-	-	-	-	-
2	0	0	0	0	0	-	-
3	0	0	0	0	0	0	-
4	0	0	0	0	0	0	-
5	0	0	0	0	0	-	0
6	0	0	0	0	0	0	-

experiments 12 and 13 (Tables 7 and 8) in which the fish were exposed to 7.5 mg/l Zn^{++} shows that stress detection occurred in a shorter period of time at the lower concentration. The quicker response time at the lower concentration is probably the result of two factors. The resistance of different individuals of the same species of fish are known to be highly variable and this may have contributed to the more rapid response. However, more than likely it was the result of an attempt to meter toxicant into the continuous flow system during this experiment. The results of zinc analysis showed that the variance in concentrations reported for this experiment to be about 10 times greater than the variances reported for those experiments in which batch mixed zinc was used. The high variance in zinc concentrations was probably responsible for the comparatively early stress detection reported in this experiment.

Experiment 18 (13) shows the effects of maintaining fish under continuous flow test conditions for eight days without feeding. Although some data were lost near the end of this experiment the results indicate no tendency toward increased abnormal movement through time.

Tables 14-A, 14-B, 15-A, 15-B, and 15-C represent the results from two experiments in which the procedures for testing were modified to answer specific questions concerning the practical use of the monitoring system. In experiment 19 (Tables 14-A and 14-B) three basic questions were posed; (1) could the time period between the transfer of fish to the test chambers be reduced from the normal four-day period to two days, (2) what effects would feeding have on the results, and (3) what effects would a short term exposure to zinc over a six and one half hour period have on the movement patterns.

In this experiment the time interval between fish transfer and initiation of data recording was reduced to two days. The results show that the two-day readjustment period is probably sufficient. During this experiment the test fish were fed two pellets of Purina Trout Chow developer daily at 11:00 a.m. Based on the results obtained feeding had no detectable effect on the movement patterns recorded.

To answer the question concerning short term exposure to zinc, a batch mixed zinc solution of 10 mg/l Zn^{++} was started through the system at 7:00 a.m. on day 5 of the experiment. At 1:00 p.m. the flow was returned to the normal dilution water. The effluent samples collected at 1:00 p.m. showed the following zinc concentrations in mg/l Zn^{++}: Tank one, 7.88, tank two, 7.91; tank three, not detected; tank four, 7.88; tank five, 7.96; and tank six, 7.79. By 12:00 a.m. on day 5 zinc analyses showed all effluents contained < 0.5 mg/l Zn^{++}. The results from this short term exposure showed that during exposure or after there were no abnormal movement patterns recorded.

Experiment 20 (Tables 15-A, 15-B, and 15-C was run for a total of twenty days during which the effects of only two days' adjustment after handling, feeding during the experiment, and response to short term stress were

TABLE 14-A. STATISTICAL ANALYSIS OF LIGHT BEAM INTERUPTIONS RECORDED DURING DAYS 1-8 OF CONTINUOUS FLOW EXPERIMENT 19. BLUEGILL INTERMITTENT Zn^{++} STRESS.

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values			
Fish							
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3-C	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				Second Half Day Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3-C	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				First Half Night Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3-C	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				Second Half Night Values			
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3-C	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	*	0(1vs3)	0	0	0	0	0
6	0	0	0	0	0	0	0

Zn^{++} Added

TABLE 14-B. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING DAYS 8-10 OF CONTINUOUS FLOW EXPERIMENT 19. BLUEGILL INTERMITTENT Zn^{++} STRESS.

	Day 8 vs Day 9	Day 9 vs Day 10
		First Half Day Values
Fish		
1	0	0
2	0	0
3-C	0	0
4	0	0
5	0	0
6	0	0
		Second Half Day Values
1	0	0
2	0	0
3-C	0	0
4	0	0
5	0	0
6	0	0
		First Half Night Values
1	0	0
2	0	0
3-C	0	0
4	0	0
5	0	0
6	0	0
		Second Half Night Values
1	0	0
2	0	0
3-C	0	0
4	0	0
5	0	0
6	0	0

re-examined. In addition, during one segment of the experiment the fish were exposed to zinc until stress detection occurred. After stress detection flow was returned to the normal dilution water and percent survival and recovery patterns were examined.

The results given in Tables 15-A, 15-B, and 15-C agree with those presented in Tables 14-A and 14-B concerning both feeding during the experiment and the time between handling and data recording. In both cases the effects of these changes in procedure were not detected in the movement patterns. At 1:00 p.m. on day 7 of this experiment zinc flow was started into the test chambers and continued until 7:30 p.m. of the same day. The concentrations of zinc in mg/l recorded in the effluent at the time flow was returned to the normal dilution water were: tank one, 13.32; tank two, <.08; tank three, 11.39; tank four, 12.72; tank five, 13.32; and tank six, 12.59. By 8:30 a.m. on day 8 of this experiment the effluent zinc concentrations were less than 0.300 in all cases. As was the case in experiment 19, this short term stress did not cause stress detection during the period of zinc addition or for the period following zinc addition.

To determine the percent survival and recovery patterns of the fish once stress detection occurred, zinc flow was re-initiated at 1:00 p.m. on day 13 of this experiment (experiment 20). Between 8:00 and 9:00 p.m. on day 13 the zinc concentration reached a maximum of: 7.51 for tank one; less than .05 for tank two; 7.49 for tank three; 7.52 for tank four; 7.49 for tank five; and 7.54 for tank six. The concentrations remained at the above values until the statistical analyses showed stress detection during the first half night values on day 14. As soon as stress detection occurred the flow was returned to normal dilution water. At 10:00 a.m. on day 15 zinc analyses showed the following effluent concentrations: 0.70 for tank one, 0.09 for tank two; 0.62 for tank three; 0.57 for tank four; 0.67 for tank five; and 0.44 for tank six. Stress detection continued to be registered for two consecutive time intervals following the initial detection, but after that no stress detection was registered and the frequency of abnormal movement patterns returned to pre-stress levels within 48 hours. To determine how the light beam interruptions recorded for day 1 compared to those after the two stress periods, a comparison was made between day 1 and day 20. The results showed no abnormal movement patterns, indicating a complete recovery to pre-stress levels. This does not, however, mean that the fish would respond to a third stress period in the same way, nor does it indicate an increase or decrease in sensitivity. In practical application after stress detection the system should probably receive a new set of test fish, added two a day until a complete exchange of fish had been made. In this way the system need not be shut down, and the problems of increased or decreased resistance to stress need not become a serious consideration.

For the first ten days of experiment 20 from 1:00 p.m. to 7:00 p.m. the number of light beam interruptions occurring during consecutive ten-minute intervals was recorded manually for each photocell. The data

TABLE 15-A. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING DAYS 1-8 OF CONTINUOUS FLOW EXPERIMENT 20. BLUEGILL (Zn^{++} ADDITION TO STRESS DETECTION).

	Day 1 vs Day 2	Day 2 vs Day 3	Day 3 vs Day 4	Day 4 vs Day 5	Day 5 vs Day 6	Day 6 vs Day 7	Day 7 vs Day 8
				First Half Day Values			
Fish							
1	0	0	0	0	0	0	0
2-C	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	*	0(4vs6)	*	*(6vs8)
6	0	0	0	0	0	0	0
				Second Half Day Values			
1	0	0	0	0	0	0	0
2-C	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	*
5	0	0	0	0	0	0	0
6	*	0(1vs3)	0	0	0	0	0
				First Half Night Values			
1	0	0	0	0	0	0	0
2-C	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
				Second Half Night Values			
1	0	0	0	0	0	0	0
2-C	0	0	0	0	0	0	0
3	0	0	0	0	0	*	0(6vs8)
4	0	0	0	*	0(4vs6)	0	0
5	0	0	0	0	0	0	*
6	0	0	0	0	0	0	0

Zn^{++} Added

TABLE 15-B. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING DAYS 8-14 OF CONTINUOUS FLOW EXPERIMENT 20. BLUEGILL (Zn^{++} ADDITION TO STRESS DETECTION).

Fish	Day 8 vs Day 9	Day 9 vs Day 10	Day 10 vs Day 11	Day 11 vs Day 12	Day 12 vs Day 13	Day 13 vs Day 14
			First Half Day Values			
1	0	0	0	0	0	0
2-C	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0(6vs9)	0	0	0	*	0(12vs14)
6	0	0	0	*	0(11vs13)	0
			Second Half Day Values			
1	0	0	0	0	*	0(12vs14)
2-C	0	0	0	*	0(11vs13)	0
3	0	0	0	0	0	*
4	0(7vs9)	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
			First Half Night Values			
1	0	0	0	0	0	*
2-C	0	0	0	0	0	0
3	0	0	0	0	0	*
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
			Second Half Night Values			
1	0	0	0	0	0	*
2-C	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0(7vs9)	0	0	0	0	*
6	0	0	0	0	0	0

Zn^{++} Added

TABLE 15-C. STATISTICAL ANALYSIS OF LIGHT BEAM INTERRUPTIONS RECORDED DURING DAYS 14-20 OF CONTINUOUS FLOW EXPERIMENT 20. BLUEGILL (Zn^{++} ADDITION TO STRESS DETECTION).

	Day 14 vs Day 15	Day 15 vs Day 16	Day 16 vs Day 17	Day 17 vs Day 18	Day 18 vs Day 19	Day 19 vs Day 20
			First Half Day Values			
Fish						
1	*	0(14vs16)	0	0	0	0
2-C	0	0	0	0	0	0
3	*	0(14vs16)	0	0	0	0
4	0	0	0	0	0	0
5	*	0(14vs16)	0	0	0	0
6	0	*	*(15vs17)	0(15vs18)	0	0
			Second Half Day Values			
1	*	*(14vs16)	*14vs17)	0(14vs18)	0	0
2-C	0	0	0	0	0	0
3	0(13vs15)	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
			First Half Night Values			
1	0(13vs15)	0	0	0	0	0
2-C	0	0	0	0	0	0
3	0(13vs15)	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
			Second Half Night Values			
1	0(13vs15)	0	0	0	0	0
2-C	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0(13vs15)	0	0	*	0(17vs19)	0
6	*	*(14vs16)	*(14vs17)	0(14vs18)	0	0

collected during this ten-day period were used to indicate the desirability of reducing the time lag from the minimum five and one half hour period used in the previous analyses to one hour. The results of this initial limited analysis are sufficiently promising to warrant further study. The monitoring system as it presently exists is not sophisticated enough to monitor time intervals less than the hourly intervals readily handled by the camera recording system. However, plans are presently under way to completely automate the data acquisition and analysis components of the system through direct interfacing with a mini-computer. When complete automation is completed all time intervals and photocell levels can be thoroughly investigated.

The monitoring equipment used in these experiments is limited by turbidity. Turbidimetric determinations made under normal operating conditions showed that arroneous counts were registered when the turbidity of the water reached 15 APHA units with the 650 mu filters in place and 27 APHA units without the filters. This limited operating range presented no problems in the laboratory studies but would require careful consideration in field studies. The levels of turbidity over which the system could operate can be increased significantly by increasing the lamp output and providing better light beam-photocell alignment. Even with a significant increase in operating range there would be conditions under which effective measurements could not be made. The advantages gained from continuous monitoring by this method would have to be weighed against the potential loss of toxicity resulting from the reduction or removal of suspended material.

SECTION V

FISH REPRODUCTION AND GROWTH

Methods and materials: Bluegill sunfish were seined from a local pond and held for several months in the laboratory in the same dilution water, with the same photoperiod and water temperatures, as for the experiments described in Section IV.

Starting April 13, approximately 200 fish (approximate total lengths: 8-15 cm; weights: 10-80 gms.) were brought into breeding condition by exposing them to a photoperiod of 16 1/2 hours of light, water temperatures of 31-32°C, and by feeding them twice daily with frozen Gordon Formula (Axelrod, 1952) and once daily with live mealworms. The dimming system described earlier simulated a 1/2 hour dawn starting at 6 a.m. and a 1/2 hour dusk starting at 10 p.m.

On May 4 most of the fish could be sexed by gently squeezing the sides and observing whether eggs of milt was extruded, and three females and one male were placed in each of twenty 20-gallon tanks (standard aquaria, long type, Ramfab Aquarium Products cat. no. RA-20L).

One standard clay flowerpot (upper rim-to-rim diameter = 6 inches) was placed on its side in each tank for the females to hide from the aggressive attacks of the males. An artificial nest, described by Eaton (1970) was also placed in each tank and five smooth pebbles, 2-3 cm in diameter, were scattered on the bottom of each nest.

One toxicant delivery apparatus was used for each set of five tanks receiving one concentration, and one water delivery apparatus was used for five control tanks which received no added zinc. The toxicant delivery apparatus combined a toxicant dipper and needle valve described by Mount and Brungs (1967) with a water delivery system described by Brungs and Mount (1970). The dilution water was the same as that used in the experiments described in Section IV. The zinc concentrations for the reproduction study were based on the lowest concentration used in the fish breathing experiments; i.e. 2.5 mg/l. Tanks 6-10 received .250 mg/l zinc (1/10 of 2.5 mg/l), tanks 16-20 received .025 mg/l zinc (1/100 of 2.5), tanks 11-15 received no added zinc and served as controls. In addition, tanks 1-5 received 1/100 the 96-hour TL50 (median tolerance limit) for adult bluegill sunfish exposed to zinc in municipal tapwater: .075 mg/l. The initial zinc concentration used in the stock jug of each toxicant delivery apparatus was calculated to yield the desired concentration in the tanks. The concentrations in the tanks were measured by atomic absorption spectrophotometry and were lower than desired, so the concentrations of the stock solutions were adjusted until the correct concentrations were obtained in the tanks.

The flow rate to each tank was approximately 100 ml/min. The water entered at the top and front of the tank and was removed from the

bottom carrying some detritus with it, by means of a sheathed standpipe at the rear of the tank.

A plastic egg hatching box (20.5 cm long, 7.0 cm wide, and 15.5 cm deep) hung on the front of each aquarium and was large enough to accept three egg cups. The egg cups were made from Turtox plastic jars (5.5 cm o.d., 6.8 cm tall), with the bottoms removed. In use, each end of the cup was covered with a piece of ladies' woven nylon hose held in place with rubber bands. Each cup rested on an airstone cemented to the bottom of the hatching box. A piece of plexiglas (20 cm long, 6.3 cm wide, and 0.6 cm thick) fit into grooves in the hatching box and rested on top of the cups to keep them from floating.

Water siphoned from each tank into the hatching boxes and was returned to the tank by an air lift. Another air lift delivered water from the hatching box to a plastic rearing chamber (38.0 cm long, 30.5 cm wide, and 17.5 cm deep) for rearing newly-hatched fry. Thus the eggs were hatched and the fry reared in the same water as their parents. Water drained from each rearing chamber through a rectangular opening (8.8 cm wide, 1.8 cm high) into a trough. The bottom of the opening was 14.5 cm from the bottom of the pan, and the opening was covered by .8 mm mesh nylon netting.

The fish in each tank were fed two grams of frozen Gordon Formula (Axelrod, 1952) twice a day and eight live mealworms once a day. The tanks were cleaned once a week by siphoning detritus from the bottom.

At 1 p.m. every day, the pebbles in each nest were removed and examined closely for eggs. If eggs were present, a plastic chamber (same dimensions as above) was filled with water from the tank and the nest was removed from the tank and placed upside down in the chamber over an airstone. A new nest was substituted immediately for the old one.

A subsample of 200 eggs was removed from the nest and placed in a hatching cup, which in turn was placed in the hatching box. After 48 hours, the number of fry in both the egg cup and the chamber were counted, by pipetting them into petri dishes and using a Dazor model M209 fluorescent magnifier and a hand tally counter. The hatch in the subsample of eggs in the cup was assumed to be proportional to the hatch in the nest, and the numbers of fry and eggs in the cup were used to back-calculate the number of eggs spawned in the nest:

$$\text{Total No. Eggs In Nest} = \text{No. Fry In Nest} \times \left(\frac{\text{No. Eggs in Cup}}{\text{No. Fry in Cup}}\right) + \text{No. Eggs In Cup}$$

When less than 200 eggs were spawned, the number of eggs in the nest and the number of fry in the hatching chamber were counted directly, without removing a subsample.

Fish that were dead or that had lost their equilibrium were removed as soon as they were noticed. In addition, six fish had an eye disease that started as a white spot and gradually consumed the entire eye, and these fish were also removed. Fish that were removed before the end of the experiment were weighed, measured and sexed--unless they were too decomposed. Fish that were removed and could be sexed were replaced by a fish of the same sex from a stock of ripe fish kept in dechlorinated tapwater containing no added zinc, until July 20, when no further replacements were made. The breeding portion of the experiment terminated August 19, when all the remaining adult fish were killed, weighed, measured and sexed. The condition and weight of the gonads was also recorded.

Fifty fry from the first spawning in each tank were placed in the rearing chamber for that tank. Newly-hatched brine shrimp were rinsed in dechlorinated tapwater and placed in each rearing chamber twice a day for the fish to feed upon.

A census of the rearing chambers of July 8 revealed that very few fish were surviving, so changes were made in the apparatus and methods. Some of the fry may have washed through the netting of the rearing chambers, so the chambers were modified by drilling five 1.1 cm holes on centers 11.5 cm above the bottom of the chambers and covering them with a piece of woven nylon. All surviving bluegills were transferred to the modified chambers on July 8 and 9, and all fry hatched after July 9 were placed in chambers of the new design.

In addition, we learned that brine shrimp were too large for bluegill fry and that the National Water Quality Laboratory, Duluth, Minnesota, was successfully feeding plankton to baby bluegills (James M. McKim, III, personal communication). Consequently, starting July 23, plankton was obtained regularly from nearby ponds, and fed, after straining through a 0.8 mm mesh net, twice a day to the fish. Samples of the plankton were examined regularly under the microscope and never appeared to be very rich, so the diet was supplemented with a pinch of TetraMin powdered baby fish food twice a day. Newly-hatched brine shrimp were fed to the fish starting approximately the third week of growth.

Since no spawnings ever occurred in some tanks, the rearing chambers for these tanks received fry from other tanks. In addition to transfers of fry made within chambers at the same zinc concentration, some fry were taken from high zinc concentrations and put into chambers containing low concentrations, and vice-versa.

The fry in each chamber were counted and total lengths determined 30, 60 and 90 days after introduction to the rearing chamber. Total lengths were determined by placing each fish in a glass petri dish over a metric ruler.

Dissolved oxygen concentrations in the breeding tanks were determined by a YSI oxygen meter, and temperatures by a mercury thermometer. Since

water from the breeding tanks was delivered directly to the rearing chambers by air lifts, we assumed that the water characteristics in the tanks and chambers were the same. This assumption was confirmed for zinc by measuring zinc concentrations in the tanks and chambers at random intervals, but D.O. and temperature were not measured in the rearing chambers.

Results and discussion: Zinc concentrations, dissolved oxygen concentrations (D.O.), and temperatures in the breeding tanks are shown in Table 16. On August 4, 1971, a new central air conditioning system began operating, and the room temperature was lowered 5.6°C in 12 hours. As a result, the temperatures in the breeding tanks reached a new steady state, approximately 4°C lower than the mean temperatures recorded earlier. Since a spawning occurred for the first time in tank 9 after the drop in temperature, and six additional spawnings occurred in other tanks, the temperature drop did not seem to affect spawning. However, eggs from a spawning on August 4 that were exposed to a drop in water temperature of 12°C showed a very low percentage hatch (2%) and were not included in the results.

The dechlorinated tapwater delivered to the control tanks (tanks 11-15) contained a zinc concentration ranging from .002 - .062 mg/l. The mean zinc concentrations and the standard diviations in all the tanks are shown in Table 16. Nominal zinc concentrations will be used in the rest of the text.

Data on the adult bluegills used as breeders are shown in Table 17. Although males and females were approximately the same size when they were introduced to the breeding tanks on May 4, Table 17 shows that the surviving males were generally heavier and longer than the surviving females when the breeding portion of the experiment terminated August 19. Also, mortality among females was proportionally greater than it was among males. The disparity in growth and survival between males and females was probably due to biting and butting attacks by the males. Most of the dead females had tattered fins and scales missing from their sides. Females that were in tanks with very aggressive males would feed hesitantly, even when food was placed near their flowerpot shelters. Most of the fish at all zinc concentrations were still ripe on August 19, and there were no trends in adult fish weights, lengths, survival, or gonad weights that could be attributed to the effects of zinc.

The variation in female mortality from tank to tank probably indicates some variation in aggressiveness from male to male, and variation in aggressiveness among males may explain why there were some tanks at all concentrations where no spawning occurred (Table 18). For example, the male in tank 17 (a tank receiving .025 mg/l zinc) killed, or contributed to the death of eight females in succession, and no spawning ever occurred in this tank. Once a spawning did occur in a tank, it was highly likely that several more would occur. In contrast to the multiple spawnings obtained in the control tanks and tanks receiving zinc con-

Table 16. Zinc and dissolved oxygen concentrations, and temperatures in breeding tanks

		Measured zinc concentrations (mg/l)			D.O. (mg/l)			Temperature (^{o}C)		
Tank	Zn*	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
1	.075	14	.071	.043	5	6.1	0.5	4	30.8	1.0
2	.075	14	.081	.047	5	6.3	0.2	4	31.2	0.5
3	.075	14	.079	.039	5	5.9	0.7	4	31.6	0.5
4	.075	14	.076	.032	5	6.0	0.7	4	31.6	0.5
5	.075	14	.074	.028	5	5.8	0.5	4	31.6	0.5
6	.250	15	.231	.055	5	5.5	0.6	4	31.0	0.4
7	.250	15	.232	.048	5	5.3	0.7	4	30.6	0.8
8	.250	15	.230	.045	5	5.4	0.7	4	30.6	0.8
9	.250	15	.234	.043	5	5.1	0.9	4	30.1	0.9
10	.250	15	.249	.044	5	5.3	0.8	4	30.1	0.8
11	.000	14	.028	.023	5	5.5	0.6	4	30.5	0.9
12	.000	14	.019	.007	5	5.7	0.5	4	30.5	0.6
13	.000	14	.019	.010	5	5.5	0.6	4	30.5	0.7
14	.000	14	.020	.014	5	5.7	0.5	4	30.5	0.9
15	.000	14	.017	.011	5	5.4	0.8	4	30.4	0.9
16	.025	14	.028	.012	5	5.6	0.7	4	30.5	0.6
17	.025	14	.040	.019	5	5.4	0.7	4	30.4	0.5
18	.025	14	.041	.018	5	5.8	0.5	4	30.4	0.5
19	.025	14	.035	.017	5	5.7	0.5	4	30.4	0.8
20	.025	14	.033	.012	5	5.4	0.7	4	29.9	0.5

* Nominal zinc concentrations (mg/l)

N-number of readings

Table 17 Survival of adult bluegills and weights, lengths, and condition of gonads of adults at end of breeding experiment

Tank	Zn (mg/l)	[a]No. males removed before end of experiment	[a]No. females removed before end of experiment	Males		
				Wts. (gms)	Standard lengths (cm)	No.
1	.071	0	*3	61.8	11.5	1
2	.081	0	4	69.6	11.5	1
3	.079	*1	0	48.2	10.5	1
4	.076	0	*1	60.9	11.6	1
5	.074	0	1	37.6	10.2	1
6	.231	0	5	69.7	12.1	1
7	.232	0	0	71.7	12.0	1
8	.230	0	2	34.0	10.0	1
9	.234	0	3	62.7	11.7	1
10	.249	0	0	26.1	8.9	1
11	.028	0	4	61.3	11.7	1
12	.019	1	1			0
13	.019	0	3	78.4	11.9	1
14	.020	0	*2	67.6	12.1	1
15	.017	0	*2	72.6	12.3	1
16	.028	0	0	72.5	12.3	1
17	.040	0	*9	70.0	11.8	1
18	.041	0	2	93.4	12.9	1
19	.035	0	2	84.8	13.0	1
20	.033	0	5	31.6	9.6	1

*One fish removed because eye was diseased. No more than one fish per tank contracted this disease.

[a]Fish with eye disease, dead fish, and fish that had lost their equilibrium were removed immediately. A ratio of three females and one male was maintained per tank by replacement of fish until July 20, 1971.

[b]Mean values (with ranges in parentheses) are shown for tanks containing more than one female.

Fish removed at end of experiment

Gonads		Females[b]			Gonads	
Wt(gms)	Condition[c]	Wts. (gms)	Standard lengths(cm)	No.	Wt(gms)	Condition[c]
1.2	R	13.1	7.2	1	1.6	1R
1.5				0		
0.9		24.2(16.6-33.6)	9.1(8.5-9.8)	3	1.6(0.1-2.6)	2R, 1N
1.7		31.2(23.0-39.4)	9.6(8.9-10.2)	2	2.3(1.3-3.3)	2R
< .1	N	29.4(23.7-35.0)	9.6(9.4-9.7)	2	2.8(1.5-4.1)	2R
1.0				0		
1.2		29.1(22.0-35.4)	9.4(8.3-10.0)	3	1.3(0.5-2.2)	3R
0.2		44.6(38.3-51.0)	10.4(10.0-10.8)	2	2.7(0.6-4.8)	1R, 1N
0.9	R	35.2(29.2-41.3)	9.9(9.5-10.3)	2	2.4(2.3-2.6)	2R
0.5		36.2(25.2-46.4)	9.7(9.0-10.3)	3	3.2(2.4-4.1)	3R
0.8	R			0		
		31.7(24.6-35.2)	9.5(9.0-9.8)	4	2.6(2.0-3.3)	4R[d]
0.9	R	38.4(34.2-42.7)	10.1(9.6-10.6)	2	3.0(3.0-3.1)	2R
1.6		39.1	10.3	1	1.7	1R
1.0	R	21.6	8.7	1	0.6	1R
0.8	R	28.6(28.1-29.0)	9.5(9.3-9.7)	2	1.6(1.3-1.9)	2R
2.1	R			0		
1.0	R	13.4	7.7	1	0.1	1N
1.0		26.8	9.3	1	2.9	1R
< .1	N	20.6(7.8-32.1)	8.5(6.6-9.7)	4	1.6(0.4-3.5)	4R[d]

[c]R = ripe, and indicates that milt was extruded when the sides of the males were squeezed; or in the case of females, that the ovaries were swollen and filled with pinhead-size eggs. N = not ripe, and indicates that the testes weighed less than .1 gm and no milt was extruded from males; in the case of females, no eggs were visible. The number of ripe and unripe females in each tank is shown.

[d]An extra female was added to tanks 11 and 20.

centrations of .075 and .025 mg/l, however, only a single spawning in one tank occurred at a concentration of .250 mg/l zinc (Table 18). Since eggs or milt could be extruded from all the breeders at the beginning of the experiment, and since most of the fish had ripe gonads when the experiment terminated, the results in Table 18 indicate that a zinc concentration of .250 inhibits spawning in ripe fish.

Table 18 also shows the percentage hatch in each tank. Where more than one hatching was used, the mean percentage hatch is shown, with the range in parentheses. Hatching data from eight spawnings were not used because some of the eggs were hatching in the breeding tanks in less than 24 hours at temperatures of 30-31 C. Attempts to remove eggs from these nests by pipetting generally caused the egg membranes to rupture, releasing the fry. In these cases, the percentage hatch in 48 hours of the eggs that were transferred without repturing was confounded with the 48-hour survival of the fry that were also unavoidably transferred to the egg hatching cups. A hatch of 21% from one spawning in tank 16 that was heavily fungused was also excluded. The hatch obtained from the one spawning at the highest zinc concentration was low (43%), but within the range of values in the other zinc concentrations.

The number of fry introduced to the old rearing chambers with 0.8 mm mesh outlets and the number introduced to the modified chambers with smaller mesh outlets are shown in Table 19. The introductions are shown in chronological sequence from left to right across the rows. For example, two introductions of fry were made to rearing chamber 4: one introduction of 50 fry from tank 4, and a later introduction of 51 fry from tank 1, after inspection revealed that there were no survivors from the first introduction. Growth and survival data in the right-hand portion of the table were always obtained on fish from the last introduction reported in the left side of the table. Survival of young bluegills at all zinc concentrations was poor. Mortality was highest during the first weeks, and can probably be attributed to starvation. The plankton collections that were fed to the young bluegills starting August 24 were never very rich, and the powdered baby fish food may not have been utilized. Once the young were large enough to feed on brine shrimp, survival improved as shown by the reduction in mortality between 30, 60 and 90 days as compared to the mortality between day 0 and day 30. At a zinc concentration of .250 mg/l, however, no bluegills survived longer than 30 days. Fry obtained from eggs spawned in a zinc concentration of .250 mg/l and fry obtained from eggs spawned in other zinc concentrations were placed in .250 mg/l zinc. Most of the fry from all these sources died within three days in 250 mg/l zinc and were visible on the bottom of the rearing chambers. In addition, fry taken from .250 mg/l zinc and placed in control tank 11 showed poor survival: only four fish survived for 30 days, one for 60 days, and none for 90 days.

An experiment on the effects on young bluegill of momentary exposure to a high zinc concentration was inadvertently conducted when a hose

Table 18. Spawning of adult bluegills and percentage hatch of eggs at four zinc concentrations

Tank	Mean zinc concentration	Total no. of eggs spawned[a]	Total no. of spawnings	Mean no. of eggs per spawning[a]	Percentage hatch[a, b]
1	.071	8414	3	2805	72 (71-72)
2	.081	0	0	0	0
3	.079	10647	8	1331	66 (49-78)
4	.076	4736	5	1184	57 (44-68)
5	.074	0	0	0	0
6	.231	0	0	0	0
7	.232	0	0	0	0
8	.230	0	0	0	0
9	.234	1009	1	1009	43
10	.249	0	0	0	0
11	.028	7188	4	1797	62 (35-76)
12	.019	0	0	0	0
13	.019	0	0	0	0
14	.020	227	2	114	33
15	.017	5849	7	985	73 (47-96)
16	.028	4274	3	1425	86
17	.040	0	0	0	
18	.041	0	0	0	
19	.035	10202	5	2040	78 (65-90)
20	.033	0	0	0	

[a]Number of eggs and percentage hatch were not determined for all spawnings because of premature hatching, fungus infestation, etc. (see text).

[b]Where more than one hatch was used, the mean percentage hatch is shown, with the range in parentheses.

Table 19. Survival and growth of bluegills in four zinc concentrations

Rearing chamber	Zn mg/l	No. of fry introduced to old rearing chambers	No. of fry introduced to new rearing chambers	Mean total lengths (mm) Days 30	60	90
1	.071	50[1][a]	50[1]	21.8(5)[b]	28.8(5)	38.8(5)
2	.081		50[4]	13.2(4)	20.3(3)	34.3(3)
3	.079	50[3]	50[3],45[19]	(0)	(0)	(0)
4	.076	50[4]	51[1]	21.0(4)	24.5(4)	36.6(4)
5	.074		50[3]	12.2(19)[c]	19.2(18)	26.9(14)
6	.231	50[15],51[15]	53[9]	(0)	(0)	(0)
7	.232		58[9]	(0)	(0)	(0)
8	.230	50[11]	50[1],58[19]	(0)	(0)	(0)
9	.234	50[11]	51[9]	(0)	(0)	(0)
10	.249	50[15]		(0)	(0)	(0)
11	.028	50[11]	50[11],51[9]	6.8(4)	10.0(1)	(0)
12	.019					
13	.019					
14	.020		50[15],40[14]	9.5(2)	21.2(2)	27.8(2)
15	.017	50[15]		- - -[d]	20.0(2)	33.0(1)
16	.028	50[16]	56[16]	(0)	(0)	(0)
17	.040					
18	.041		51[19]	(0)	(0)	(0)
19	.035	50[19]	50[19]	14.4(7)	19.3(6)	30.6(6)
20	.033					

[a] Numbers in brackets indicate the number of the tank where the fry were obtained.
[b] Numbers in parentheses indicate the number of fish.
[c] At least 2 fish died as a result of a zinc spill on day 22 (see text).
[d] The fish in chamber 15 were not counted or weighed on day 30.

separated from a connector and fell into rearing chamber 5 while a stock bottle was being filled with concentrated zinc solution. The hose was removed from the tank in a fraction of a second and the young bluegill were transferred to the proper zinc concentration within two minutes, but two of the 21 fish died within 15 minutes. During the two minute period, the 22-day-old bluegills had been exposed to a zinc concentration of 9.18 mg/l, although the concentration initially may have been higher in portions of the chamber. There were 19 survivors on day 30, 18 on day 60, and 14 on day 90. After the initial deaths, survival in chamber 5 was comparable to survival in other tanks at the same concentrations.

In summary, it appears that a zinc concentration of .250 mg/l inhibits spawning in adult bluegills brought into breeding condition in dechlorinated municipal water containing no added zinc, and causes complete mortality of bluegill fry. Water containing no added zinc and zinc concentrations of .075 and .025 mg/l do not have these effects.

The lowest zinc concentration tested in the apparatus for monitoring fish breathing was 2.55 mg-l. This concentration was detected by the monitoring method, and the reproduction and growth experiment shows that 1/100 or 1/34 of this concentration might be safe for chronic exposure of bluegills, but that 1/10 of this concentration certainly is not.

SECTION VI

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SECTION VII

LITERATURE CITED

American Public Health Association. 1965. Standard Methods for the Examination of Water and Wastewater. 12th ed., 769 pp. New York.

Axelrod, J. H. R. 1952. Tropical Fish as a Hobby. George Allen and Unwin, Ltd. London. 264 pp.

Brett, J. R. 1968. Personal communication.

Brungs, William A. 1969. Chronic toxicity of zinc to the fathead minnow, Pimephales promelas Rafinesque. Trans. Amer. Fish. Soc. 98(2): 272-279.

Brungs, W. A., and D. I. Mount. 1970. A water delivery system for small fish-holding tanks. Trans. Amer. Fish. Soc. 99(4): 799-802.

Cairns, J., Jr. 1970. New concepts for managing aquatic life systems. Jour. Water Poll. Contr. Fed. 42(1): 77-82.

Cairns, J., Jr., K. L. Dickson, Richard E. Sparks, and William T. Waller. 1970. A preliminary report on rapid biological information systems for water pollution control. Jour. Water Poll. Contr. Fed. 42(5): 685-703.

Cairns, Jr., Jr. (in press). Management problems in multiple use of aquatic ecosystems. River Ecology and the Impact of Man. Clarence A. Carlson (Ed.).

Davis, R. E. and J. E. Bardach. 1964. Time co-opdinated prefeeding activity in fish. Animal Behaviour XIII (1): 154-162.

Eaton, S. G. 1970. Chronic malathion toxicity to the bluegill (Lepomis macrochirus Rafinesque). Water Research. 4: 673-684.

Moore, J. G., Jr., Commissioner. 1968. Water Quality Criteria. Report of the National Technical Advisory Committee to the Secretary of the Interior. U.S. Govt. Printing Office. 234 pp.

Mount, Donald I. and Charles E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish- Malathion and the butoxyethanol ester of 2,4-D. Trans. Amer. Fish. Soc., 96(2): 185-193.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research. 1: 21-29.

Mount, Donald I. 1968. Chronic toxicity of copper to fathead minnows (Pimephales promelas Rafinesque). Water Research, 2: 215-223.

Scheier, A. and J. Cairns, Jr. 1966. Persistence of gill damage in Lepomis gibbosus following a brief exposure to alkyl benzene sulfonate. Not. Nat. Acad. Nat. Sci. Philadelphia, No. 391: 1-7.

Shirer, H. W., J. Cairns, Jr., and William T. Waller. 1968. A simple apparatus for measuring activity patterns of fishes. Water Resources Bull. 4(3): 27-43.

Sokal, R. R. and F. James Rohlf. 1969. Biometry. W. H. Freeman and Co. 776 pp.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. Water Research. 3: 793-821.

Sparks, Richard E. (in prep.). Using the respiratory and cardiac responses of bluegills (Lepomis macrochirus Rafinesque) to monitor zinc concentrations in water.

Spoor, W. A., T. W. Neiheisel, and R. A. Drummond. 1971. An electrode chamber for recording respiratory and other movements of free-swimming animals. Trans. Amer. Fish. Soc. 100(1): 22-28.

Train, R. E. (Ed.). 1970. The First Annual Report of the Council on Environmental Quality. U.S. Govt. Printing Office. 326 pp.

SECTION VIII

PUBLICATIONS

Sparks, R. E., W. T. Waller, J. Cairns, Jr. and A. G. Heath. 1970. Diurnal variation in the behavior and physiology of bluegills (Lepomis macrochirus Rafinesque). The ASB Bulletin. 17(3): 90 (Abstract).

Cairns, J., Jr., R. E. Sparks and W. T. Waller. 1971. The relationship between continuous biological monitoring and water quality standards for chronic exposure. American Chemical Society, Division of Water, Air and Waste Chemistry. Preprints of Papers Presented at the 162nd National Meeting. 11(2): 55-62.

Cairns, J., Jr., R. E. Sparks and W. T. Waller. 1971. The relationship between continuous biological monitoring and water quality standards for chronic exposure. Abstracts of Papers. 162nd National Meeting, September 12-17, 1971. Washington, D. C. WATR division, abstract no. 19.

Cairns, J., Jr., R. E. Sparks and W. T. Waller. 1971. (manuscript in press) The relationship between continuous biological monitoring and water quality standards for chronic exposure.

Cairns, J., Jr., R. E. Sparks and W. T. Waller. 1971. (manuscript in press) The use of fish as sensors in industrial waste lines to prevent fish kills.

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16. Abstract The feasibility of using fish movement patterns measured by light beam interruption as a technique for continuous monitoring of the response of fish to zinc was investigated. In conjunction with the monitoring studies the growth and reproductive success of the Bluegill sunfish (Lepomis macrochirus) exposed to various fractions of the lowest concentration of zinc detected by the monitoring apparatus were studied. The monitoring apparatus does not in any way interfere with fish movement within the test chamber and allows for the maintenance of fish for long time periods. Under the conditions described the system detects premortal aberrations in fish movement caused by zinc. The detection of stress occurs in sufficient time to permit survival of the test fish if stress conditions are reversed at the time of detection. The lowest concentration of zinc detected by the system during a 96-hour exposure was between 3.64 and 2.94 mg/l Zn^{++}. The system's range of effective measurement as related to turbidity is discussed. This method should detect other toxicity equally well. The growth and reproductive success of the bluegill was tested in concentrations approximately equal to 1/10 and 1/100 the lowest concentration of zinc detected by the monitoring system and 1/100 of the 96 hour TL50 (median tolerance limit) determined under continuous flow conditions. The growth and reproductive success in 1/100 the lowest detected zinc concentration and 1/100 the 96 hour TL50 value did not differ appreciably from the controls while a concentration of approximately 1/10 the lowest detected zinc concentration in effect eliminated reproduction in the bluegill.

17a. Descriptors

*Water pollution control, *Industrial wastes, *Bioindicators, Fishkill, Fish physiology

17b. Identifiers

*Biological monitoring, Bluegill, Zinc, Lepomis macrochirus Rafinesque

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