THE DEVELOPMENT OF CONTINUOUS AUTOMATIC BIOLOGICAL MONITORING SYSTEMS FOR WATER QUALITY CONTROL

by

William Stephen Gilbert Morgan
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Department of Zoology,
UNIVERSITY OF CAPE TOWN.

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DECLARATION OF CANDIDATE

I, William Stephen Gilbert Morgan, declare that this thesis is my own work and that it has not been submitted for a degree at another University.

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automatic - self acting, having the power of action within itself.

biological - relating to the science of physical life, dealing with organized beings or animals or plants; their morphology, physiology, origin and distribution.

continuous - uninterrupted in time, sequence or essence.

monitoring - pertaining to something that reminds or gives warning.

SHORTER OXFORD ENGLISH DICTIONARY

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SYNOPSIS

During the past decade, South Africa has experienced an unprecendented degree of industrial expansion. Although this has enhanced the material wealth and personal comfort of all the nations' peoples, it has also produced an undesirable consequence - ever increasing pollution of the aquatic environment. Viable systems for continuously monitoring water quality are, therefore, of critical importance for the future management and use of our watersheds.

The value of regional monitoring programs using physical and chemical measurements is already well established. 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 such as dissolved oxygen, pH, temperature and conductivity. Further, it is difficult, if not impossible, to predict the biological effects of a complex continuously changing industrial effluent from chemical analyses alone. A possible solution to these problems involves the use of biological monitoring techniques to complement physico-chemical ones. It is important, however, that such techniques are able to provide a continuous assessment of water quality and automatically indicate when deleterious conditions are developing. A biomonitoring system should, therefore, provide continuous information on the effects of industrial effluents on aquatic organisms, prevent industrial spills from damaging aquatic ecosystems and help prevent expensive over-treatment of industrial waste when a lesser degree of treatment would suffice.

Previous research has shown that abnormal responses appear in the breathing and activity of fish exposed to acute and sub-lethal pollution. The intention of this study has been to design means by which these responses may be continuously measured in such a way as to provide information which may be fed to an automatic data analysis system which would provide notice of developing toxic conditions.

Several techniques have been utilized to monitor fish breathing rate. The method found to be simplest and causing the least stress on fish was to monitor the electrical signals generated by the opercular muscles

during ventilatory movements by means of dual external electrodes affixed to opposite ends of a test chamber. With sufficient amplification this electrical signal was recorded using an oscilographic recorder. Initially breathing rate was analyzed visually from the records, a procedure which required a great deal of time and may have introduced subjectivity into the analyses. These problems were alleviated by the development of a fully automated electronic system wherein the electrical signals elicited by sensor fish, in individual chambers, were monitored by an apparatus that produced a d.c. voltage proportional to the breathing rate every minute.

Measurement of total fish activity was also accomplished with little stress on sensor fish. Two independent techniques were employed. Initially locomotor behaviour was monitored by recording the breaks, by a single fish, of light beams traversing a test chamber. Data were stored in mechanical counters which were reset every hour. The light beams were found to have an adverse effect upon natural diurnal variation and, therefore, a second generation system used infra-red beams to provide data. Automation of the system was achieved when data from electronic counters was stored and fed to a printer at hourly intervals. Certain difficulties were experienced with the system which made it impractical for use in industrial situations. The light beam arrangement restricted ones choice of sensor, beams were blocked in turbid water and the precise alignment of light source and light detector was upset by slight disturbances.

A monitoring system which sensed fish locomotory activity by means of ultrasonic echoes was found to be an improvement in all respects. Not only was it more stable and, therefore, more acceptable for use in an industrial environment, but it also functioned successfully in detecting toxicity in the marine environment. This was not possible using dual external electrodes to detect breathing rate, as high salt concentrations prevented the transmission of the electrical signals. The system operated on the Doppler effect. An ultrasonic sound wave pattern was set up in a test chamber by a transmitter. A receiver detected any shift in frequency of the sound waves caused by fish movement within the chamber. This frequency shift, after mixing in the receiver with other reflections, caused an amplitude modulation of the total signal. The

modulation was processed and interfaced with an automatic counter which advanced every time the fish moved. The activity count was totalled every hour and recorded, in digital form as a printout, for analysis.

All fish species, employed as sensors in the biomonitoring systems, elicited two normal sources of variation in both breathing rate and locomotor activity: diurnal and individual variation. Both factors were taken into account in developing appropriate methods for data analysis. Variations between individual sensors made it advisable, in all experimental series, to use several individuals and to have each serve as its own control. Breathing and activity rates were also found to be different at different times of the day in individual sensor fish. Critical rates were, therefore, established, for each sensor fish, for each hour of the day during a standarization period of between five and seven days before any experimental toxicant addition. Control data obtained during this period, after acclimation to test conditions, was used to generate confidence intervals by which abnormal responses could subsequently be detected. An upper critical limit was calculated for the 95% level, and any individual fish was deemed to show a response when the breathing or activity rates, observed in the test period, exceeded the corresponding critical limits. A detection of toxicity was defined as responses by a majority of the test fish (60%) within an arbitrarilly set period, usually 24 h, subsequent to toxicant addition. This level of detection was built into all monitoring systems in such a way that responses by a majority of sensors activated alarm signals.

Laboratory simulations showed that the biomonitoring systems would effectively detect acute and sub-lethal concentrations of a number of toxicants, and would do so in sufficient time to protect fish in receiving ecosystems. The sensitivity of the systems depended largely on the species of sensor fish used, the toxicant concentration as well as its mode of action. Loss of sensitivity, to certain toxic effects, occurred following long-term exposure to very low levels of toxic materials. Decreased system sensitivity during night-time was most likely a statistical effect since respiration and activity rates were lower and less variable at that time, the elapsed time between introduction of a toxic effect and response at maximal critical levels being artificially extended. In both cases, however, the losses in sensiti-

vity were not so great that ecosystem protection would be seriously impaired. On the other hand, the systems proved to be incapable of detecting chronic levels of cummulative toxicants rapidly enough to prevent inhibitory effects upon growth and reproduction of species in receiving waters, and this fact must be taken into account when such biomonitoring systems are utilized at industrial in-plant situations.

This study has shown that biological monitoring systems can give warning of potentially hazardous conditions which may arise in industrial effluents being discharged to aquatic ecosystems, as well as detecting toxic conditions that may pass physical sensors undetected. Further, they would provide continuous, immediate information on the toxicity of effluents to aquatic organisms. Therefore, it appears likely that biological monitoring will develop and techniques for automatically analyzing data, and even decision making, will make it feasible to use such systems to help control water quality in industrial effluents and watersheds.

SECTION I

GENERAL INTRODUCTION
AND
ANCILLARY INFORMATION

GENERAL INTRODUCTION

"And the fish that was in the river died;
and the river stank, and the Egyptians
could not drink of the water of the river."

Exodus, chap. 7, v 21.

Contamination of the environment is an inevitable consequence of the activities of man. The deposition, by both industrial and agricultural societies, of waste material into the environment has been both *historically* and *socially* a characteristic of mans' activities.

Historically, throughout the development of the industrial age, waste products of manufacturing processes have been returned to the earth. The maintenance of mans' material wealth and personal comfort has thus produced an undesirable consequence - ever increasing contamination. The communal activities of man as a social animal produces by-products which have grown in volume at a faster rate than population. These by-products are concentrated by mans' tendency to congregate in cities and towns, resulting in extensive contamination surrounding mans' habitations to a degree where natural purification systems are becoming overstressed.

Although contamination, being simply a consequence of living, is neither right nor wrong; excessive contamination, produced by industrial processes, which interferes with the health and happiness of a population is *pollution*, and not only is this wrong - it is immoral.

Most polluting materials will eventually find their way into some phase of the hydrologic cycle, thereby having some impact on the aquatic environment. It is imperative that the effect of these materials on the environment is minimized. Good industrial and water management practice dictates that waste materials be discharged into a receiving water in such a way that nature's ability to assimilate these wastes is utilized without any deleterious effects on water quality. Obviously, the ability of surface waters to accept waste materials without causing harmful effects must be considered as a beneficial use of water. To do otherwise can only result in a higher cost to the user and ultimately to a lower standard of living for society. If we are to maintain our present standard of living and economic growth, the water we do have must be adequately managed.

The capability of successfully managing the aquatic environment for its many uses depends, to a great extent, upon the ability to predict the effects of waste products upon the environment. Both industrial and water management personnel require information on the general quality of a waste being discharged at a fairly constant rate, and also must be warned of any extreme variations in general quality which would adversely effect the receiving system. For example, many of the fish kills which have resulted from the release of slugs of highly toxic substances could have been prevented had these conditions been detected before the waste had entered the aquatic environment.

The control and treatment of such wastes, for safe release into receiving waters, does present industrial personnel with problems of considerable magnitude. Much effort has been expended by some industries to solve this problem, primarily through chemical analyses of their effluents. Unfortunately, many wastes are chemically complex and the necessary techniques for separating individual constituents and analytic methods for determining all components have not been developed. Further, materials may be toxic at concentrations which chemical methods are not sensitive enough to detect. As well as the, often appreciable, delay between the sampling of a waste and its analysis, chemical monitoring has a further disadvantage in that it cannot appreciate the probable harmful effects of pollutants which may either increase or decrease the toxicity of others. These phenomena of synergism and antagonism require a large degree of expert assessment even when the concentrations of individual pollutants are known.

In recent years the problems of discontinuity associated with chemical sampling and analysis have been overcome, to some extent, by the development of automatic monitoring equipment employing physical and chemical sensors. The value of such equipment installed in industrial environments and field situations is being increasingly recognised. However, despite these advances, the range of pollutants which may be detected, by automatic monitors of this kind, remains small. At the same time the variety of potential pollutants has rapidly increased due, in no small measure, to advances in the chemical manufacturing industry. Therefore, a clear need remains for a system which has the ability to monitor on a broad basis for unusually high concentrations of a wide range of pollutants. It is impractical and economically not feasible to do this by means of specific

sensors, even if they existed. In seeking to develop a broad spectrum sensor for monitoring wastes the most obvious system to consider is one which employs such a sensor.

For centuries, fish have been recognised as being widespread and important indicators of water quality. Some progressive industrial plants have met the need to determine effluent quality by installing aquaria in which fish are exposed to the plant effluent on a continuous flow-through basis. A "satisfactory" effluent quality is demonstrated by the survival of the fish. This process is biomonitoring (Jackson and Brungs, 1966).

The standard fish bioassay, which also uses death as a response, is used by many organizations to predict the toxicity of industrial wastes. It has been suggested (Henderson and Tarzwell, 1957) that such tests solve the problems outlined above as they may be used to evaluate directly the toxicity of chemically complex wastes. In many cases it may provide the quickest and most economical approach to identifying and solving waste disposal problems. One limitation of the standard bioassay is that it uses a grab sample which represents the quality of a waste at a single point in time. The dilution water is also taken at one point in time. At an actual industrial site, the quality of both waste and river water vary *through* time. A composite sample partially overcomes this limitation, but may mask variations that are biologically important. The placing of fish in a continuous flow of waste diluted with river water would obviate the problem, but then there is one further limitation of the standard bioassay: death is used as the response. In order to prevent damage to the aquatic ecosystem, it is necessary to have an early warning of dangerous conditions, so that corrective action may be taken. Accordingly, symptoms of ill health, which occur before death, must be detected to allow time for diagnosis and treatment. In this respect biologists have been somewhat dilatory in developing systems comparable with those now in use which automatically and continuously monitor physical and chemical parameters.

In order to function successfully and usefully in combination with modern physical and chemical techniques, a biological system, which monitors pre-mortal physiological or behavioral symptoms, must meet certain requirements. The sensor animals should respond to the greatest number of toxic substances, and be able to be maintained within the testing system for long

periods of time without change in behaviour, metabolism or sensitivity. The test organisms, their reaction to toxic substances and the measuring method must be suited to continuous and automatic recording whereby, in cases of emergency, a warning system will be put into operation. It is advisable that the method should be very simple so that it can be applied in both industrial and field situations without using specially trained personnel.

This study describes the investigative procedures, feasibility analyses and developmental aspects involved in the production of continuous automatic biological monitoring systems, using fish as sensors, which meet the above requirements.

A CONSTANT FLOW APPARATUS FOR LONG TERM TESTS ON FISH PHYSIOLOGICAL AND BEHAVIOURAL RESPONSE TO INTOXICATION

Automatic biological systems established to monitor surface waters or industrial effluents should use water organisms present in and exposed to a continuous flow of the water being monitored. Control of such parameters in the research laboratory, so that they relate to field conditions, cannot be obtained using static tests. Further, in order to examine fish physiological and behavioural responses to the effects of both lethal and sublethal toxicant concentrations, a high rate of flow needs to be established through experimental aquaria and a constant level of toxicant must be maintained. Therefore, some kind of proportionating device is necessary to enable a concentrated toxic solution to be mixed in uniform proportion with dilution water.

Recent years have seen the advent of a number of mechanical devices for regulating flow of test water and/or toxicants. Mount and Brungs (1967) described a "proportional" diluter, simplified from an earlier serial diluter (Mount and Warner, 1965). Both have long-term dependability and failsafe characteristics, but successive test toxicant concentrations are somewhat dependant upon each other in magnitude. Other designs are simpler but are either not as fail safe (Burke and Ferguson, 1968), or offer little advantage over a simple Mariotte bottle (Solon *et al.*, 1968).

An automatic dosage apparatus developed by Abram (1960) was found inconvenient for the present study, being very fragile and requiring specialized glass blowing for its construction. A comparable apparatus described by Grenier (1960) may be criticized on the grounds that it is difficult to adjust.

The device created for the present study (Plate 1), being an extension of an appliance designed by Stark (1967), is sturdy and is assembled primarily from standard laboratory equipment. It consists intrinsically of a 2-dm^3 aspirator bottle, a siphon, a float assembly and a hypodermic syringe.

Dilution water under constant head enters the aspirator through activated carbon columns. Its flow is controlled and measured by a stopcock and flow meter. The aspirator outlet carries a curved tube siphon which can be

adjusted to regulate the overflow volume to between 1,5 and 2,0 dm^3. The siphon serves to empty the aspirator when the water rises to the aspirator neck. A fairly continuous flow of solution to the experimental aquarium is thus ensured.

Stock solution of toxicant is added to the aspirator from a volumetrically calibrated syringe which is filled and emptied via solenoid valves. The solenoid valves are operated, through a microswitch, by a float assembly as the water in the aspirator rises and falls.

When the float is in the position shown, stock solution flows into the syringe by hydrostatic head, and raises the weighted piston until it is arrested by an adjustable stop, which is set to control the volume in the syringe. During this event the solenoid valve between the stock solution of toxicant and the syringe is switched open by the action of the microswitch, the other solenoid valve being closed. As the float rises the microswitch acts to close the former and open the latter solenoid valves, so that the weighted piston discharges the contents of the syringe into the aspirator. Discharge is arranged to occur before the siphon starts so that stock solution mixes with the water, which is then carried over by the siphon action into the experimental aquarium.

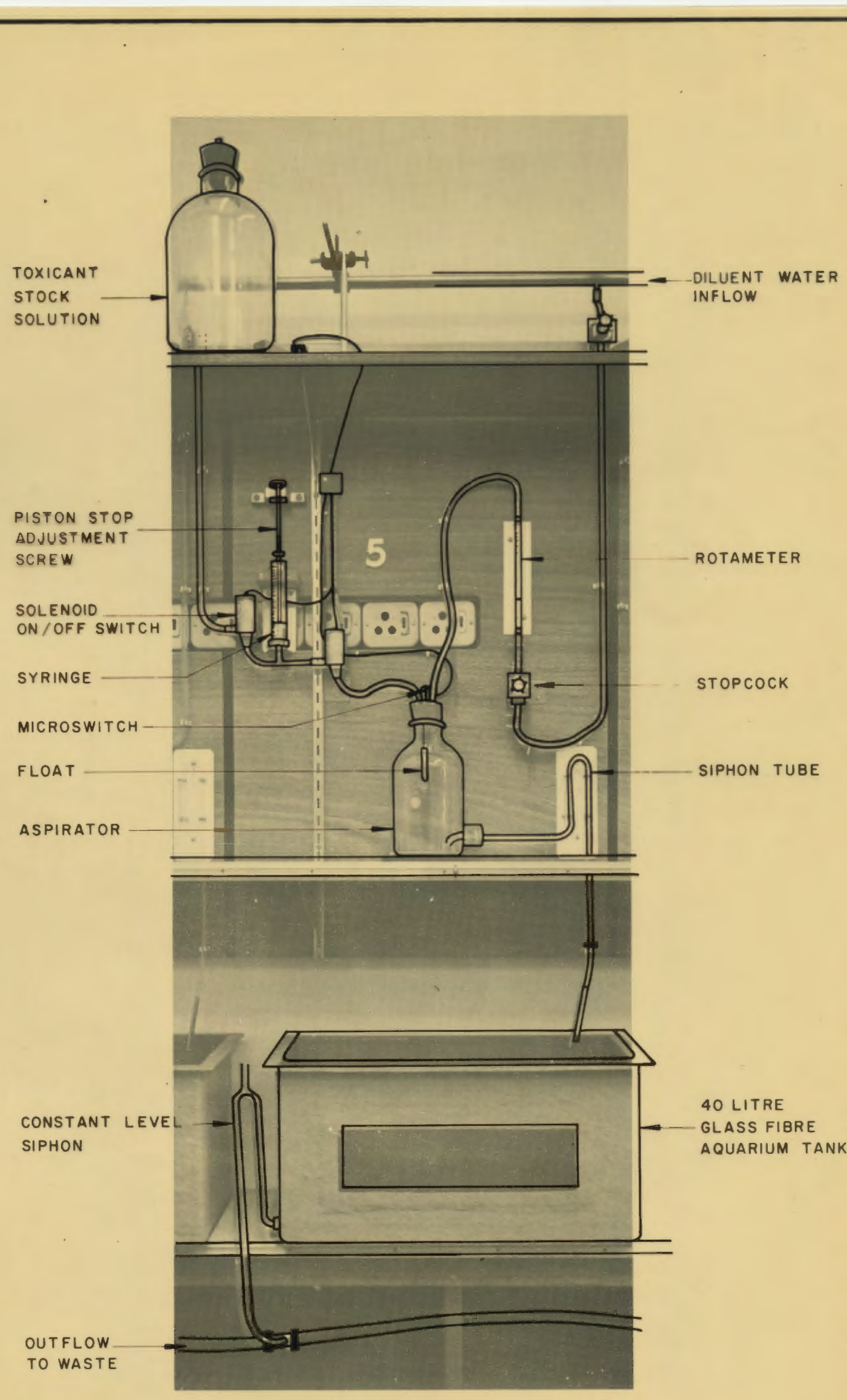

PLATE 1: *AUTOMATIC DOSING APPARATUS FOR USE IN FISH TOXICITY TESTS.*

ACUTE, CHRONIC AND CUMULATIVE EFFECTS OF SELECTED TOXICANTS UPON FISH PHYSIOLOGICAL AND BEHAVIOURAL PARAMETERS

Adequate management of surface waters polluted by industrial chemical wastes implies, among other things, the control of concentrations of toxic substances to levels at which damage to the aquatic ecosystem is demonstrably avoided. For an industrial effluent control system to function successfully in this respect a wide range of toxic effects must be monitored. Environmental protection can only be achieved, when using a monitoring system, if the system has the ability to assess, timeously, concentrations of toxicants which would prove harmful.

The toxicants used to assess the efficacy of fish physiological and behavioural responses to intoxication as sensor bases for monitoring, were chosen primarily as being representative of common industrial pollutants. Further, the fact that a multiplicity of adverse toxic effects upon the physiological well being of fish, as described below, give rise to single parameters which are easily measured, validates the use of those parameters for monitoring a broad spectrum of pollution.

Ammonia - The occurrence of ammonia in natural waters and its effect on fish have been extensively documented. Ammonia is discharged from a wide variety of industrial processes and operations that use ammonia or ammonium salts. Ammonia behaves in water as a Brønsted acid or base dissociating readily into ammonium and hydroxyl ions as follows:

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$$

The unionized fraction (NH_3) has long been recognized as the component toxic to fish (Wuhrmann and Woker, 1948; Downing and Merkens, 1955) and, therefore, the toxicity of ammonia solutions increases with increasing pH. However, evidence has been presented which indicates that the toxicity of solutions of ammonia to fish in different dilution waters showed a variation which was not entirely related to the concentration of the unionized ammonia molecule (Lloyd and Herbert, 1960). This variation was attributed to the increase of free carbon dioxide at gill surfaces, which caused a decrease in pH and, consequently, a decrease in the concentration of unionized ammonia present. The toxicity of unionized ammonia to fish is also increased by a decrease in dissolved oxygen (Wuhrmann, 1952; Merkens

and Downing, 1957). On the other hand the resistance of estuarine fish increases with salinity up to that of about 30 per cent sea water (Herbert and Shurben, 1965).

Embryonic and early larval stages of fishes are often considered to be especially sensitive to toxicants. Burkhalter and Kaya (1977) have documented the effects of continuous exposure to ammonia on the fertilized eggs and resulting sac fry of rainbow trout (*Salmo gairdneri*). They found that concentrations as low as 0,05 mg/dm^3 NH_3-N caused retardation of early growth and development. Histopathological changes, including hypertrophy, karyolysis and karyorrhexis of secondary gill lamelae occurred at concentrations of between 0,19 and 0,28 mg/dm^3. Recently Burrows (1964) has observed that exposure of chinook salmon fry to sublethal levels of ammonia produces considerable hyperplasia of the gill epithelium, with concomitant decreased resistance to bacterial gill disease. More extensive studies with carp (Flis, 1968) have shown that not only the gills but also the tissues of the skin, intestines, liver and kidneys can be damaged, and that this damage can be severe after prolonged exposure to sub-lethal concentrations. Using several species of fish, Reichenbach-Klinke (1967) showed that gill damage caused by ammonia was associated with a reduction in the number of blood erythrocytes. Impairment of oxygen carrying capacity of the blood of trout followed an exposure to a concentration of total ammonia nitrogen of 0,3 mg/dm^3 (Brockway, 1950).

Fromm (1970) investigated the effect of ammonia toxicity on excretion by rainbow trout and found that at a toxicant concentration of 5,0 mg/dm^3 excretion was inhibited. Whereas Lloyd and Orr (1969) suggested that the rate of urine excretion by rainbow trout increases with a rise in the concentration of ambient unionized ammonia, possibly caused by an increase in the permeability of the fish to water. No increased production of urine was found at an ammonia concentration of 12 per cent of the lethal threshold (46 µg/dm^3 NH_3-N).

Cadmium - This metal is an extremely dangerous cumulative poison. In its acute lethal action on rainbow trout (*Salmo gairdneri*) Ball (1967) found cadmium to be unusually slow. The lethal threshold of 0,01 mg/dm^3 was not discernable until seven days exposure. Results from the work of Pascoe and Cram (1977) confirm the high toxicity of cadmium, which is now seen to

cause death at concentrations as low as 0,001 mg/dm^3, and, presumably, causes toxic sublethal effects at lower concentrations. In view of their findings the upper limit of cadmium conducive to health of cold and warm water fish has been suggested to be 0,003 mg/dm^3 in hard water and 0,0004 mg/dm^3 in soft water (Wedemeyer and Wood, 1974). It is clear that these values would be unacceptable to fish as sensitive as rainbow trout (Ball, 1967; Kumada *et al.*, 1972). The pattern of mortality found by Ball (1967) and Pascoe and Cram (1977) indicates that above 10,0 mg Cd^{2+}/dm^3 the median period of survival decreases with increasing cadmium concentration, whilst below this concentration and above 0,003 mg Cd^{2+}/dm^3 the median period of survival is independant of concentration. It would appear, therefore, that the mode of toxic action of cadmium on fish varies at high and low concentrations.

The precise mechanism of cadmium poisoning in fish has not been established. However, Schweiger (1957) reported damage to the gills of carp, *Cyprinus carpio*, brook trout, *Salvelinus fontinalis*, rainbow trout and tench, *Tinca tinca*, exposed to high concentrations of cadmium and concluded the toxic action of cadmium was due to suffocation resulting from the deposition of dead cellular material on the gills and body surface. Schweiger further proposed that the varying response of the different species was related to the opercular rate of the individual species, and consequently to the oxygen demand. That cadmium is accumulated in gill tissue has been demonstrated by Mount and Stephan (1967) and direct damage to the gills by Gardner and Yevich (1970), where it was found that, although all gill filaments were not uniformly affected, corrosive action to the gill epithelia of several gill filaments was evident. In the instance of necrotized gill epithelium, the underlying basal cells were also destroyed. The impairment of these cells, which generate the gill epithelium, might essentially nullify the extrarenal activity of the gills. Further, they found that the respiratory function of the gill was impaired, following hypertrophy and hyperplasia of the interlamellar epithelia, by the mere reduction of respiratory surface area. Under conditions of high concentrations of cadmium and short exposure, the gill appears to be the primary site of damage as other tissues and organs appear to be relatively unaffected before death occurs. Mount and Stephan (1967) reported that there was a virtual absence of cadmium uptake by the liver of bluegills, *Lepomis macrochirus*, during acute lethal exposures of a few days but there

was a consistent and large increase in the cadmium during prolonged sub-lethal exposures.

Under conditions of prolonged exposure to comparatively low cadmium concentrations the intestine, kidney and liver of fish are measurably affected. Cellular damage to intestinal mucosa and kidney tubules has been reported (Gardner and Yevitch, 1970), as well as changes in liver enzyme activity (Jackim *et al.*, 1970) and changes in blood composition (Gardner and Yevitch, 1970). Eaton (1974) recorded cadmium residues in the liver, intestine, kidneys and gills of bluegills and observed major physical deformities in young fish reared in cadmium solutions. He also observed abnormal behaviour of bluegills exposed to acute cadmium intoxication, the fish showing convulsions and frenzied swimming movements terminating in tetany.

Middaugh and Dean (1977) compared the sensitivity to cadmium of specific developmental stages of the estuarine teleosts *Fundulus heteroclitus* and *Menidia menidia* and found that the larval forms of both species were more sensitive than were the eggs or adults. They emphasized the need for utilizing different developmental stages for establishing water quality criteria. Eaton (1974) suggested that the maximum acceptable toxicant concentration (MATC) for cadmium should be attributed to that concentration which has no effect upon survival, growth and reproduction. Eaton found that a concentration of 31 $\mu g/dm^3$ caused no adverse effects. This figure compares favourably with that found for brook trout by Benoit *et al.* (1976) who suggested a MATC of 34 $\mu g/dm^3$.

Copper - Toxicity of copper to fish has been the subject of many research papers, some of which have been reviewed by Doudoroff and Katz (1953) and McKee and Wolf (1963). However, despite these many studies the prediction of copper toxicity to fish is uncertain. Published 96-h LC50's for fish have a wide range, from 0,01 to 10,0 mg/dm^3 for total copper (O'Hara, 1971). Some of the variability is because the physical and chemical characteristics of surface water modifies the toxicity of copper. A number of research papers have addressed themselves to the physical and chemical variables that have an effect upon the toxicity such as water hardness, temperature, dissolved oxygen and pH. Of these factors hardness was considered to be of primary importance by Lloyd (1965). Stiff (1971b),

using the equilibrium equations of Scaife (1957), investigated the formation of complex carbonate species in copper-bicarbonate solutions and suggested that the difference in toxicity of copper to fish, with differences in water hardness, might be dependent upon carbonate complexation. At pH and bicarbonate concentrations of most fresh waters, cupric copper reacts with bicarbonate and hydroxide to give malachite:

$$2Cu^{2+} + HCO_3^- + 2OH^- \rightleftarrows Cu_2(OH)_2CO_3 + H^+$$

and this is quickly precipitated from solutions in which copper is present at concentrations greater than about 0,5 mg/dm³. Thus, for example, it was found that in an unrenewed solution of copper in hard water at pH 8,0 the concentration of soluble copper could fall within 2 h from an initial level of 1,1 mg/dm³ to 0,5 mg/dm³ (Shaw and Brown, 1974). Consequently, tests made with concentrations of copper greater than 0,5 mg/dm³, even at pH values somewhat below 8,0, without continual replacement, are likely to give misleading results.

At lower concentrations of copper and in solutions having a pH of less than 8,0, the reaction between cupric copper and hydroxide is unimportant in comparison with that occurring between cupric copper and bicarbonate, and Stiff (1971b) has shown that the stability constant of the reaction:

$$Cu^{2+} + HCO_3^- \rightleftarrows CuCO_3 + H^+$$

is such that, except in acidic surface waters, the concentration of carbonate-complexed copper will exceed that of cupric copper.

Investigations into the toxicity of copper to fish seem largely to have been made by adding some simple salt, such as copper sulphate, to waters in which the major chemical constituents were calcium and magnesium carbonates and from which other complexing agents were effectively absent. In this type of water, the total concentration of copper (Cu_T) is given by:

$$[Cu_T] \rightarrow [Cu^{2+}] + [CuCO_3]$$

It has been widely observed for many years that the calcium bicarbonate concentration of water has a marked effect on the toxicity of copper to

aquatic organisms, and the toxicity of copper to rainbow trout has been conveniently described in terms of the total hardness of water (Lloyd and Herbert, 1962), a characteristic which, in general, reflects the bicarbonate concentration, although, as Stiff (1971b) has pointed out, hardness alone might not be sufficient to define such systems.

Work researching the toxicity of copper to fish has usually been carried out with hardness as a controlled variable and at a stabilized pH. This approach is acceptable for most natural waters, but in an altered environment, such as one affected by mining wastes, combinations of hardness and pH must be taken into account. Howarth and Sprague (1978) found that the 96-h median lethal concentration (LC50) of total dissolved copper, for rainbow trout, varied from 20 $\mu g/dm^3$ in soft acid water to 520 $\mu g/dm^3$ in hard alkaline water, in tests with hardness ranging from 30 to 360 mg/dm^3 as $CaCO_3$ and pH from 5,0 to 9,0. They considered ionic copper (Cu^{2+}) and two ionized hydroxides ($CuOH^+$ and $Cu_2OH_2^{2+}$) to be the toxic species of copper. The sum of these ions produced LC50's ranging from 0,09 $\mu g/dm^3$ of copper in soft alkaline water to 230 $\mu g/dm^3$ in hard acid water. The ions were different in relative toxicity, or became more highly toxic at high pH, or both.

Laboratory studies on the toxicity of copper to fish have invariably been made in waters of low organic content, using relatively simple chemical systems in which the only complexing agent is carbonate. However, studies have been carried out on the effects of additions of a sewage effluent, an amino acid, humic substances, and suspended organic matter on the acute lethal toxicity of water containing copper sulphate to rainbow trout (Brown, Shaw and Shurben, 1974). The authors realized that in all cases the toxicity of a given total concentration of copper was quantitatively reduced. It was concluded that neither the total concentration of copper nor that of "soluble" copper in a water could be used to determine the toxicity to fish which was attributable to copper. It was also concluded that data from toxicity tests with copper in which natural surface waters are used for dilution purposes cannot define the true toxicity of copper nor have application to other natural waters except when the concentrations of the toxic chemical species are known. Such a study, involving acute and chronic toxicity tests conducted with the fathead minnow produced a MATC between 0,066 and 0,118 mg/dm^3 based on survival, growth, reproduction and hatchability of eggs (Brungs, Geckler and Gast, 1976).

The chronic toxicity of copper to fish has been extensively investigated with both partial and complete life cycle tests on all developmental stages of three species of freshwater fish, fathead minnow (*Pimphales promelas*) (Mount, 1968; Mount and Stephan, 1969); brook trout (*Salvelinus fontinalis*) (McKim and Benoit, 1971; 1974); and bluegill (*Lepomis macrochirus*) (Benoit, 1975). In all these long-term studies the most sensitive stage, or one of the most sensitive stages, in the life cycle was the embryo-larval or early juvenile period. In addition, several investigators have studied the sensitivity of embryos, larvae and early juveniles of freshwater fish to copper in acute exposures and have found that newly hatched larvae are more sensitive to copper than embryos or early juveniles. The chronic effect of prespawning exposure to various concentrations of copper on fathead minnow reproduction was investigated by Pickering, Brungs and Gast (1977). They found that prespawning exposure time had no significant effect on reproduction. However, the number of eggs produced per female decreased with increasing copper concentrations. The MATC was estimated to be 32 $\mu g/dm^3$, which is 0,07 of the 96-h LC50.

McKim, Christensen and Hunt (1970) investigated the short and long term effects of copper on the blood of brook trout (*Salvelinus fontinalis*). Seven blood characteristics, red blood cell count (RBC); haematocrit (Hc); haemoglobin (Hb); plasma chloride (Cl); plasma glutamic oxalacetic transaminase (PGOT); osmolarity (Os) and total protein (TP) were measured in brook trout that had been exposed to three concentrations (67,5 to 69,2; 38,2 to 39,0 and 22,8 to 24,0 $\mu g/dm^3$) of copper for six and 21 days. Concentrations of 67,5 to 69,2 and 28,2 to 39,0 $\mu g/dm^3$ caused statistically significant increases in RBC, Hc, Hb, PGOT and TP, whereas Cl and Os decreased. Five blood characteristics (RBC, Hb, Hc, Cl and PGOT) were also measured in brook trout which were exposed for 337 days to copper concentrations of 32,5; 17,4; 9,4; 5,7 and 3,4 $\mu g/dm^3$. After this long-term exposure, no changes were observed in the blood except for a measurable decrease in PGOT values at 32,5 and 17,4 $\mu g/dm^3$. Nevertheless, the authors expressed the belief that the measurement of specific physiological and biochemical changes in the blood of fish exposed for short periods to sublethal environmental stress may provide a sensitive method for predicting the effects of chronic exposure on survival, reproduction and growth. They maintained that this would allow a relatively rapid evaluation of the chronic toxicity of a compound.

Cyanide - The cyanide radical is a constituent of many compounds or complex ions that may be present in industrial wastes. Cyanide-bearing wastes derive from gas works, coke ovens, steel plant scrubbing operations, metal finishing and chemical industries.

The toxicity of cyanide to fish may be mainly attributed to the action of undissociated molecules of HCN, which has a much greater power of penetrating living material than dissociated ions (Brinley, 1927). Alkaline cyanides form hydrogen cyanide on solution in water as follows:

$$KCN + H_2O \rightarrow K^+ + OH^- + HCN$$

The extent of this hydrolysis is influenced to a large degree by the pH of the solution, becoming greater by an increase in acidity and depressed by the action of alkalis. It is not surprising, therefore, that cyanide toxicity varies with the pH of a solution, a fact that has been convincingly demonstrated by the experiments of Wuhrmann and Woker (1948). They found that in a solution containing 0,66 mg/dm³ CN the concentration of molecular HCN was 0,45 mg/dm³ at pH 9,84. On reducing the pH to 8,12 the molecular HCN increased to 0,62 mg/dm³, and at pH 7,58 it reached 0,66 mg/dm³. An increase in toxicity accompanied the fall in pH, overturning times for chub (*Squalus cephalus*) were 94, 70 and 54 minutes at pH values of 8,84; 8,12 and 7,58 respectively.

"Free cyanide" (CN ion and HCN) concentrations from 0,05 to 0,01 mg/dm³ as CN have proved fatal to many sensitive fish (Jones, 1964), and levels much above 0,2 mg/dm³ are rapidly fatal for most species of fish. A level as low as 0,01 mg/dm³ is known to have a pronounced, rapid and lasting effect on the swimming ability of salmonid fishes.

The toxic action of cyanide is known to be accelerated markedly by increased temperature (Wuhrmann and Woker, 1955; Cairns and Scheier, 1963), but the influence of temperature during long exposure has not been demonstrated. Numerous investigations have shown that the toxicity of free cyanide increases at reduced dissolved oxygen concentrations (Downing, 1954; Wuhrmann and Woker, 1955; Burdick, Dean and Harris, 1958; Cairns and Scheier, 1963), but not one of the authors has suggested an explanation for this. The gills of fish poisoned by cyanide become considerably brighter

than those of normal fish owing to the inhibition, by cyanide, of the cytochrome system, so that the tissues become incapable of utilizing the oxygen brought to them by the blood. It would seem, therefore, that a reduction in the amount of oxygen available would make little difference if the fish cannot utilize it, but this argument would apply only if cyanide inhibited oxidation completely. There is evidence that cyanide does not completely suppress protoplasmic oxidation, even at high concentrations, and it has been claimed that the respiratory enzyme system which can be inactivated by cyanide only accounts for about two-thirds of the oxidation going on in animal tissues, the remainder being accounted for by a system stable to cyanide. According to Commoner (1940) the cyanide-sensitive system is most active in connection with the oxidation of carbohydrates.

Extensive investigations into the long-term effects on fish of sub-lethal concentrations of cyanide have not been conducted. It has, however, been demonstrated that mammals administered small doses of cyanide over a period of time show a significant decrease in Vitamin B12 content of the liver. The possibility that prolonged exposure of fishes to sub-lethal concentrations of cyanide is accompanied by vitamin disturbances should not be ignored. A suggestion of neuronal damage as a result of mild chronic exposure to cyanide has also been reported. Vitamin deficiencies are not readily apparent in aquatic organisms but may express themselves in subtle failures, i.e. decreased reproduction, shortened life span and decreased capacity to adjust to environmental stress.

Southgate (1953) considers that the safe concentrations quoted for the tolerance of fish to cyanide are too high, and has suggested that in waters where fish are expected to live for long periods the amount of cyanide present should not exceed 0,01 mg/dm^3. Until further information is available regarding the tolerance of fish to cyanide and the extent to which they can become acclimatized to it, this would appear to be a good standard at which to aim. Whether fish can detect the presence of cyanide in solution does not appear to have been tested.

Mercury - Until recently, the major source of environmental mercury has been produced by the erosion and leaching of mercury by rainfall from geological formations which is transported to rivers and lakes by ground water runoff.

The industrial revolution, however, has produced increasing amounts of mercury which has been lost to the environment from waste products as a result of manufacturing processes that utilize mercury, or from the improper disposal of industrial and consumer products which contain mercury compounds. Mercury is consumed in the manufacture of electrical apparatus, chlorine, caustic soda, industrial control instruments, paint, pharmaceuticals, cosmetics, paper, pulp, agricultural fungicides and dental amalgams. Moreover, the amalgamative and catalytic properties of mercury and its compounds are responsible for many additional industrial uses.

With respect to the toxicity of inorganic mercury in the form of mercuric ion, in freshwater aquatic ecosystems, short-term studies indicate that concentrations in the region of 1,0 mg/dm^3 are fatal to fish (Boetius, 1960; Jones, 1939). For long-term exposures, mercury levels as low as 0,01 mg/dm^3 have been shown to be fatal to fish. Doudoroff and Katz (1953) established that concentrations of 0,01 and 0,02 mg/dm^3 of mercury were fatal to minnows in 80 to 92 days. Belding (1927) established that the lowest concentration of mercuric chloride fatal to brook trout within 24 h was 12,5 mg/dm^3. However, Schaut (1939) reported that 12,6 mg/dm^3 of mercuric chloride killed minnows in only 15 minutes. According to Jones (1947) fish show no special ability to detect or avoid toxic concentrations of mercuric chloride.

The acute toxic action of mercuric ions is a result of the damage caused to gill tissues as well as the formation of a film of coagulated mucus that occludes the spaces between the gill filaments, preventing their normal movement. Thus, the necessary contact between respiratory surfaces and the oxygen carrying medium is interrupted, gaseous exchange being impeded to such an extent that fish die from asphyxiation (Carpenter, 1925). Lloyd (1960) has attributed this suffocation to a breakdown and swelling of the gill epithelium which obstructs the ability of the gills to exchange gases. In addition, mercuric ion inhibits the active uptake of sodium into the gills of fish and thereby causes an increased sodium loss from the fish, resulting in a harmful osmotic imbalance in fish tissues.

The wide range of concentrations at which mercury has been reported to be toxic to aquatic organisms indicates that chemical and physical factors as well as normal environmental variables affect the degree of toxicity. In general, mercury toxicity has been shown to be a function of several water

quality parameters: temperature, pH, organic load, hardness, alkalinity, heavy metal load and dissolved oxygen. Further, the relative toxicity of mercury to fish varies widely with the species, its life stage and the state of adaptation of the animals' acclimation to environmental conditions in the aquatic ecosystem. Carpenter (1927) stated that there is no theoretical lower limit for the toxicity of mercury, and even trace amounts of mercuric chloride would be toxic to fish if the exposure were long enough. This conclusion has been supported by Boetius (1960). However, he suggested that, since the poisonous nature of mercury is accumulative, an absolute quantity of mercury must be accumulated in order to produce a lethal effect upon a given organism. Boetius also established that the product of the concentration of mercuric ion and the survival time of a given species is a constant which is a function of the species, body weight, life stage and the physical and chemical properties of the aquatic ecosystem.

Levels of mercury as low as 0,1 mg/dm^3 cause structural alterations in fish epidermal mucus, thus deleteriously affecting the swimming speed which is regulated by the hydrodynamic resistance of the skin surface (Varanasi, Robisch and Malins, 1975). The deleterious alteration may also affect the fish's capacity to resist attack by pathogenic organisms.

Mercuric chloride in water containing developing eggs of *Paracentrotus lividis* brought about a severe disturbance of development at 10 µg/dm^3 (Soyer, 1963) and when *Fundulus heteroclitus* (killifish) embryos were exposed to inorganic mercury at a concentration of 0,03 mg/dm^3 at the early blastula stage, the percentage of successful axis formation was reduced and a significant proportion of embryos developed cyclopia or intermediate conditions leading to cyclopia (Weis and Weis, 1977). Studies conducted on developing salmon eggs (*Oncorhynchus nerka* and *O. gorbuscha*) showed that concentrations of mercury exceeding 0,3 µg/dm^3 led to severe deformities. Accordingly it has been suggested that concentrations of mercury equal or exceeding 0,1 µg/dm^3 constitute a hazard in the aquatic environment.

Phenol - The toxicity of phenols to freshwater fish has been a subject of study for a number of years. Sources of phenol pollution in freshwater include: waste from the refining of oil and the distillation of coal or wood, phenolic chemicals used in livestock dips, discharges of domestic

sewage and the rotting of vegetation. The literature has been critically reviewed by the European Inland Fisheries Advisory Commission (EIFAC, 1972) who reported that a wide range of phenol concentrations (0,08 to 1 900 mg/dm^3) had been recorded as being harmful to fish. The Commission considered salmonids to be more sensitive to phenol poisoning than coarse fish, such as carp, and that newly hatched fish are generally more sensitive than adults. The concentrations which have been found to be lethal within a few days lie between 4,0 and 25,0 mg/dm^3.

Fish exposed to such concentrations of phenol soon become excited, swimming rapidly and becoming more senstive to outside stimuli and showing increased respiration (Veselov, 1957). In addition there may be colour changes (Wuhrmann and Woker, 1950) and increased secretion of mucus (Greven, 1953). Death may occur quickly or follow after a stage of depressed activity and loss of equilibrium interrupted by occasional convulsions.

Measurements of the effects of various environmental factors have shown that the toxicity of phenol to fish is increased by a decrease in dissolved oxygen (Herbert, 1962), an increase in water salinity (Brown, Shurben and Fawell, 1967) and a decrease in water temperature (Brown, Jordan and Tiller, 1967). Within the range of pH values from 6,5 to 8,5 there was little or no difference in toxicity of phenol to rainbow trout (Herbert, 1962) and similar results have been found for carp within the range of pH 4,0 to 11,0 (Lukanenko, 1967). Phenol toxicity to fish is not markedly affected by variations in water hardness.

Numerous pathological effects have been described in fish acutely poisoned by phenol. Linhardt (1951) found clotting of blood, gastroenteritis, increased leucocytosis and cyanosis of the blood vessels. Phenol affects the nervous system by solubilizing lipids, and the circulatory system by dissolving erythrocytes. Havelka and Effenberger (1957) recorded that in both acute and chronic poisoning bleeding from the gills did not occur and that fish showed the usual symptoms of nerve poisoning. Suffocation occurred from vasodilation and stasis of blood in the gills, with coagulation of blood in the heart cavity. Lammering and Burbank (1960) described deterioration and discoloration of gill membranes, brain damage and liver degradation in bluegill sunfish exposed to concentrations of phenol at

about the 48-h LC50 value. Mitrovic *et al.* (1968), also working with concentrations of phenol approximating to the 48-h LC50 value, found that rainbow trout killed within a few hours revealed, at autopsy, gross changes which included inflammation and necrosis of the pharynx and gills, internal haemorrhages, with blood in the body cavity and swelling of the spleen. Extensive, but less severe, damage, involving the gall bladder, liver and kidney, also occurred in fish surviving for seven days in lower concentrations. Waluga (1966) concluded that fatality in fish poisoned by phenol is caused through the diminution of the respiratory field and a decline in pressure in the circulatory system leading to respiratory paralysis.

Less attention has been given to the measurement of physiological changes in fish exposed to sub-lethal concentrations of phenol. Kuba (1969) investigated the action of phenol on the neuromuscular transmission of the red muscle of silver carp and showed that phenol increased the production of acetylcholine so facilitating the transmission of impulses to the muscle. Kristofferson *et al.* (1973, 1974) found no significant changes in the blood parameters of pike (*Esox lucius* L.) exposed to phenol, although the concentrations of some serum enzymes were increased. Swift (1978) found that phenol at non-lethal levels had no effect on the urine flow rate or haematocrit of rainbow trout for exposure times of 24 h .

There is no strong evidence that fish avoid low concentrations of phenol in laboratory tests; avoidance reactions reported in the field could result from the reaction of fish either to components of phenolic wastes or to low dissolved oxygen or both.

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SECTION II

THE DEVELOPMENT OF AN AUTOMATIC CONTINUOUS BIOLOGICAL MONITORING SYSTEM BASED ON FISH RESPIRATORY RESPONSE TO INTOXICATION

INTRODUCTION

That the respiratory system of fish is adversely affected by environmental change has been widely appreciated for a long time. A little over a century ago Bert (1871a,b) concluded that freshwater fish transferred to sea water died as a result of arrest of the branchial circulation caused by gill shrinkage. Further studies have emphasized this osmotic effect and it seems clear that hypertonic solutions of heavy metals may be fatal for this reason. Isotonic solutions of various salts, however, vary in the rate of their action, presumably due to the property of differential permeability of gill membranes. Furthermore, there are many examples of salts of metals being harmful at very low concentrations, well below isotonicity. The nature of this toxic action continues to be investigated, but there is evidence that the effects are exerted primarily on the external surface of fish and especially the gills.

As a result of the close relationship between environmental change, including pollution, and respiratory function, there has been increasing interest in the effects that variations in environmental conditions such as temperature, hypoxia and photoperiod have on the respiratory activity of fish (Randall and Shelton, 1963; Roberts, 1964; Marvin and Heath, 1968; Hughes and Roberts, 1970; Hughes and Saunders, 1970). In particular respiratory activity change has been suggested as a rapid bioassay method for the detection of pollutants (Schaumberg *et al.*, 1970; Cairns *et al.*, 1970). Physiological methods directed at observing respiratory changes in fish were performed many years ago by Jones (1938, 1939) and in recent years several studies have been carried out on the effects of pollutants on fish respiratory activity (Lee, 1969; Skidmore, 1970; Spoor, Neiheisel and Drummond, 1971; O'Hara, 1971), particular attention having been given, in these studies, to the measurement of oxygen utilization or opercular (breathing) rate.

Since Keys (1930) the most widely used experimental technique, when measuring oxygen utilization, has been to place the fish in a chamber through which water flows slowly and to measure oxygen consumption by analyzing the inflowing and outflowing water together with the rate of water flow. The development of the oxygen sensing probe has enabled quick and precise determinations of dissolved oxygen to be made without the

laborious titration of the Winkler method. O'Hara (1971a,b) developed an apparatus that provided a continuous recording of the oxygen consumption rates of fish which was utilized to investigate the effect of copper upon oxygen utilization. Although the method was found to provide a fast and sensitive tool in evaluating water quality, the work of Jones (1964) indicated increasing oxygen consumption by fish exposed to acute toxicity to be of transient nature and not as easily appreciated as breathing rate. The observed increases in oxygen uptake, under these circumstances, was probably due to enhanced locomotory activity rather than a direct result of toxic action upon the respiratory system itself.

Respiratory activity of fish involving, as it does, the movement of water through the gills by the muscular contraction of the buccal and opercular cavities (Hughes and Shelton, 1962), can be observed. It is, therefore, a simple matter to measure breathing frequency by direct observation. The technique requires no special equipment and has been used by several researchers (Belding, 1929; Ellis, 1937; Jones, 1947; Skidmore, 1970). The method has obvious disadvantages. Not only would the presence of an observer entail many man hours in long-term experiments, but his movements would also influence the fish adversely. It is, in any case, very difficult to make accurate counts when the breathing frequency rises above 100/minute, even should the fish remain quiescent. This problem was made quite apparent by Jones (1938) in his statement: *'The fish usually remained sufficiently still for the opercular movements to be counted for at least half a minute, but in some cases it was found necessary to hold the fish gently in a blunt pair of forceps while making the count'.*

The technical difficulties encountered during visual observation methods and the sensitivity of fish to such treatment has induced researchers to investigate techniques whereby the opercular rhythm may be measured without undue disturbance to the fish. Accordingly, opercular rates have been measured by means of transducers or electrodes attached to the fish, by recording pressure changes in the buccal cavity and by placing the fish between fixed immersed electrodes not attached to the fish.

A technique described by Sutterlin (1969) involved soldering a bead on the end of two very thin insulated wires and inserting these through each

of the opercular flaps. The bead lies in the opercular cavity and the movement of the operculae causes the beads to move back and forth in relation to each other producing a potential change which may be amplified and displayed on an oscillograph. Sutterlin utilized this technique to measure opercular frequency in swimming fish.

The expansion and contraction of the buccal and opercular cavities of fish produce pressure changes of from 5 to many mm of water. These pressure changes during breathing are responsible for the movement of water over the gills and through the opercular openings. The detection of these pressure changes is accomplished by introducing fine plastic tubing into the opercular and buccal cavities through holes in the clythrum and snout. The tubing is attached to sensitive pressure transducers and the pressure changes recorded on an oscillograph (Saunders, 1961). Using this method Hughes and Roberts (1970) and Hughes and Saunders (1970) examined the alterations in breathing rate produced by temperature and hypoxia respectively. The method yields an accurate measure not only of breathing frequency but also of depth of breathing. However, in order to use this technique the fish must be restrained to such an extent that it cannot turn around and twist the plastic tubing. For long-term monitoring a more useful system would be one that will allow the fish to swim freely in a relatively large volume of flowing water, feed, and at the same time let respiratory movements be recorded without disturbance.

Possibly the simplest method of detecting and recording fish breathing movements, other than visual observation, is accomplished by placing electrodes in the water where a fish is contained. The breathing movements of the fish cause a cyclic potential change between the electrodes which can be displayed on a suitable oscillographic recorder. Of the methods utilizing this technique those described by Halsband (1955), Roberts (1964) and Camougis (1960) seem to be the most readily adaptable for the purpose of continuous monitoring, although none is suitable without modification.

In Halsband's (1955) method a yoke-like metal electrode fits around, without touching, the opercular region of a fish confined to a tube, and electrical currents passing between this and a second electrode ahead of

the fish cause the indicator of a sensitive galvanometer to move synchronously with opercular movements. Halsband, citing Charmandarjan and Perwuschin (1930), attributed the current to the disturbance caused at the metal water interface of the yoke-electrode by the pulses of water issuing from the opercular chambers. The method has several disadvantages, however, the most important one being that the fish has to be confined to a narrow tube and restricted to one end of it so that the operculae remain between the arms of the yoke-electrode. Roberts (1964) placed a fish into a cylindrical chamber with a stainless steel mesh electrode at each end. Potential changes of from 20 to 400 microvolts developed between these electrodes with each opercular movement, and, after amplification, were recorded on a strip chart recorder. The potential differences were attributed to changes in the resistance of the water related to the opercular movements. The fish was not confined as strictly as in Halsband's apparatus but a closed cylinder proves difficult to use for all but short-term experiments. Camougis (1960) placed copper electrodes at opposite ends of an open vessel of water containing a fish and detected potential differences of 50 to 100 microvolts between them on an oscilloscope. This arrangement complies more favourably with the requirements for long-term monitoring, and for industrial situations where rapid biological monitoring of effluents is desired, the external electrode system is preferable because of its simplicity.

To accomplish the objectives of this research, namely the design and construction of an automatic monitoring system for industrial effluent control, preliminary tests were carried out to determine whether the external electrode technique for fish opercular rhythm measurement would provide an adequate input signal to the proposed system. Further, it was considered desirable to investigate the degree to which fish would respond to harmful conditions. In other words, would symptoms of stress, in this case increased respiratory activity, occur in sensor fish in sufficient time that corrective action may be taken to prevent immediate damage to experimental fish and, presumably, to the freshwater ecosystem.

RESEARCH PROCEDURES

The research was designed to expose test fish to selected concentrations of a number of common industrial toxicants. For each compound, either three or four fish were exposed to predetermined acute or sub-lethal concentrations of the toxicant, whilst one test fish was maintained in fresh-water as a control. Alternative methods of analysis were considered necessary in the collection of data to provide for a statistical appreciation of respiratory response to intoxication.

SENSOR FISH

The success of any biological test and the significance of the results depend largely on the normal performance of the experimental animal during the test period under laboratory conditions. The test species should be able to acclimate to laboratory conditions easily in a relatively short period of time, and should be in a healthy condition. In laboratory aquaria, the presence of uneaten food and waste products necessitate frequent cleaning to suppress fungal and microbial forms. The growth of micro-organisms may be harmful and when coupled with experimental intoxication may provide misleading results. Careful consideration was, therefore, given to ensure that the sensor fish would remain healthy in the laboratory environment.

The rectangular fish stock tanks were constructed of a non-toxic fibreglass material of 350 dm^3 capacity. All tanks were provided with a corner drain and stand pipe constructed so that the lowest layers of water were removed. The floor of the tanks sloped toward the drain to assist the exit of food and faecal particles. Fish stocks were maintained under natural photoperiod and a controlled temperature of 23 ± 1°C. Fresh-water flow through the tanks was adjusted to provide for 90 % replacement every 8 h . Not more than ten fish, of an average weight of 100 g, were maintained in a stock tank at any one time. The fish were fed trout pellets (Epol) to approximately 3 % of body weight daily and were regularly examined for signs of ill health or disease. During the period of the research, the mortality rate of experimental fish stocks never exceeded 5 % .

The following species of fish were tested initially for use in the monitoring system: *Cyprinus carpio* (Carp), *Carassius auratus* (Goldfish), *Sarotherodon mossambicus* (Blue kurper), *Tilapia sparmanni* (Lake kurper), *Tilapia melanopleura* (Red-breasted kurper), *Micropterus salmoides* (Large-mouth bass) and *Barbus holubi* (Small-mouth yellowfish). Yellowfish, kurper and largemouth bass responded to environmental stress with a notable change in breathing frequency, and of these the largemounth bass consistently gave the best results. This species was used, therefore, almost exclusively for the experimental tests. In addition, largemouth bass were readily available from the Transvaal Provincial Administration Fish Hatchery at Lydenburg in very good condition at all seasons of the year.

The experimental fish were acclimated to the dilution water and test conditions by being transferred individually to 50 dm^3 fibreglass aquaria, where they were maintained for at least two weeks before experimental use.

THE ELECTRODE CHAMBER

Chambers the size of that represented in Figure 1 were used for large-mouth bass between 150 and 200 mm in length. The electrodes were constructed of stainless-steel wire arranged in the form of a grid (Plate 2) and set across the chamber at each end of the animal compartment. The electrodes were fixed to perspex backing sheets with stainless-steel screws and soldered to a piece of stainless-steel wire connected to pin-

jacks. The pin-jacks were connected by insulated wire to a shielded cable leading to a low noise amplifier of variable gain. The perspex backing sheets, supporting the electrodes, were suspended from a lid so that the pair of electrodes could be lifted from the chamber by raising the lid. The lid was designed to cover the top of the chamber and was provided with a number of location points from which the electrodes could be suspended in order to accommodate experimental fish of different lengths.

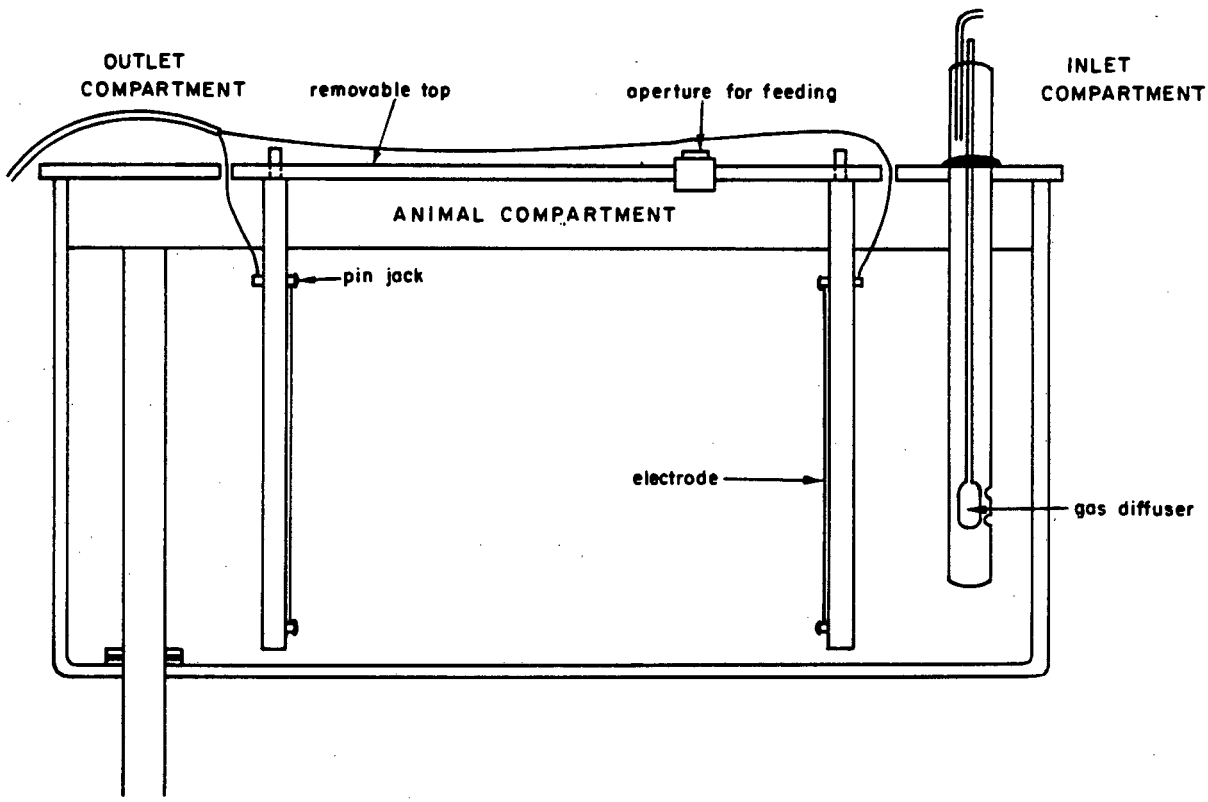

FIGURE 1. Diagram of an electrode chamber. This one was 600 mm long by 300 mm wide by 300 mm high. The animal compartment could be adjusted to provide a length of between 200 to 450 mm . The outflow standpipe could be raised or lowered to provide a volume of 45 to 50 dm^3.

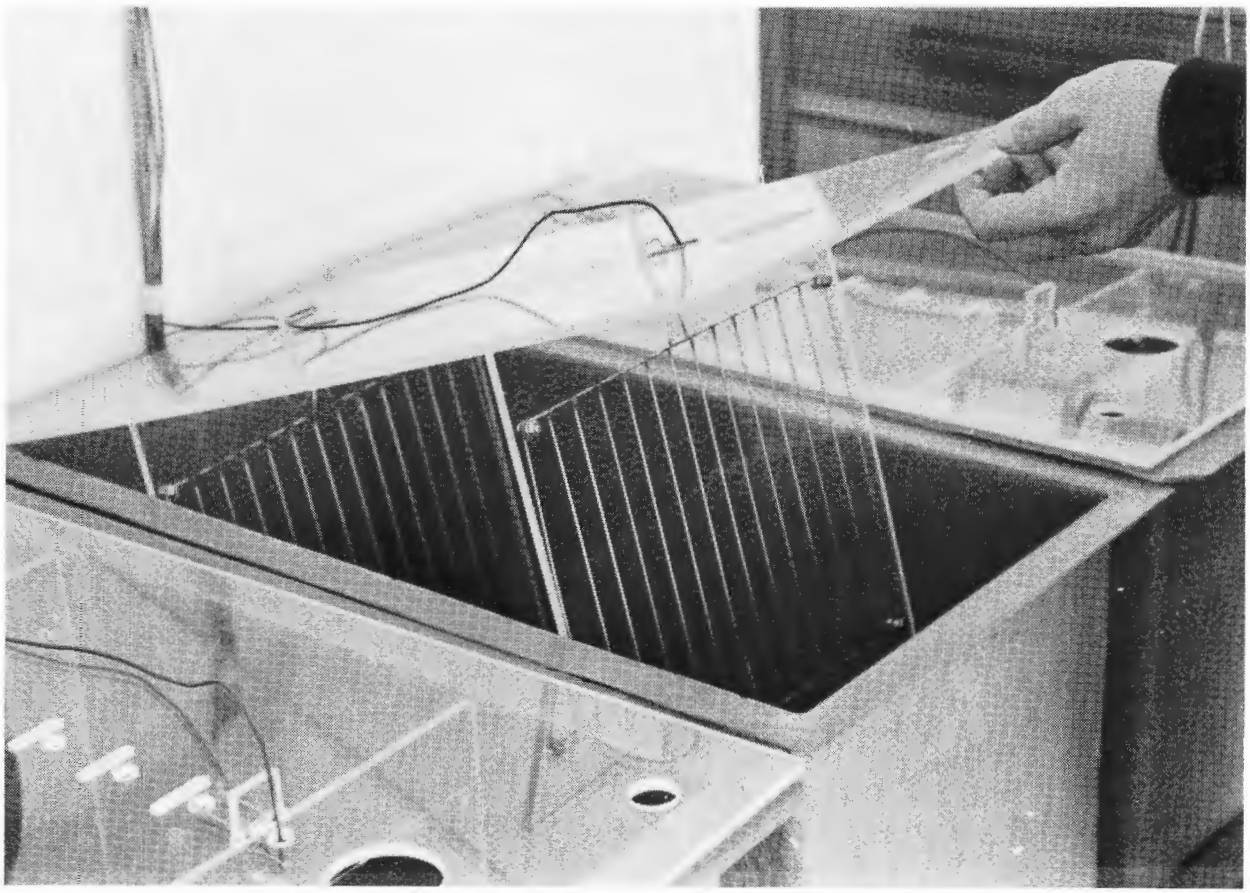

PLATE 2. Electrode chambers showing design of the stainless steel grid electrodes.

The equilibrium potential, in municipal tap water, of the type of stainless-steel electrode used, was approximately +0,1 V relative to a saturated calomel electrode, but the conditions of the water-metal interface were so complex (Pourbaix, 1966) that the equilibrium potentials of the two electrodes, being of the same composition and in the same body of water, differed from each other by a few millivolts, occasionally as much as 20. However, differences of this magnitude were not so important with regard to their use, as the method depended only upon departures from the normal steady state. It was important that the electrodes were of the same composition so that the potential difference between them remained small enough for there to be no problems with corrosion or the complication of the potential differences being amplified. It was also necessary that the solder used in the construction of the electrodes was of the same type, as some solders change the equilibrium potential of stainless-steel electrodes by as much as 0,2 V .

A study of the literature indicated that it was unlikely that the small potential differences which occurred between the electrodes would affect the test fish adversely. Regnart (1931) found goldfish to show a mild response to the making and breaking of a current of 3 milliamperes (a density of 5 microamperes/cm^2), but to appear to have been unaffected by continuous current. Dijkgraaf (1963) reported fish to be sensitive to current densities from 5 to 15 microamperes/cm^2. The current between the stainless-steel electrodes in the chamber was very much smaller, approximately 0,3 to 1,0 microampere (a density of 0,005 to 0,01 microamperes/cm^2), and although it might have varied slightly was not interrupted. McMillan (1928) concluded that young salmon in fresh water can sense a voltage gradient of between 40 and 80 millivolts/cm, and according to Lamarque (1967) the first sign of response by a number of species of freshwater fish occurs in a gradient of about 100 millivolts/cm . The gradient in the system described here was not more than 1 millivolt/cm, and was usually much less than that. The behaviour of the test fish supported the belief that they were not affected by the electrical conditions. Regnart (1931) found that repeated electrical stimulation caused codfish to be come sluggish and stop feeding. Largemouth bass, however, have been maintained in the chambers for over two months without signs of changes in respiratory and feeding behaviour or in activity.

The electrodes covered most of the cross-sectional area of the chamber, thus maintaining as uniform an electrical field as possible. This arrangement also served to retain the test fish between them. The chamber itself was divided into three compartments in order that irregularities in flow would be minimized. It was considered that most of these irregularities in flow would arise at the inlet and outlet where, in the first case, turbulence of the incoming stream may cause fluctuations, and in the second, interruptions in drainage may have caused the level of water to rise and fall slightly. A gas disperser was placed in the inlet compartment which effectively broke up the incoming stream of water so that it could not impinge directly on the electrode. The opening between the inlet and animal compartments was partly baffled by the electrode backing plate which directed the water towards the side. The passage to the outlet compartment extended across the chamber at the bottom of the partition so that debris could pass easily from the animal compartment. The outflow standpipe had a diameter large enough to

prevent the entraining of air bubbles which would cause irregularities in flow and thus impede drainage. The outlet was placed diametrically opposite the inlet and adjusted to mainain a volume of approximately 47 dm^3 in the chamber.

WATER FLOW CONDITIONS

The fish stock room and experimental chambers received aerated municipal tap water from a 23 000 dm^3 constant head tank, where the temperature was adjusted, and chlorine removed by passing the water through a series of activated carbon columns. At least once a day the following characteristics of the water were determined: total hardness, calculated from calcium and magnesium concentrations; total alkalinity, using the bromocresol green - methyl red indicator method; electrical conductivity, by means of a Radiometer conductivity meter; pH, using a Radiometer pH meter; chemical oxygen demand, with the dichromate reflux method; ammonia nitrogen, using the phenate method adapted to auto-analysis; and orthophosphate, using the vanadomolybdophosphoric acid colorimetric method. Dissolved oxygen concentrations were measured at random intervals by the azide modification of the iodometric method (American Public Health Association *et al*, 1965). Calcium, magnesium, sodium, potassium, mercury, cadmium and copper concentrations were measured by atomic absorbtion spectrophotometry, and cyanide and phenol concentrations by colorimetric techniques.

The effects of various levels of intoxication upon opercular rhythm were investigated by the addition of predetermined volumes of 1 g/dm^3 solutions, chosen arbitrarily to establish acute and sub-lethal levels, to the diluent water flowing continuously through the experimental chambers. Thereafter the nominal toxicant concentrations were maintained using a dosing unit similar to that of Stark (1967), wherein solenoid valves operated through a microswitch activated by a perspex float replaced the suggested pneumatic valve arrangement. A flow rate of approximately 50 dm^3/h was maintained throughout. Throughout the present study the toxicants tested were added as reagent grade chemicals. Copper was added as copper sulphate ($CuSO_4.5H_2O$), cadmium as cadmium chloride ($CdCl_2$), mercury as murcuric chloride ($HgCl_2$), ammonia as ammonium hydroxide (NH_4OH), Phenol as ortho-pheno (C_6H_5OH) and cyanide as potassium cyanide (KCN). Toxicant concentrations were measured at regular intervals from samples taken at the outflow from the chambers (Table 2).

TABLE 1. Routinely determined characteristics of dilution water

Water characteristic	Number of analyses	Mean	S.D.	Range
pH	84	7,5	0,06	7,4 - 7,6
Total hardness (mg/dm^3 as $CaCO_3$)	86	85,51	8,77	82,1 - 91,2
Electrical conductivity (mS/m)	80	23,75	0,17	23,4 - 24,1
Chemical oxygen demand	89	10,52	0,26	10 - 11
Sodium (mg Na^+/dm^3)	84	12,88	1,33	12 - 14
Potassium (mg K^+/dm^3)	84	2,53	0,26	2,4 - 2,7
Calcium (mg Ca^{2+}/dm^3)	84	23,88	0,56	23 - 25
Magnesium (mg Mg^{2+}/dm^3)	84	6,55	0,27	6 - 7
Ammonia (mg N/dm^3)	86	always less than 0,2*		
Orthophosphate (mg P/dm^3)	86	always less than 0,2*		
Dissolved oxygen (mg/dm^3)	84	always more than 7,0		
Cadmium ($mg/Cd^{2+}/dm^3$)	84	always less than 0,005*		
Copper (mg Cu^{2+}/dm^3)	84	always less than 0,025*		
Cyanide (mg CN^-/dm^3)	84	always less than 0,05*		
Mercury (mg Hg^{2+}/dm^3)	84	always less than 0,001*		
Phenol (mg C_6H_5OH/dm^3)	84	always less than 0,01*		

* limit of detection

S.D. - Standard deviation

TABLE 2. Toxicant concentrations during experiments

Toxicant	Nominal concentration (mg/dm^3)	Measured concentration (mg/dm^3) mean	range	pH
Copper (Cu^{2+})	5,0	4,8	nd	5,9
	1,0	1,23	0,89 - 1,57	6,1
	0,1	0,07	0,04 - 0,12	6,2
Cadmium (Cd^{2+})	1,0	1,12	0,88 - 1,36	6,1
	0,5	0,53	0,51 - 0,55	6,2
	0,1	0,09	0,08 - 0,11	6,2
	0,05	0,05	0,04 - 0,06	6,2
Phenol (C_6H_5OH)	10,0	9,4	nd	6,2
	5,0	4,87	4,75 - 4,99	6,2
	1,0	0,8	0,79 - 0,81	6,2
	0,5	0,51	0,49 - 0,53	6,2
Ammonia (as N)	10,0	9,79	nd	8,5
	5,0	4,97	4,76 - 5,18	8,2
	1,0	1,13	0,79 - 1,47	7,3
	0,5	0,47	0,26 - 0,68	7,1
Cyanide (CN^-)	0,5	0,48	nd	6,7
	0,1	0,09	0,05 - 0,13	6,6
	0,05	0,04	0,03 - 0,05	6,6

nd - Not determined. Experimental fish died within 24 h and thus only one sample was taken for analysis.

It has been suggested (Pourbaix, 1966) that equilibrium potentials of electrodes are affected by various environmental changes such as temperature, oxygen concentration, pH and salt content. The sensitivity of the method over the range of environmental parameters likely to be significant was tested. Variations in temperature from 10 to 30 °C, in oxygen concentration from 1,0 to 7,0 mg/dm^3 and in pH from 3 to 11 (varied by adding hydrochloric acid or sodium hydroxide to the diluent water) were found not to be limiting. The range of toxicant concentrations utilized were also found to have no effect upon the equilibrium potential of the electrodes used. However sensitivity was markedly reduced when the electrodes were immersed in water having a salt content equivalent of that of sea water.

PROPAGATION OF THE OPERCULAR SIGNAL

The source of the change in electrode potential, which arises when a fish is placed between the immersed electrodes, has been ascribed to changes

in the resistance of the water between the electrodes produced by the water being ejected from the opercular cavities (Roberts, 1964). Charmandarjan and Perwuschin (1930) attributed the current to the disturbance caused at the metal-water interface of the electrodes by the pulses of water issuing from the opercular chambers.

If water movement were the source of the electrode potential change, it ought to be possible to induce changes in the potentials by purely mechanical means, but stirring the water with a paddle did not cause a significant change. Spurting water from a tube under the surface directly upon the electrodes also caused little effect.

Camougis (1960) considered the potential differences to be caused by muscle action potentials. A finding suggestive of this was obtained by placing a hand in the water and moving a finger back and forth. A wave form, somewhat similar to that produced by a fish, was obtained. This was not due to water movement as there was little water displaced.

It seems evident, therefore, that the electrode system does not respond to the spurt of water from the branchial cavities of the fish, yet it does detect respiratory movement as a changing potential between the electrodes. It is probable that the small bioelectric current that is produced when fish breathe results in a potential change caused by a summation of electrical signals generated by the muscles which operate the buccal and opercular components of the fish breathing mechanism.

Accordingly, as well as signal amplification (the potential change ranged from 50 to 100 μ volts), care was taken to filter out muscle action potentials arising from body movement, which would have interfered with the opercular recordings.

The breathing signals from the electrodes were fed into a low noise d.c amplifier of variable gain (Figure 2). The amplified signal then passed, via a high-pass filter network, to a potentiometric recorder. The filter was found necessary to suppress large variations in the level of the baseline on the recorder caused by body movements of the fish. Without this filter, the amplitude of potential variation due to body movement was sufficient to drive the recorder off scale. The resistor R2 served merely to terminate the filter in the correct impedance.

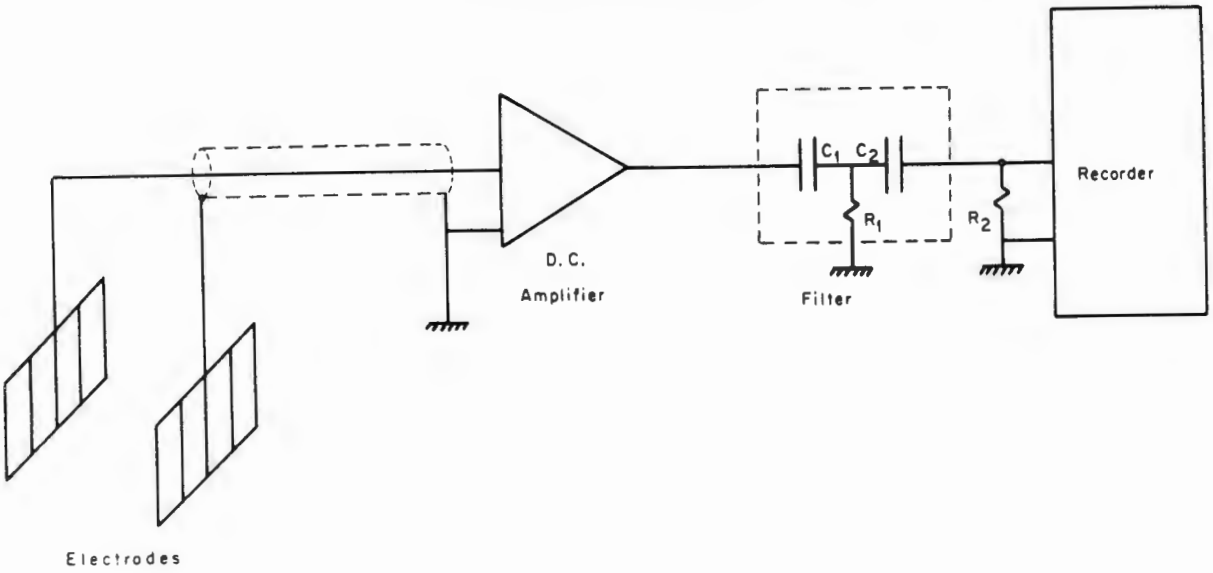

FIGURE 2. Schematic diagram of the monitoring system.

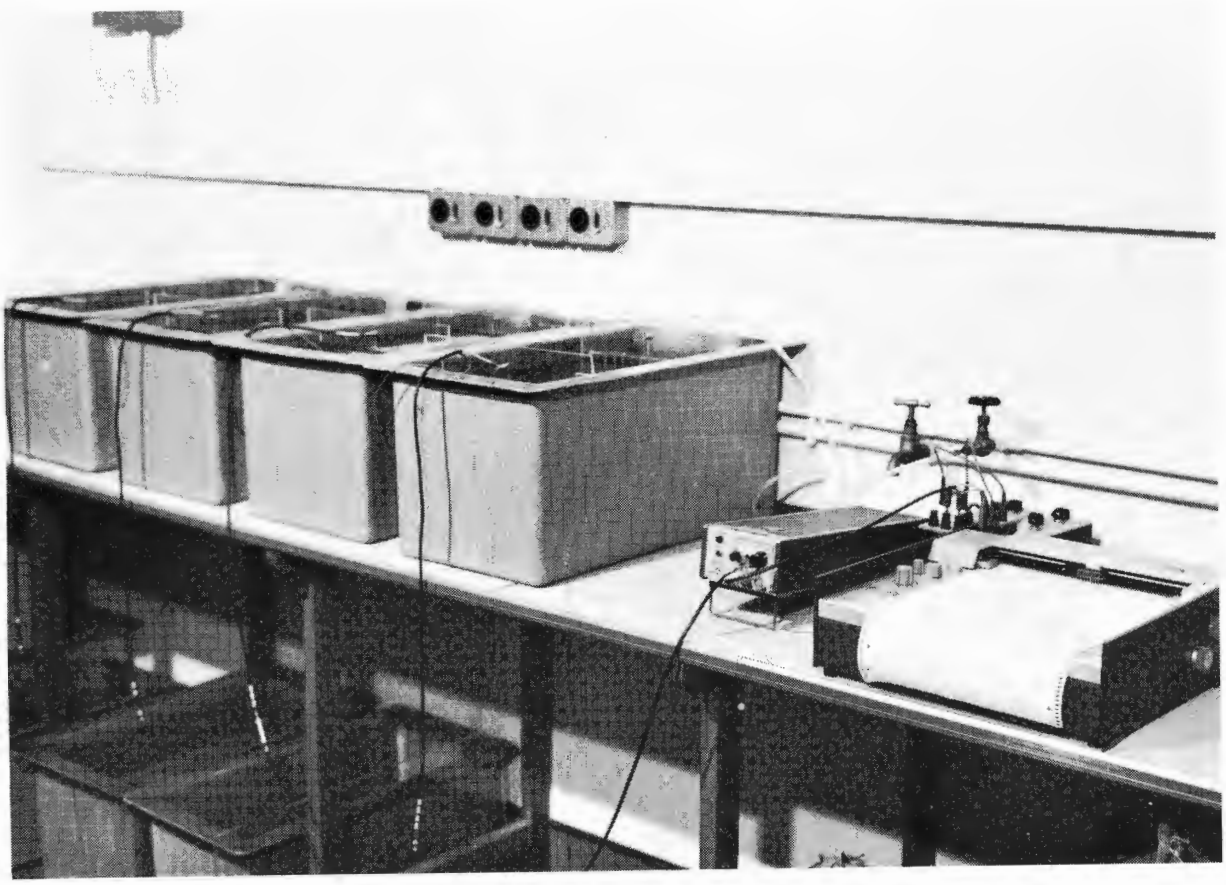

PLATE 3. Preliminary equipment set-up showing, from left to right, electrode chambers, Dymec Model 2460A amplifier, high pass filter components and Phillips PM 8100 potentiometric recorder. The electrode chambers were enclosed within a screen during experimental analyses.

The amplifier used was a Dymec Model 2460A, the amplification of this instrument being variable between 1 and 1 000 times, via a ten-turn potentiometer. The amplifier was operated at a gain of approximately 900. The values of the components in the high pass filter were: R1 = 1MΩ; C1 = C2 = 0,47 μF. This gave a cut-off frequency of 0,5 Hz, which was found to provide the required suppression without adversely affecting the desired signal. The terminating resistor, R2, had a value of 1 MΩ. The input impedance of the recorder was sufficiently high so as to have a negligible effect upon this termination.

The recorder (Phillips PM 8100) operated at a sensitivity setting of 500 mV full-scale, providing a signal deflection of approximately 10 mm .

All signal leads were screened to reduce hum and noise pick-up. This precaution was particularly important in the connections between electrodes and the input of the amplifier, where the signal amplitude was in the order of microvolts. Co-axial cable and BNC connectors were used to provide adequate screening.

Various activities by observers were found to have an adverse effect upon the breathing rate of experimental fish (Figure 3). Therefore the electrode chambers (Plate 3) were enclosed within a screen so that any movements of research personnel were unobserved by the fish.

The records of opercular rate (Figure 4) were seen to be repetitive and diphasic potentials of long period (0,5 - 1,5 s). The breathing frequency was, therefore, easily determined by a visual count against time. Certain changes in the amplitudes of the signals resulted from the position of the fish relative to the electrodes. The strongest signals occurred when the fish was perpendicular to the plane of either electrode, and weakest when parallel. Since breathing frequency was being monitored the significance of such amplitude changes was minimal.

DATA ANALYSIS

Breathing rates at different times of the day for the same fish, and at the same time of day for different fish were heterogeneous. These normal sources of variation, individual and duirnal, had to be taken into account in the analysis of data. To allow for individual variation each experimental sensor fish served as its own control.

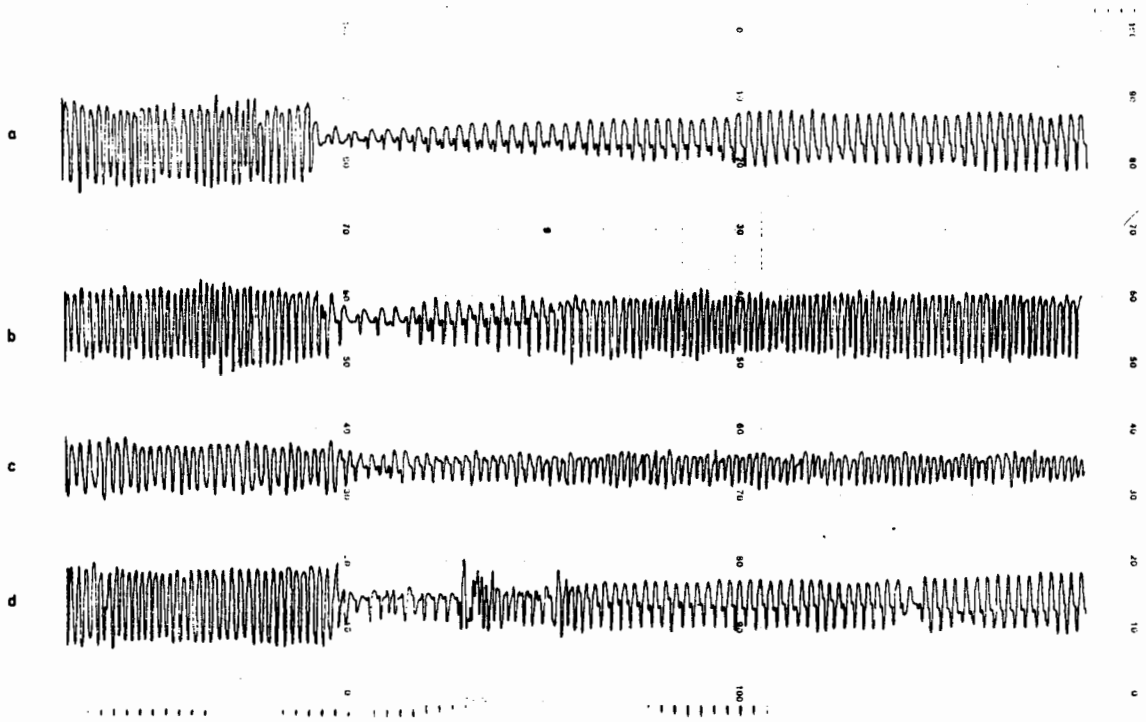

FIGURE 3. The effects of various stimuli on respiratory activity in a largemouth bass.

a - the door leading to the monitoring laboratory was opened and closed,

b - an observer passed through the field of view of the fish,

c - the observer remained within the field of view of the fish, and

d - the observer within the field of view of the fish raised an arm.

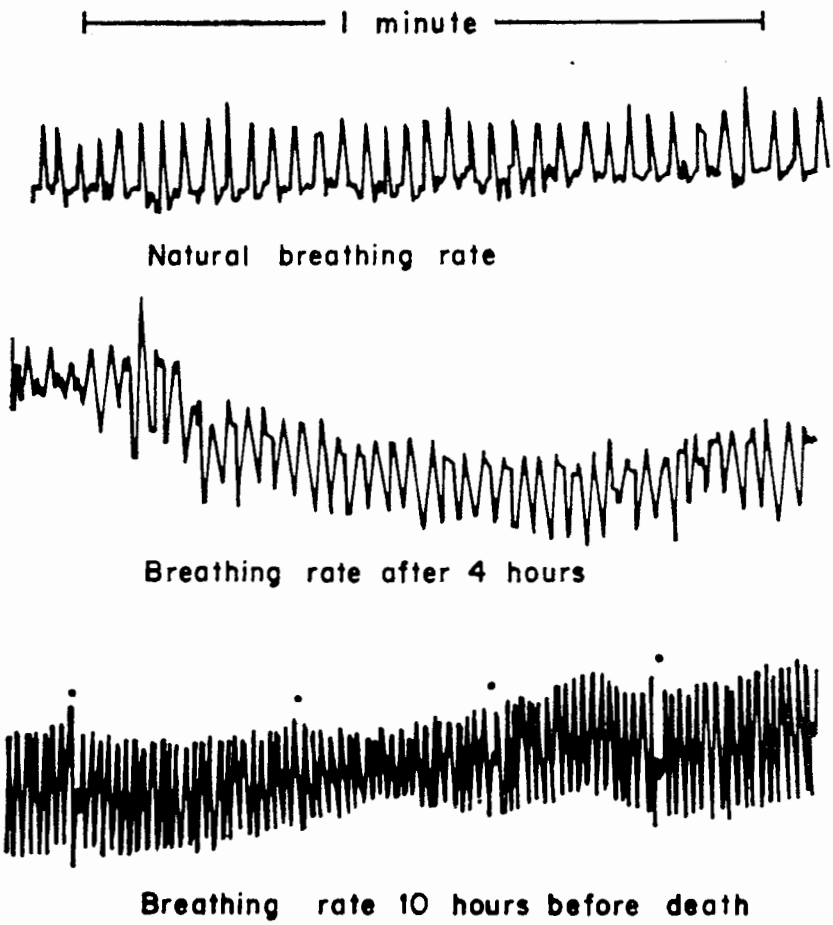

FIGURE 4. Respiratory records of a largemouth bass exposed to 10 mg/dm^3 of ammonia.

Two methods of analysis were used to define the response levels for sensor fish exposed to intoxication. The initial definition of response was based upon the observation that, during the period of acclimation to the electrode chamber, the breathing rate was high because the sensor fish were recovering from the stress of being transferred to an unfamiliar environment. Throughout the acclimation period breathing rates, however, rarely exceeded those recorded during the first 24 h after transfer. Any increases in the breathing rate of sensor fish which exceeded the maximum recorded during the first 24 h after transfer were, therefore, regarded as a response to an environmental stress condition over and above the stress of capture and transfer to an electrode chamber.

An alternative method of analyzing the data was based on the observed diurnal variation in breathing rate. Post-acclimation breathing rates for each sensor fish were recorded, for five successive days, at hourly

intervals. Mean and standard deviation values were calculated for every hour of the day and utilized to provide maximum 95 and 99 % confidence limits. The values obtained were used as critical limits to provide delimitation values for the designation of a definitive response.

EXPERIMENTAL PROCEDURE

1. Sensor fish were transferred individually from stock tanks to 50 dm^3 fibreglass aquaria and maintained in contimuously flowing municipal tap water for four weeks under conditions of natural photoperiod and a temperature of 23 ± 1 °C . Fish were fed trout pellets (Epol) to approximately 3 % of body weight daily throughout this period.

2. One sensor fish was placed in each of four electrode chambers and allowed at least four to five days acclimation. During this period the conditions were the same as in the holding facility and records of breathing rate were sampled once every hour throughout the first 24 h . These records were utilized to provide critical limits for respiratory response to intoxication according to the method for stress response outlined.

3. Sensor fish were 'standardized' for a further five days in dechlorinated municipal tap water and hourly records of breathing rate sampled for use in determining critical limits from mean and standard deviation values.

4. Sensor fish were exposed to varying concentrations of toxicants by the initial addition of predetermined volumes of 1 g/dm^3 solutions, thereafter the toxicant concentration was maintained using a dosing system similar to that of Stark (1967) and described previously. Hourly records of breathing rate were taken, for use as a basis for the future recognition of responses due to toxicant addition, for a further seven days or until death of the sensor fish.

DISCUSSION OF RESULTS

Figure 5 shows the effect that exposure to a nominal concentration of 1,0 mg/dm^3 of copper had upon the breathing rate of an experimental fish. As stated, breathing rate varied in an individual fish during different

periods of the day, and also between one fish and another. However, the normal breathing rate presented in Figure 5 may be taken as a typical example for the species. A low level of respiration was maintained throughout the night, rising gradually during the dawn period. During daylight hours one or two peaks of increased respiratory activity were noted; a slight increase at or about noon and another, more pronounced, toward dusk.

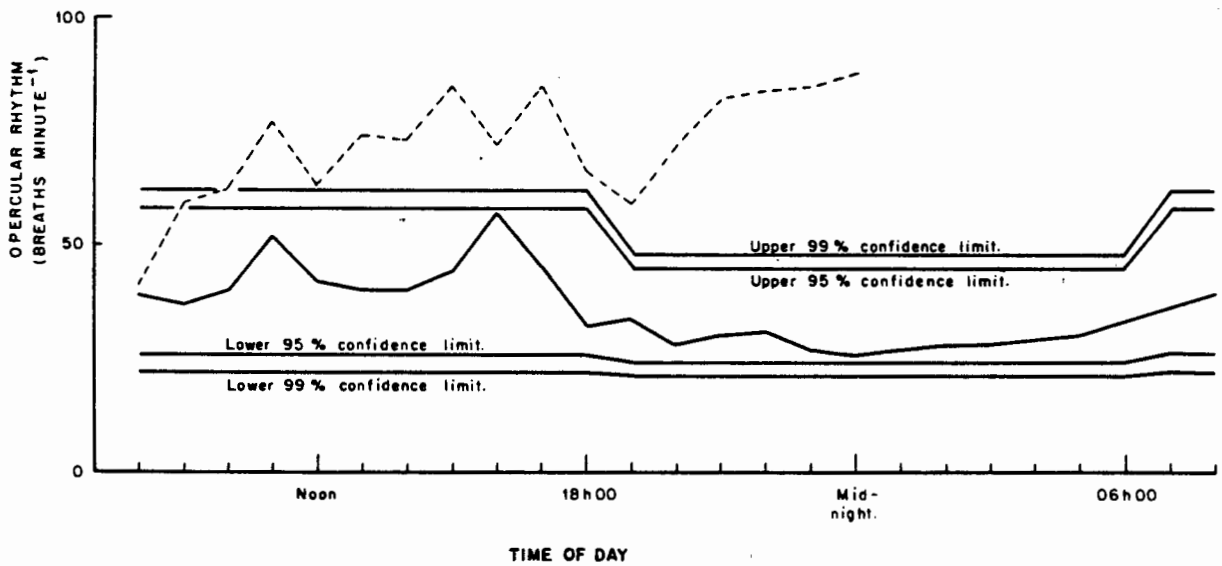

FIGURE 5. Breathing rate of *Micropterus salmoides* exposed to a nominal concentration of 1,0 mg/dm^3 of copper (broken line). The continuous line represents the hourly average breathing rate for five days before exposure.

The respiratory response to a lethal concentration of toxicant also shows a fairly typical reaction. One or two sharp increases in opercular rate, followed by partial recovery to a normal level, were observed. Subsequently, the breathing rate rose to a level well above designated critical response limits, continuing at this very high level until death.

It would be reasonable to assume that near death the respiration rate should decrease quite rapidly. Whilst this was observed in one case only (1,0 mg Cd^{2+}/dm^3), the interval of one hour between successive recordings probably caused the decreasing respiratory activity to remain unnoticed in the majority of cases. This assumption was confirmed in a supplementary experiment where a sensor fish was exposed to 5,0 mg Cu^{2+}/dm^3 (Figure 6) and a continuous record of opercular rhythm was charted. The record was utilized to calculate average respiratory rates for each six minute interval from two hours before the introduction of the toxicant until death.

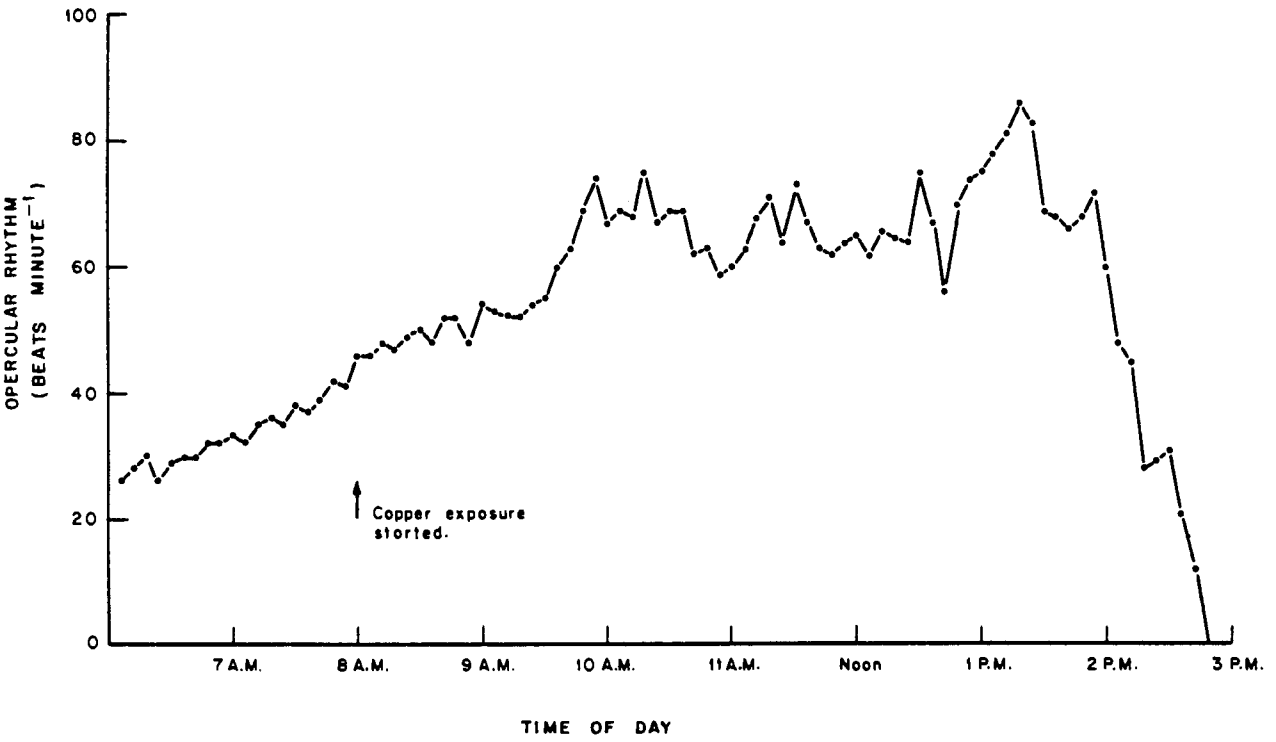

FIGURE 6. Opercular frequency of *Micropterus salmoids* exposed to 5,0 mg/dm^3 of copper. Each point being an average of the preceding six minute period.

In all cases where death ensued, a response was easily recognized beforehand. It would, however, have been of interest to establish whether, on receipt of a response to a lethal toxicant concentration, sensor fish would have recovered if immediately transferred to fresh water. Responses

were also observed in all sub-lethal toxicant concentrations, excepting 0,05 mg Cd^{2+}/dm^3 and 0,5 mg ammonia (as N)/dm^3 utilizing the stress response, i.e. a breathing rate exceeding the highest achieved during the first 24 h after transfer of the experimental fish to an electrode chamber, and with the latter toxicant concentration utilizing the statistic based upon maximum variance.

The gradual rise in breathing rate, which occurred in many sub-lethal toxicant concentrations before a critical response level was attained, may be noted for use as a preliminary warning of toxic action.

Copper sulphate is widely used as an algicide in all types of natural waters, and because of this its toxicity to freshwater organisms has been studied by many investigators. Concentrations of copper sulphate ranging from 0,25 to 1,0 mg Cu^{2+}/dm^3 have been reported as being lethal or maximum safe concentrations for largemouth bass (Kellerman, 1912). Ellis (1937) considered the toxic action of copper to be directed primarilly at the gills, and pointed out that the toxic process is threefold in its attack on the respiratory apparatus. Firstly, the spaces between the gill filaments become filled with a precipitate of coagulated mucus, so that water flowing through the branchial chambers cannot reach the gill filament cells, a layer is, therefore, established between the respiratory surface and the water, interrupting the oxygen diffusion gradient. Secondly, the spaces between the gill lamellae become filled so that movement of the gill filaments becomes impossible, thus constraining the circulation of blood in the gill capillaries; thirdly, this stasis affecting the blood circulation causes the heart action to drop to approximately half normal rate. This "coagulation film anoxia" theory has been supported by Dilling, Healey and Smith (1926), Westfall (1945) and Schweiger (1957).

The severity of the toxic effect upon the gill apparatus produced sudden increases in breathing rate when the sensor fish were exposed to lethal copper concentrations (Tables 3 and 4). Despite the animals' efforts to maintain their oxygen supply, indicated by the high breathing rates, the sensor fish rapidly became exhausted and the opercular movement rate decreased precipitously throughout the hour before death. Sensor fish response to the sub-lethal copper concentration was somewhat more delayed

TABLE 3. Hourly breathing rates for a sensor fish before and after exposure to 5,0 mg Cu^{2+}/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								38	39	44	38	38	37	42	43	41	45	48	33	30	29	27	30	32	37
2	32	32	29	30	35	48	44	39	39	43	35	35	37	39	33	40	44	36	35	39	36	34	34	29	36
3	34	33	29	30	30	41	39	40	41	42	43	38	37	41	43	37	43	48	28	32	25	25	30	30	36
4	31	31	34	35	35	44	37	41	37	46	47	43	38	41	33	44	41	34	36	34	35	31	31	26	37
5	34	33	30	33	30	40	44	35	38	45	44	39	37	34	36	38	39	46	35	39	32	32	32	29	37
	30	29	29	34	39	42	43	*54*	*87*	*73*	*68*	*69*	*63*												
a	32	32	30	32	34	43	41	39	39	44	41	39	37	39	38	40	42	42	33	35	31	30	31	29	
b	1,8	1,7	2,2	2,3	3,8	3,2	3,2	2,3	1,5	1,6	4,8	3,0	0,5	3,2	5,1	2,7	2,4	6,8	3,2	4,1	4,5	3,7	1,7	2,2	
c	36	35	35	37	41	49	48	43	42	47	51	44	38	46	48	45	47	56	37	43	40	37	35	34	
d	37	36	36	38	44	51	50	45	43	48	54	46	38	48	51	47	49	60	42	45	43	39	36	35	

a, b, c and d are, respectively; the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 69.

TABLE 4. Hourly breathing rates for a sensor fish before and after exposure to 1,0 mg Cu^{2+}/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								44	43	43	40	42	41	48	47	47	56	39	41	41	38	37	35	30	39
2	30	28	27	37	26	33	39	42	54	40	41	43	39	46	46	46	42	51	37	32	36	38	29	28	38
3	31	27	27	32	29	34	38	44	39	45	48	44	41	43	48	50	48	39	39	39	39	35	36	32	39
4	30	32	28	29	29	36	44	44	38	45	45	49	43	39	42	46	42	38	38	33	39	32	30	29	38
5	31	31	31	30	32	36	38	39	37	40	40	42	40	40	44	57	45	32	32	28	30	31	27	26	35
6	27	28	28	29	30	33	36	*58*	*62*	*77*	*64*	*74*	*73*	*85*	*72*	*85*	*68*	*55*	*82*	*83*	*87*	*82*	*68*	*53*	
	42																								
a	30	30	28	31	29	34	39	43	42	43	45	44	41	43	45	49	47	40	36	35	36	35	31	29	
b	1,6	2,2	1,6	3,4	2,2	1,5	3,0	2,2	7,0	2,5	5,0	2,9	1,5	3,8	2,4	4,7	5,8	6,9	4,3	5,3	3,8	3,1	3,9	2,2	
c	33	34	31	38	34	37	45	47	56	48	55	50	44	51	50	58	58	53	45	45	44	41	39	33	
d	34	35	32	40	35	38	47	48	60	49	58	52	45	53	52	61	62	58	47	48	46	43	42	35	

a, b, c and d are, respectively; the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 73.

TABLE 5. Hourly breathing rates for a sensor fish before and after exposure to 0,1 mg Cu^{2+}/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								32	33	38	32	32	31	36	37	35	39	42	27	24	23	21	24	26	31
2	26	26	23	26	29	42	38	33	34	34	31	33	30	37	37	37	33	42	28	23	27	29	20	19	29
3	21	19	18	28	17	24	30	35	35	39	31	31	32	35	29	36	40	32	31	35	31	30	30	25	32
4	30	29	25	26	26	27	35	34	35	36	37	32	31	35	37	31	37	42	22	26	19	19	24	24	30
5	25	25	28	29	29	38	31	33	45	31	32	34	30	37	37	37	33	42	28	23	27	29	20	19	29
6	22	18	18	23	20	25	29	*35*	*46*	*48*	*50*	*49*	*51*	*54*	*58*	*65*	*64*	*65*	*67*	*61*	*54*	*50*	*47*	*38*	*55*
7	*37*	*36*	*54*	*64*	*79*	*76*	*65*	*61*	*64*	*63*	*58*	*52*	*48*	*46*	*46*	*51*	*45*	*39*	*53*	*52*	*50*	*47*	*44*	*38*	*51*
8	*46*	*48*	*54*	*60*	*61*	*51*	*48*	*50*	*55*	*62*	*65*	*71*	*73*	*68*	*69*	*54*	*48*	*49*	*52*	*60*	*48*	*53*	*46*	*44*	*58*
9	*56*	*64*	*62*	*58*	*63*	*63*	*58*	*55*	*49*	*43*	*42*	*50*	*53*	*56*	*58*	*56*	*59*	*50*	*43*	*42*	*42*	*41*	*40*	*39*	*48*
10	*44*	*45*	*46*	*47*	*48*	*49*	*52*	*50*	*44*	*39*	*40*	*48*	*45*	*53*	*51*	*51*	*53*	*54*	*45*	*38*	*37*	*35*	*36*	*37*	*43*
11	*34*	*41*	*40*	*39*	*42*	*43*	*44*	*45*	*39*	*35*	*34*	*40*	*54*	*47*	*46*	*48*	*51*	*40*	*33*	*33*	*31*	*31*	*31*	*32*	*39*
12	*37*	*36*	*36*	*38*	*39*	*41*	*44*	*35*	*29*	*25*	*24*	*30*	*33*	*36*	*36*	*38*	*39*	*30*	*23*	*22*	*22*	*21*	*20*	*19*	*28*
	24	*25*	*26*	*27*	*28*	*29*	*33*																		
a	25	23	22	26	24	31	33	33	36	36	32	32	31	36	35	35	36	40	27	26	25	26	24	23	
b	3,6	4,7	4,4	2,3	5,5	8,2	3,8	1,1	4,9	3,2	2,5	1,1	0,8	1,0	3,6	3,5	3,3	4,5	3,3	5,1	4,6	5,2	4,1	3,4	
c	32	33	31	31	35	47	40	36	46	42	38	35	33	38	42	42	43	49	34	36	34	36	32	29	
d	34	36	34	32	38	52	42	36	49	44	39	35	33	39	45	44	45	52	36	39	37	39	34	31	

a,b,c and d are, respectively; the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 76.

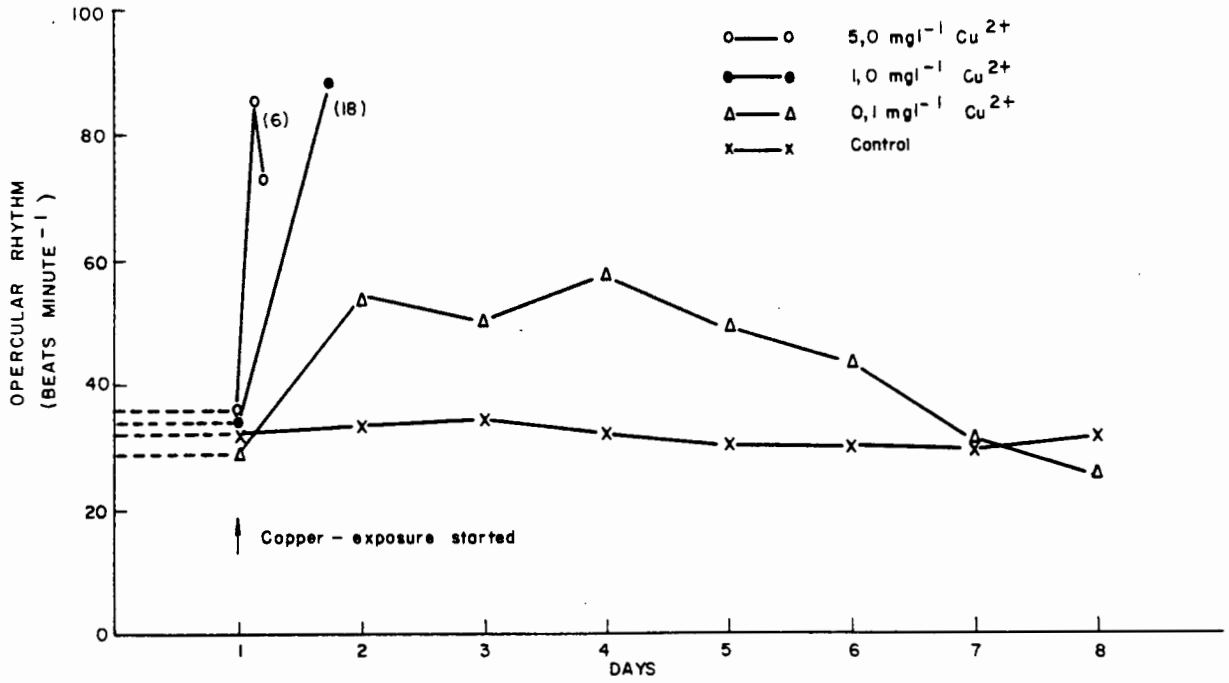

FIGURE 7. Daily average opercular frequencies of *Micropterus salmoides* exposed to different concentrations of copper. Each point represents the 24-h period preceding it. Numbers in brackets denote times to death (h) for individual fish.

(Table 5, Figure 7), but was still attained more rapidly than in any other sub-lethal toxicant concentration, indicative of the direct effect that this metal had upon the respiratory apparatus. The breathing rate remained at a high level until the seventh day of exposure. However, a diurnal pattern was re-established after four days. O'Hara (1971) found no alteration in the rate of oxygen consumption of bluegills exposed to 0,1 mg Cu^{2+}/dm^3. This would be the expected result if the increased opercular rate was established purely to maintain oxygen supplies in the face of decreasing respiratory efficiency and confirms opercular rhythm response to be a more sensitive indicator of toxic stress than oxygen consumption.

Pathological changes attributable to cadmium poisoning have been observed in the intestinal tract, kidney and gills of fish. The toxic effect of

this metal is recognized as being cumulative. Lethal levels of cadmium, however, produce an increase in the number of red blood cells as early as four hours after exposure (Gardner and Yevitch, 1970), indicating that high concentrations of cadmium do induce respiratory disfunction. The results indicated that cadmium had a less severe effect upon the gill apparatus than copper. When exposed to lethal concentrations of cadmium sensor fish responded within a short time, nevertheless the time-lag between exposure and response for cadmium was longer than for equivalent concentrations of copper by a factor of three (Tables 6, 7 and 8, Figure 8). It is possible that high concentrations of cadmium cause death by direct damage to gill filaments rather than by the formation of a coagulated film of mucus, and this can be a quick or slow process depending upon concentration. The insidious cumulative toxic effect of cadmium was shown in sensor fish exposed to 0,1 mg Cd^{2+}/dm^3, which concen tration proved lethal whereas an equivalent concentration of copper did not, even though the sensor fish exposed to cadmium took longer to respon A response was also obtained from sensor fish exposed to a sub-lethal con centration of cadmium (Table 9). As with copper intoxication at sub-lethal levels, the animal resumed a normal diurnal breathing pattern afte approximately four days exposure.

Fish poisoned by phenol concentrations near the 48-h median tolerance level reveal, at autopsy, gross changes. These include inflammation and necrosis of the pharynx and gills, internal haemorrhage with blood in the body cavity, and swelling of the spleen. Extensive, but less severe, damage has been noted in the gall bladder, liver and spleen of fish surviving for seven days (Mitrovic, Brown, Shurben and Berryman, 1968). The deleterious effects of phenol upon membraneous structures would naturally affect the respiratory surfaces, and in lethal concentrations (Table 10) led to an immediate increase in breathing rate. Responses wer also obtained in all sub-lethal concentrations of phenol (Tables 11, 12 and 13). A high level of respiration was maintained for a longer period than with either copper or cadmium (Figure 9), the normal diurnal respiratory pattern becoming re-established on the fifth or sixth day after intoxication.

TABLE 6. Hourly breathing rates for a sensor fish before and after exposure to 1,0 mg Cd^{2+}/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1										46	50	38	39	42	40	46	44	44	40	39	38	34	36	28	36
2	27	30	27	27	28	29	33	31	30	45	44	41	40	39	43	44	44	37	41	41	42	31	38	27	36
3	26	28	27	26	26	31	40	37	29	49	46	39	43	48	49	46	46	38	39	39	40	32	31	31	38
4	31	29	30	26	32	34	41	39	35	47	47	40	43	47	43	48	47	38	36	37	38	37	36	33	38
5	24	27	26	24	31	38	45	41	36	45	48	44	41	43	48	50	48	39	39	39	39	35	36	32	39
6	30	32	28	29	29	36	44	35	37	*47*	*47*	*45*	*53*	*51*	*48*	*48*	*47*	*55*	*57*	*54*	*64*	*60*	*62*	*72*	*57*
7	*62*	*59*	*62*	*61*	*58*	*66*	*65*	*64*	*66*	*62*	*71*	*61*	*67*	*77*	*67*	*88*	*90*	*83*	*83*	*73*	*76*	*72*	*83*	*81*	
8	*91*	*76*	*77*	*68*	*83*	*43*	*40*																		
a	28	29	28	26	29	34	41	37	33	46	47	40	41	44	45	47	46	39	39	39	39	34	35	30	
b	2,9	1,9	1,5	1,8	2,4	3,7	4,7	3,9	3,7	1,7	2,2	2,3	1,8	3,7	3,8	2,3	1,8	2,8	1,9	1,4	1,7	2,4	2,6	2,6	
c	33	33	31	30	34	41	50	44	41	50	51	45	45	51	52	51	49	45	43	42	43	39	41	35	
d	35	34	32	31	35	43	53	47	43	51	53	46	46	53	54	53	50	46	44	43	44	40	42	37	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 71.

TABLE 7. Hourly breathing rates for a sensor fish before and after exposure to 0,5 mg Cd^{2+}/dm^3

Day	Time of day																							Daily average rate
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1										47	45	49	48	42	44	50	48	47	39	34	31	31	30	28	38
2	28	26	24	32	36	36	40	41	42	45	49	48	46	41	47	44	43	45	32	29	30	30	30	24	36
3	27	25	28	31	34	31	32	33	37	43	39	49	43	43	41	43	42	46	37	30	34	28	27	31	36
4	33	30	31	28	29	31	41	34	37	41	42	51	49	41	39	45	40	39	31	27	29	26	29	30	36
5	30	30	33	34	35	36	39	34	39	45	45	49	43	39	42	46	42	38	30	33	39	32	30	29	37
6	31	31	31	30	32	36	38	34	40	*37*	*42*	*45*	*49*	*47*	*47*	*45*	*48*	*44*	*52*	*52*	*53*	*51*	*53*	*60*	*53*
7	*58*	*60*	*60*	*59*	*61*	*60*	*58*	*69*	*65*	*70*	*73*	*67*	*72*	*74*	*78*	*74*	*75*	*74*	*73*	*81*	*78*	*65*	*76*	*78*	*73*
8	*82*	*83*	*83*	*62*	*60*	*67*	*64*	*67*	*63*	*77*	*64*	*64*	*57*	*70*	*70*	*89*									
a	30	28	29	31	33	34	38	35	39	44	44	49	46	41	43	46	43	43	34	31	33	29	29	28	
b	2,4	2,7	3,5	2,2	2,8	2,7	3,5	3,3	2,1	2,3	3,7	1,1	2,8	1,5	3,1	2,7	3,0	4,2	4,0	2,9	4,0	2,4	1,3	2,7	
c	35	34	36	35	39	39	45	42	43	49	51	51	51	44	49	51	49	51	42	36	41	34	32	34	
d	36	35	39	37	40	41	47	44	45	50	54	52	53	45	51	53	51	54	44	38	43	36	33	35	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 66.

TABLE 8. Hourly breathing rates for a sensor fish before and after exposure to 0,1 mg Cd^{2+}/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1										50	54	47	40	40	37	40	41	38	32	29	28	27	30	29	38
2	29	30	33	36	31	39	46	47	46	48	49	50	41	42	35	41	42	31	34	31	30	29	28	29	38
3	36	31	34	38	36	44	48	41	43	44	41	49	34	39	38	39	40	32	36	34	31	26	26	24	36
4	31	31	36	36	30	46	40	42	45	47	48	45	36	40	33	38	42	34	31	36	34	30	32	23	37
5	33	29	29	34	36	41	41	43	48	46	47	43	36	41	33	44	39	34	36	34	35	31	31	26	36
6	34	33	30	33	30	40	44	36	33	*37*	*43*	*32*	*37*	*40*	*48*	*40*	*40*	*32*	*32*	*36*	*47*	*58*	*41*	*52*	*44*
7	*56*	*43*	*47*	*54*	*50*	*51*	*48*	*49*	*43*	*46*	*54*	*66*	*48*	*46*	*50*	*74*	*73*	*50*	*75*	*50*	*46*	*49*	*44*	*51*	*59*
8	*72*	*60*	*64*	*67*	*65*	*62*	*64*	*66*	*68*	*68*	*69*	*78*	*74*	*66*	*65*	*91*	*65*	*74*	*75*	*85*	*71*	*72*	*73*	*83*	*73*
9	*76*	*67*	*64*	*68*	*70*	*72*	*74*	*72*	*71*	*74*	*73*	*69*	*72*	*75*	*78*	*81*	*80*	*83*	*86*	*91*	*86*				
a	33	31	32	35	33	42	44	42	43	47	48	47	37	40	35	40	41	34	34	33	32	29	29	26	
b	2,7	1,5	2,9	2,0	3,1	2,9	3,4	4,0	5,9	2,2	4,7	2,9	3,0	1,1	2,3	2,3	1,3	2,7	2,3	2,8	2,9	2,1	2,4	2,8	
c	38	34	38	39	39	48	50	50	55	51	57	52	43	43	40	45	43	39	38	38	37	33	34	32	
d	40	35	40	40	41	50	52	52	58	53	60	54	45	43	41	46	44	41	40	40	39	34	36	33	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 74.

TABLE 9. Hourly breathing rates for a sensor fish before and after exposure to 0,05 mg Cd^{2+}/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1										42	40	44	41	36	38	51	48	46	41	36	31	29	28	28	35
2	28	25	24	27	31	36	33	31	32	37	42	44	40	34	40	50	40	39	34	38	30	31	27	27	34
3	27	27	22	26	27	31	33	29	30	35	41	39	36	33	39	41	46	47	42	38	33	34	28	29	35
4	31	28	22	28	30	34	38	34	38	34	39	41	37	31	41	48	48	43	39	34	35	33	29	30	35
5	29	25	29	29	32	36	32	28	30	39	40	41	39	38	42	45	40	40	38	36	36	36	31	30	36
6	30	27	28	29	29	34	36	30	37	*33*	*28*	*32*	*39*	*33*	*30*	*34*	*35*	*35*	*30*	*35*	*43*	*45*	*41*	*48*	*42*
7	*56*	*62*	*56*	*58*	*46*	*48*	*49*	*52*	*44*	*46*	*46*	*35*	*48*	*39*	*48*	*49*	*44*	*46*	*52*	*36*	*30*	*47*	*50*	*39*	*42*
8	*41*	*33*	*33*	*41*	*40*	*40*	*42*	*44*	*37*	*36*	*37*	*41*	*42*	*41*	*41*	*44*	*40*	*42*	*46*	*44*	*42*	*40*	*43*	*45*	*42*
9	*41*	*48*	*45*	*43*	*40*	*40*	*47*	*44*	*36*	*38*	*40*	*39*	*38*	*36*	*40*	*46*	*39*	*39*	*38*	*35*	*36*	*35*	*32*	*31*	*36*
10	*29*	*28*	*27*	*27*	*38*	*38*	*39*	*40*	*38*	*38*	*41*	*38*	*37*	*38*	*42*	*44*	*41*	*37*	*36*	*36*	*34*	*31*	*30*	*31*	*36*
11	*28*	*29*	*29*	*29*	*34*	*34*	*36*	*42*	*41*	*40*	*41*	*42*	*36*	*35*	*41*	*46*	*40*	*34*	*34*	*32*	*31*	*31*	*30*	*29*	*35*
12	*27*	*26*	*26*	*26*	*31*	*31*	*38*	*46*	*44*	*42*	*40*	*40*	*38*	*38*	*37*	*42*	*41*	*37*	*37*	*36*	*36*	*35*	*32*	*29*	*35*
	29	*29*	*27*	*27*	*28*	*28*	*33*	*41*	*40*																
a	29	26	25	28	30	34	34	30	33	37	40	42	39	34	40	47	44	43	39	36	33	33	29	29	
b	1,6	1,3	3,3	1,3	1,9	2,1	2,5	2,3	3,9	3,2	1,1	2,2	2,1	2,7	1,6	4,1	4,1	3,5	3,1	1,7	2,6	2,7	1,5	1,3	
c	32	29	32	30	34	38	39	35	41	44	43	46	43	40	43	55	52	50	45	40	38	38	32	31	
d	33	30	34	31	35	40	41	36	43	46	43	47	44	41	44	58	55	52	47	41	40	40	33	32	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 69.

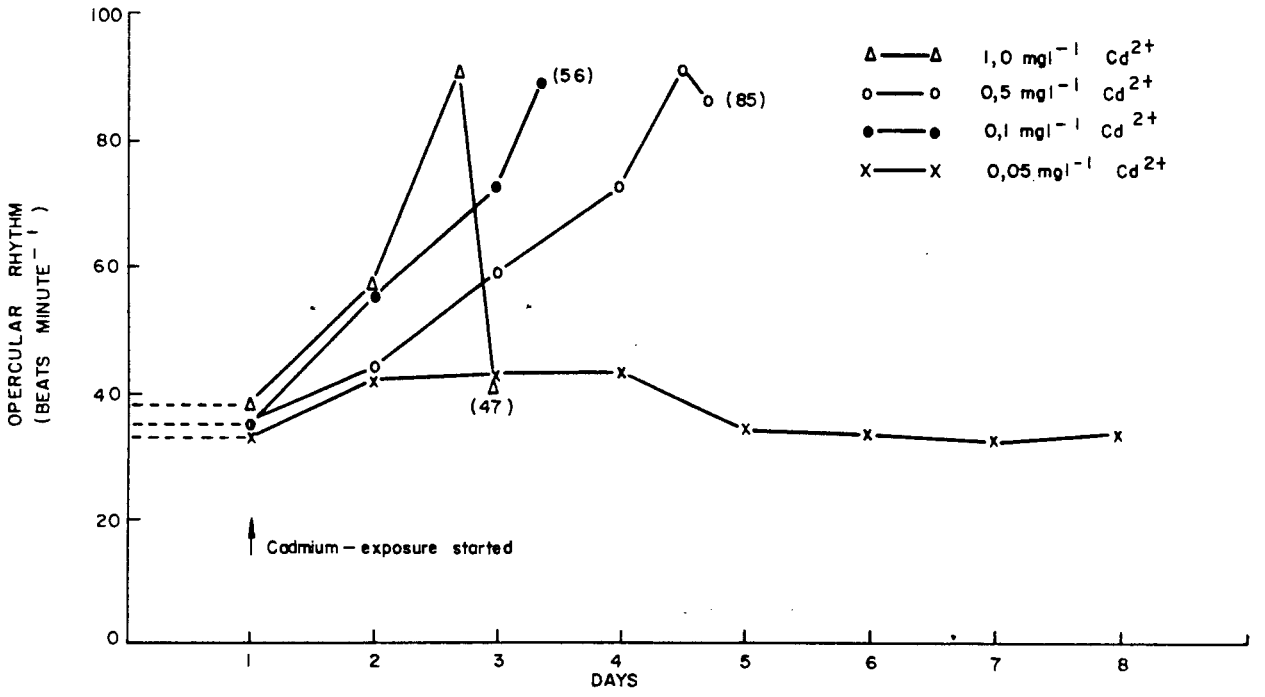

FIGURE 8. Daily average opercular frequencies of *Micropterus salmoides* exposed to different concentrations of cadmium. Each point represents the 24-h period preceding it. Numbers in brackets denote times to death (h) for individual fish.

TABLE 10. Hourly breathing rates for a sensor fish before and after exposure to 10,0 mg phenol/dm³

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								47	43	37	33	42	45	46	49	49	45	44	35	35	34	33	32	30	40
2	35	36	37	40	41	41	51	43	40	38	36	39	41	43	44	45	47	40	38	38	37	35	35	34	39
3	37	37	34	34	39	39	41	41	38	35	34	38	40	42	42	44	45	39	33	33	33	32	32	31	37
4	36	36	36	36	37	37	39	42	39	37	36	39	41	43	43	44	47	39	35	35	34	34	33	33	38
5	37	37	38	38	38	39	40	46	44	38	41	36	45	47	48	49	48	45	35	34	34	32	31	29	40
6	36	37	39	40	41	41	45	*72*	*84*	*82*	*80*	*78*	*76*	*76*											
a	36	37	37	38	39	39	43	44	41	37	36	39	42	44	45	46	46	41	35	35	34	33	33	31	
b	0,8	0,6	1,9	2,6	1,8	1,7	4,9	2,6	2,6	1.2	3,1	2,2	2,4	2,2	3,1	2,6	1,3	2,9	1,8	1,9	1,5	1,3	1,5	2,1	
c	38	38	41	43	43	43	53	49	46	39	42	43	47	49	51	51	49	47	39	39	37	36	36	36	
d	38	38	42	44	44	44	56	51	48	40	44	44	49	50	53	53	50	49	40	40	38	37	37	37	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 71.

TABLE 11. Hourly breathing rates for a sensor fish before and after exposure to 5,0 mg phenol/dm³

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								43	40	37	36	40	42	44	44	46	47	41	35	35	33	32	32	31	38
2	36	36	36	36	37	38	39	47	45	39	42	37	46	47	49	50	49	46	36	34	34	32	31	30	39
3	37	38	40	41	42	42	46	50	45	40	37	44	49	52	53	55	57	47	38	37	37	36	36	35	43
4	39	39	40	41	42	43	48	48	43	38	37	42	47	49	49	51	54	46	40	37	36	36	35	34	41
5	37	37	34	33	41	42	46	42	41	37	36	40	42	43	43	44	46	41	37	37	36	35	35	34	39
6	38	38	39	39	39	40	41	*51*	*49*	*48*	*52*	*59*	*62*	*58*	*56*	*68*	*66*	*61*	*56*	*54*	*50*	*54*	*50*	*55*	*58*
7	*57*	*59*	*66*	*58*	*64*	*74*	*72*	*76*	*78*	*81*	*83*	*84*	*84*	*81*	*76*	*79*	*75*	*76*	*72*	*77*	*71*	*74*	*78*	*84*	*75*
8	*81*	*76*	*71*	*64*	*61*	*63*	*62*	*63*	*66*	*71*	*73*	*71*	*67*	*67*	*64*	*61*	*61*	*60*	*59*	*59*	*60*	*58*	*55*	*53*	*57*
9	*50*	*42*	*38*	*38*	*38*	*43*	*51*	*56*	*52*	*46*	*42*	*51*	*54*	*55*	*58*	*58*	*54*	*53*	*44*	*44*	*43*	*42*	*41*	*39*	*49*
10	*44*	*45*	*46*	*49*	*50*	*50*	*60*	*49*	*47*	*41*	*44*	*39*	*48*	*50*	*51*	*52*	*51*	*48*	*38*	*37*	*37*	*35*	*34*	*32*	*43*
11	*39*	*40*	*42*	*43*	*44*	*44*	*48*	*40*	*39*	*35*	*34*	*38*	*40*	*41*	*41*	*42*	*44*	*39*	*35*	*35*	*34*	*33*	*33*	*32*	*37*
12	*36*	*36*	*37*	*37*	*37*	*38*	*39*	*42*	*40*	*37*	*36*	*40*	*41*	*43*	*44*	*44*	*47*	*41*	*38*	*37*	*37*	*35*	*35*	*34*	*39*
	37	*38*	*34*	*39*	*39*	*40*	*41*																		
a	37	38	38	38	40	41	44	46	43	38	38	41	45	47	50	49	51	44	37	36	35	34	34	33	
b	1,1	1,1	2,7	3,5	2,2	2,0	3,8	3,4	2,3	1,3	2,5	2,6	3,1	3,7	5,4	4,3	4,7	3,0	1,9	1,4	1,6	2,1	2,2	2,2	
c	40	40	43	45	45	45	52	53	47	41	43	46	51	54	61	58	60	50	41	39	38	38	38	37	
d	40	41	45	47	46	46	54	55	49	42	44	47	53	57	64	60	63	52	42	40	39	40	39	38	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 72.

TABLE 12. Hourly breathing rates for a sensor fish before and after exposure to 1,0 mg phenol/dm³

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								45	41	35	31	40	43	44	47	47	49	42	33	33	32	31	30	28	38
2	33	34	35	38	39	39	43	40	39	35	34	38	40	41	41	42	44	39	35	35	34	33	33	32	37
3	36	36	37	37	37	38	39	42	38	38	41	44	46	47	48	52	42	38	38	37	37	36	36	35	41
4	39	39	40	40	41	43	45	48	46	40	43	38	47	49	50	51	50	47	37	35	35	34	33	31	41
5	38	39	41	42	43	43	47	40	39	35	34	38	40	41	41	42	44	39	38	38	37	35	35	34	38
6	37	37	34	34	39	39	41	*46*	*48*	*55*	*55*	*54*	*53*	*55*	*56*	*56*	*53*	*56*	*56*	*52*	*51*	*53*	*54*	*54*	*55*
7	*57*	*58*	*59*	*59*	*59*	*58*	*56*	*63*	*65*	*67*	*64*	*60*	*59*	*59*	*62*	*63*	*59*	*57*	*54*	*53*	*51*	*53*	*51*	*50*	*58*
8	*54*	*52*	*54*	*58*	*62*	*60*	*64*	*67*	*63*	*68*	*71*	*77*	*84*	*81*	*72*	*62*	*64*	*65*	*61*	*60*	*59*	*57*	*55*	*54*	*60*
9	*48*	*47*	*45*	*41*	*39*	*46*	*54*	*53*	*49*	*49*	*52*	*55*	*57*	*58*	*59*	*54*	*53*	*49*	*49*	*48*	*48*	*47*	*47*	*46*	*52*
10	*50*	*50*	*51*	*51*	*52*	*64*	*56*	*53*	*51*	*47*	*43*	*44*	*39*	*41*	*41*	*42*	*45*	*37*	*33*	*35*	*32*	*32*	*31*	*31*	*39*
11	*35*	*35*	*36*	*36*	*36*	*37*	*38*	*42*	*39*	*36*	*35*	*39*	*41*	*43*	*43*	*45*	*46*	*40*	*34*	*34*	*34*	*33*	*33*	*32*	*38*
12	*37*	*37*	*37*	*37*	*38*	*38*	*40*	*46*	*42*	*36*	*32*	*41*	*44*	*45*	*48*	*48*	*44*	*43*	*34*	*34*	*33*	*32*	*31*	*29*	*39*
	34	*35*	*36*	*39*	*40*	*40*	*50*																		
a	37	37	37	38	40	40	43	43	41	37	37	40	43	44	45	47	46	41	36	36	35	34	33	32	
b	2,3	2,7	3,1	3,0	2,3	2,4	3,2	3,5	3,2	2,3	5,1	2,6	3,3	3,6	4,2	4,8	3,5	3,7	2,2	2,0	2,1	1,9	2,3	2,7	
c	41	42	43	44	44	45	49	50	47	41	47	45	50	51	54	56	53	48	41	39	39	38	38	37	
d	43	44	45	46	46	47	51	52	49	43	50	46	52	54	56	59	55	51	42	41	41	39	39	39	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 75.

TABLE 13. Hourly breathing rates for a sensor fish before and after exposure to 0,5 mg phenol/dm³

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								37	36	32	31	35	37	38	38	39	41	36	32	32	31	30	30	29	34
2	33	33	34	34	34	35	36	41	39	33	36	31	40	42	43	44	43	40	30	29	29	27	26	24	35
3	31	32	34	35	36	36	40	44	39	34	32	38	43	46	47	49	51	41	32	31	31	30	30	29	37
4	33	33	34	35	36	37	42	36	35	29	33	34	36	40	40	41	40	35	30	30	29	29	29	28	34
5	32	32	33	33	33	34	35	36	32	32	35	38	40	41	42	37	35	32	32	31	31	30	30	29	35
6	33	33	34	34	35	46	39	*45*	*40*	*36*	*37*	*44*	*50*	*52*	*50*	*52*	*52*	*55*	*54*	*53*	*53*	*52*	*52*	*44*	*46*
7	*35*	*36*	*37*	*40*	*41*	*41*	*51*	*56*	*58*	*54*	*50*	*44*	*40*	*49*	*52*	*54*	*56*	*56*	*42*	*42*	*41*	*40*	*39*	*37*	*47*
8	*42*	*43*	*44*	*47*	*48*	*48*	*46*	*56*	*56*	*50*	*48*	*54*	*59*	*62*	*63*	*65*	*68*	*55*	*48*	*41*	*41*	*42*	*40*	*38*	*49*
9	*39*	*39*	*40*	*41*	*42*	*43*	*48*	*46*	*44*	*38*	*41*	*36*	*45*	*47*	*48*	*49*	*48*	*45*	*35*	*34*	*34*	*32*	*31*	*29*	*40*
10	*36*	*37*	*40*	*41*	*41*	*41*	*45*	*41*	*38*	*35*	*34*	*38*	*40*	*42*	*42*	*44*	*45*	*39*	*33*	*33*	*33*	*32*	*32*	*31*	*37*
11	*36*	*36*	*36*	*36*	*37*	*37*	*39*	*39*	*38*	*34*	*33*	*37*	*39*	*40*	*40*	*41*	*43*	*38*	*34*	*34*	*33*	*32*	*32*	*31*	*36*
12	*35*	*35*	*36*	*36*	*36*	*37*	*38*	*40*	*37*	*35*	*34*	*37*	*39*	*41*	*41*	*42*	*45*	*37*	*33*	*33*	*32*	*32*	*31*	*31*	*36*
	35	*35*	*36*	*36*	*36*	*37*	*38*																		
a	32	33	34	34	35	38	38	39	36	32	33	35	39	41	42	42	42	37	31	31	30	29	29	28	
b	0,9	0,6	0,5	0,8	1,3	4,8	2,9	3,6	3,0	1,9	2,1	3,0	2,8	3,0	3,4	4,7	5,8	3,7	1,1	1,1	1,1	1,3	1,7	2,2	
c	34	34	35	36	37	47	44	46	42	36	38	41	45	47	49	51	53	44	33	33	32	32	32	32	
d	35	34	35	36	38	50	46	48	44	37	39	43	46	49	51	54	57	46	34	34	33	33	34	33	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 65.

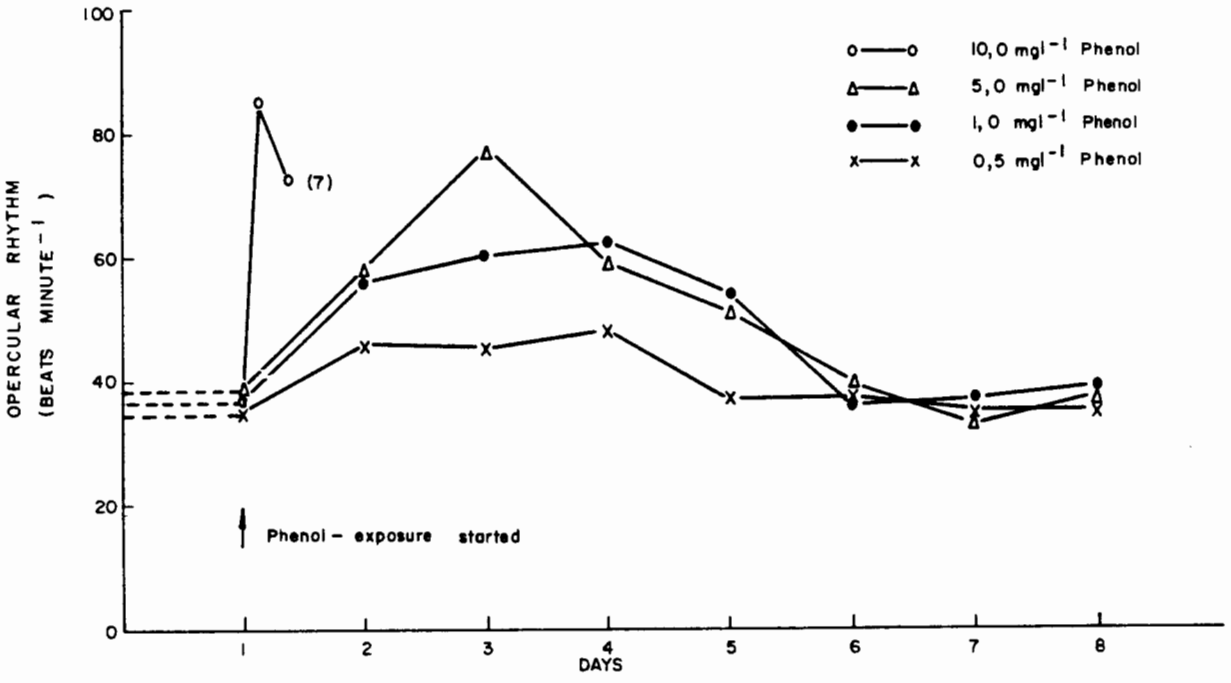

FIGURE 9. Daily average opercular frequencies of *Micropterus salmoides* exposed to different concentrations of phenol. Each point represents the 24-h period preceding it. Numbers in brackets denote times to death (h) for individual fish.

As well as the pathological stresses, the sensor fish also responded behaviourally to intoxication. All sub-lethal concentrations caused an increase in activity levels with a concommitent increase in respiratory activity allied to a longer acclimation period.

The relationship between the toxicity of ammonia solutions and the concentration of undissociated ammonia or ammonium hydroxide suggests that ammonia acts on fish as a true internal poison entering the body by way of the gills. However, the exact nature of its action is not known. It is possible that, as in man, it causes swelling and congestion of mucus membranes and that death may be caused by asphyxia (Jones, 1964). The sensor fish responded fairly rapidly to the highest concentration of ammonia (Table 14), but the effect on the gills was seen to be less severe than that of phenol. An anomalous situation was observed in the breathing rate of the sensor fish exposed to 5,0 mg ammonia (as N)/dm^3 (Table 15)

TABLE 14. Hourly breathing rates for a sensor fish before and after exposure to 10,0 mg ammonia (as N)/dm³

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1									48	42	45	40	49	51	52	53	52	49	39	38	38	36	35	33	44
2	40	41	43	44	45	45	49	50	39	39	42	45	47	48	49	44	43	39	39	38	38	37	37	36	42
3	40	40	41	41	42	53	46	43	43	37	33	42	45	46	49	49	45	44	35	35	34	33	32	30	40
4	35	36	37	40	41	41	51	47	43	38	37	42	47	49	49	51	54	46	40	38	36	36	35	34	41
5	37	37	34	33	41	42	47	48	40	37	36	40	42	44	44	46	47	41	35	35	35	34	34	33	39
6	38	38	38	38	39	39	41	43	*47*	*48*	*52*	*56*	*48*	*56*	*58*	*56*	*58*	*62*	*70*	*68*	*61*	*56*	*54*	*48*	
	50	*58*	*62*	*72*	*74*	*68*	*64*																		
a	38	38	39	39	42	44	47	46	43	39	39	42	46	48	49	49	48	44	38	37	36	35	35	33	
b	2,1	2,1	3,5	4,1	2,2	5,5	3,8	3,1	3,5	2,1	4,8	2,1	2,7	2,7	2,9	3,7	4,7	4,0	2,4	1,6	1,8	1,6	1,8	2,2	
c	42	43	46	47	46	55	54	52	50	43	48	46	51	53	54	56	57	52	42	40	40	38	38	38	
d	44	44	48	50	47	58	57	54	52	44	51	47	53	55	56	58	60	54	44	41	41	39	39	39	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 68.

TABLE 15. Hourly breathing rates for a sensor fish before and after exposure to 5,0 mg ammonia (as N)/dm³

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1									39	36	35	39	41	43	43	45	46	40	34	34	34	33	33	32	38
2	37	37	37	37	38	38	40	42	41	37	36	40	42	43	43	44	46	41	37	37	36	35	35	34	39
3	38	38	39	39	39	40	41	42	43	37	33	42	45	46	49	49	51	44	35	35	34	33	32	30	40
4	35	36	37	40	41	41	45	47	41	39	38	41	43	45	45	46	49	41	37	35	34	34	33	33	39
5	37	37	38	38	38	39	40	42	44	38	39	36	45	47	48	49	48	45	35	34	34	32	31	29	40
6	36	37	39	40	41	41	45	46	*37*	*33*	*31*	*28*	*28*	*29*	*31*	*30*	*33*	*35*	*28*	*25*	*23*	*21*	*21*	*20*	*37*
7	*37*	*43*	*55*	*56*	*61*	*65*	*56*	*49*	*54*	*63*	*68*	*63*	*62*	*67*	*72*	*74*	*74*	*76*	*79*	*79*	*81*	*63*	*61*	*61*	*65*
8	*60*	*59*	*62*	*59*	*59*	*58*	*54*	*51*	*49*	*51*	*46*	*41*	*40*	*45*	*50*	*52*	*52*	*54*	*57*	*48*	*43*	*41*	*39*	*39*	*44*
9	*38*	*37*	*40*	*40*	*37*	*36*	*44*	*45*	*43*	*41*	*42*	*39*	*45*	*48*	*49*	*56*	*50*	*45*	*39*	*38*	*38*	*37*	*37*	*37*	*43*
10	*40*	*41*	*41*	*41*	*42*	*42*	*44*	*47*	*43*	*37*	*33*	*42*	*45*	*46*	*49*	*49*	*45*	*44*	*35*	*35*	*34*	*33*	*32*	*30*	*40*
11	*35*	*36*	*37*	*40*	*41*	*41*	*51*	*47*	*42*	*38*	*35*	*34*	*38*	*40*	*42*	*42*	*44*	*45*	*39*	*35*	*33*	*33*	*32*	*32*	*37*
12	*31*	*36*	*36*	*36*	*36*	*37*	*37*	*39*	*39*	*36*	*35*	*39*	*41*	*43*	*43*	*45*	*46*	*40*	*34*	*34*	*34*	*33*	*33*	*32*	*38*
	37	*37*	*37*	*37*	*38*	*38*	*40*	*42*																	
a	37	37	38	39	39	40	42	44	42	37	36	40	43	45	46	47	48	42	36	35	34	33	33	32	
b	1,1	0,7	1,0	1,3	1,5	1,3	2,6	2,5	2,0	1,1	2,4	2,3	1,8	1,8	2,8	2,3	2,1	2,2	1,3	1,2	0,9	1,1	1,5	2,1	
c	39	38	40	41	42	42	47	49	45	40	41	44	47	47	51	51	52	47	38	37	36	36	36	36	
d	40	39	41	42	43	43	49	50	47	40	42	46	48	49	53	53	54	48	39	38	37	36	37	37	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 64.

where the opercular rhythm decreased markedly for the first 18 h after intoxication. Thereafter the breathing rate attained a very high level throughout the second day of exposure (Figure 10), recovery to a diurnal pattern being accomplished on the third day. The lesser degree of asphyxiation, caused by this sub-lethal concentration may have been responsible for the sensor fish initially assuming greater breathing depth rather than breathing rate, whereas the breathing rate increased during the second day possibly in response to the higher metabolic energy required for excretion. The fact that ammonia did not effect the behaviour pattern of sensor fish as severely as phenol was indicated by the shorter recovery period elicited by the animals exposed to 1,0 and 0,5 mg ammonia (as N)/dm^3 (Tables 16 and 17).

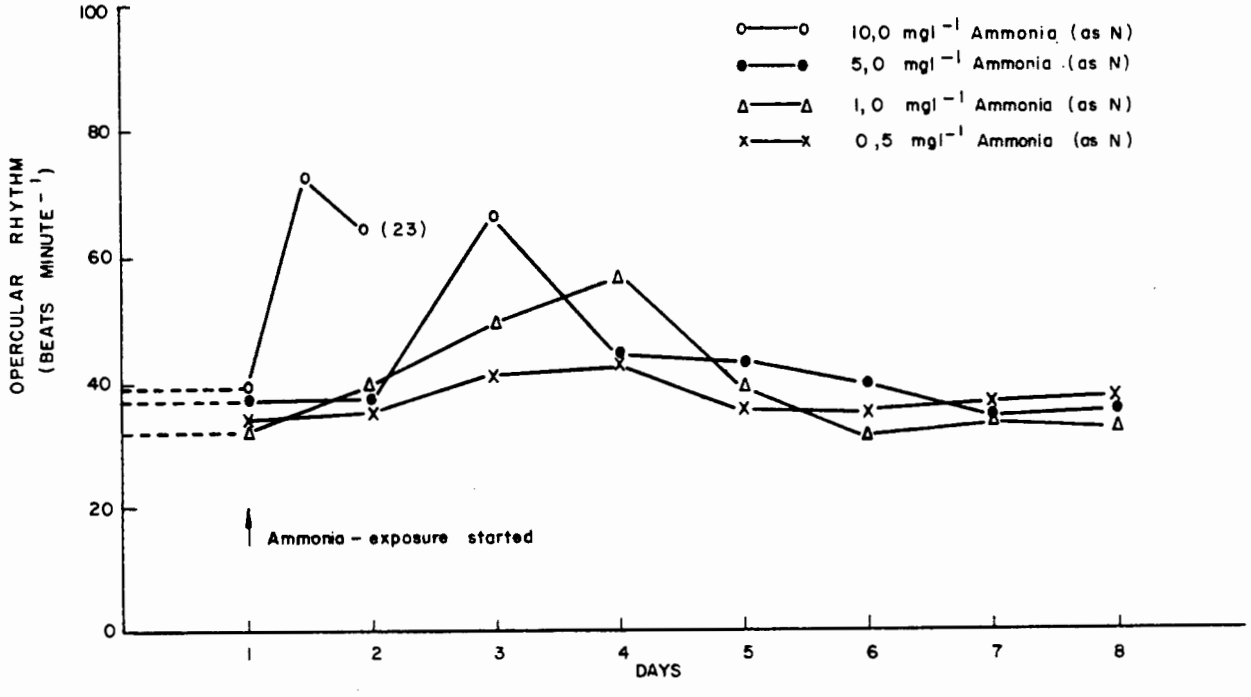

FIGURE 10. Daily average opercular frequencies of *Micropterus salmoides* exposed to different concentrations of ammonia. Each point represents the 24-h period preceding it. Numbers in brackets denote times to death (h) for individual fish.

TABLE 16. Hourly breathing rates for a sensor fish before and after exposure to 1,0 mg ammonia (as N)/dm³

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1									38	33	31	37	42	45	46	48	50	40	31	30	30	29	29	28	36
2	32	32	33	34	35	36	41	43	36	32	31	36	39	43	43	44	44	37	29	28	28	27	26	25	35
3	31	32	33	34	35	36	38	40	38	30	30	36	40	42	43	45	47	39	30	29	29	28	27	26	35
4	31	32	33	35	35	36	40	41	35	32	31	35	36	39	39	41	42	36	30	30	30	29	29	28	34
5	33	33	33	33	34	34	36	38	39	33	36	31	40	42	43	44	43	40	30	29	29	27	26	24	35
6	31	31	34	35	36	36	40	41	*39*	*34*	*35*	*39*	*41*	*43*	*43*	*45*	*46*	*40*	*34*	*34*	*34*	*33*	*33*	*32*	*38*
7	*37*	*38*	*37*	*37*	*38*	*39*	*40*	*42*	*40*	*41*	*44*	*49*	*51*	*52*	*52*	*50*	*57*	*59*	*60*	*62*	*52*	*48*	*38*	*31*	*50*
8	*36*	*48*	*56*	*66*	*58*	*50*	*42*	*52*	*56*	*54*	*52*	*55*	*57*	*59*	*60*	*61*	*63*	*56*	*54*	*54*	*53*	*51*	*51*	*50*	*55*
9	*53*	*53*	*50*	*50*	*55*	*55*	*57*	*59*	*54*	*52*	*47*	*41*	*39*	*41*	*41*	*43*	*44*	*38*	*32*	*32*	*32*	*31*	*31*	*30*	*38*
10	*34*	*34*	*35*	*35*	*36*	*36*	*38*	*40*	*34*	*30*	*29*	*33*	*35*	*36*	*36*	*37*	*40*	*35*	*30*	*30*	*29*	*28*	*28*	*27*	*32*
11	*31*	*30*	*32*	*30*	*32*	*33*	*34*	*35*	*36*	*32*	*31*	*35*	*37*	*38*	*38*	*39*	*41*	*36*	*32*	*32*	*31*	*30*	*30*	*29*	*34*
12	*29*	*31*	*34*	*36*	*36*	*35*	*36*	*37*	*37*	*31*	*34*	*29*	*38*	*40*	*41*	*42*	*41*	*38*	*28*	*27*	*27*	*25*	*24*	*22*	*33*
	26	*31*	*32*	*33*	*34*	*34*	*38*	*39*																	
a	32	32	33	34	35	36	39	41	37	32	32	35	39	42	43	44	45	38	30	29	29	28	27	26	
b	0,9	0,7	0,5	0,8	0,7	0,9	2,0	1,8	1,6	1,2	2,4	2,4	2,2	2,2	2,5	2,5	3,3	1,8	0,7	0,8	0,8	1,0	1,5	1,8	
c	33	33	34	36	36	37	43	44	40	34	37	40	44	46	48	49	52	42	31	31	31	30	30	30	
d	34	34	34	36	37	38	44	45	41	35	38	41	45	48	49	51	54	43	32	31	31	31	31	31	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 64.

TABLE 17. Hourly breathing rates for a sensor fish before and after exposure to 0,5 mg ammonia (as N)/dm³

Day	Time of day																								Daily average
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1									37	33	32	37	40	43	43	44	45	38	30	29	29	28	27	26	35
2	32	33	34	35	36	37	39	41	41	35	38	33	42	44	45	46	45	42	32	31	31	29	28	26	37
3	33	34	36	37	38	38	42	43	38	34	33	37	39	40	40	41	43	38	34	34	33	32	32	31	36
4	35	35	36	36	36	37	38	39	33	33	36	39	41	42	43	38	37	34	34	32	32	31	31	30	36
5	34	34	35	35	36	47	40	37	40	35	33	39	44	47	48	50	52	42	33	32	32	31	31	30	38
6	34	34	35	36	37	38	43	45	*40*	*34*	*37*	*32*	*41*	*43*	*44*	*45*	*44*	*41*	*31*	*30*	*30*	*28*	*27*	*25*	*36*
7	*30*	*33*	*36*	*37*	*38*	*39*	*42*	*43*	*40*	*37*	*36*	*40*	*42*	*46*	*46*	*48*	*51*	*43*	*39*	*38*	*35*	*33*	*33*	*31*	*39*
8	*35*	*36*	*36*	*38*	*38*	*38*	*41*	*43*	*43*	*37*	*33*	*42*	*45*	*46*	*49*	*49*	*45*	*44*	*35*	*35*	*34*	*33*	*32*	*30*	*40*
9	*35*	*36*	*37*	*40*	*41*	*41*	*48*	*47*	*40*	*37*	*35*	*34*	*37*	*39*	*41*	*41*	*42*	*45*	*37*	*33*	*33*	*32*	*32*	*31*	*36*
10	*31*	*34*	*35*	*36*	*36*	*36*	*37*	*38*	*40*	*37*	*33*	*33*	*36*	*39*	*41*	*42*	*43*	*38*	*37*	*34*	*33*	*32*	*32*	*30*	*36*
11	*31*	*30*	*34*	*34*	*34*	*34*	*36*	*47*	*39*	*35*	*34*	*39*	*42*	*45*	*45*	*46*	*47*	*40*	*32*	*31*	*31*	*30*	*29*	*28*	*37*
12	*32*	*35*	*36*	*37*	*38*	*39*	*41*	*43*	*42*	*44*	*39*	*34*	*33*	*38*	*43*	*45*	*45*	*47*	*50*	*41*	*36*	*34*	*32*	*32*	*37*
	31	*30*	*33*	*33*	*30*	*31*	*37*	*38*																	
a	34	34	35	36	37	39	40	41	38	34	34	37	41	43	44	44	44	39	33	32	31	30	30	29	
b	1,1	0,7	0,8	0,8	0,9	4,3	2,1	3,2	3,1	1,0	2,5	2,5	1,9	2,6	3,0	4,6	5,4	3,4	1,7	1,8	1,5	1,6	2,2	2,4	
c	36	35	37	38	38	48	45	47	44	36	39	42	45	48	50	53	55	45	36	35	34	33	34	33	
d	37	36	37	38	39	50	46	49	46	37	41	43	46	50	51	56	58	47	37	36	35	34	35	35	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 71.

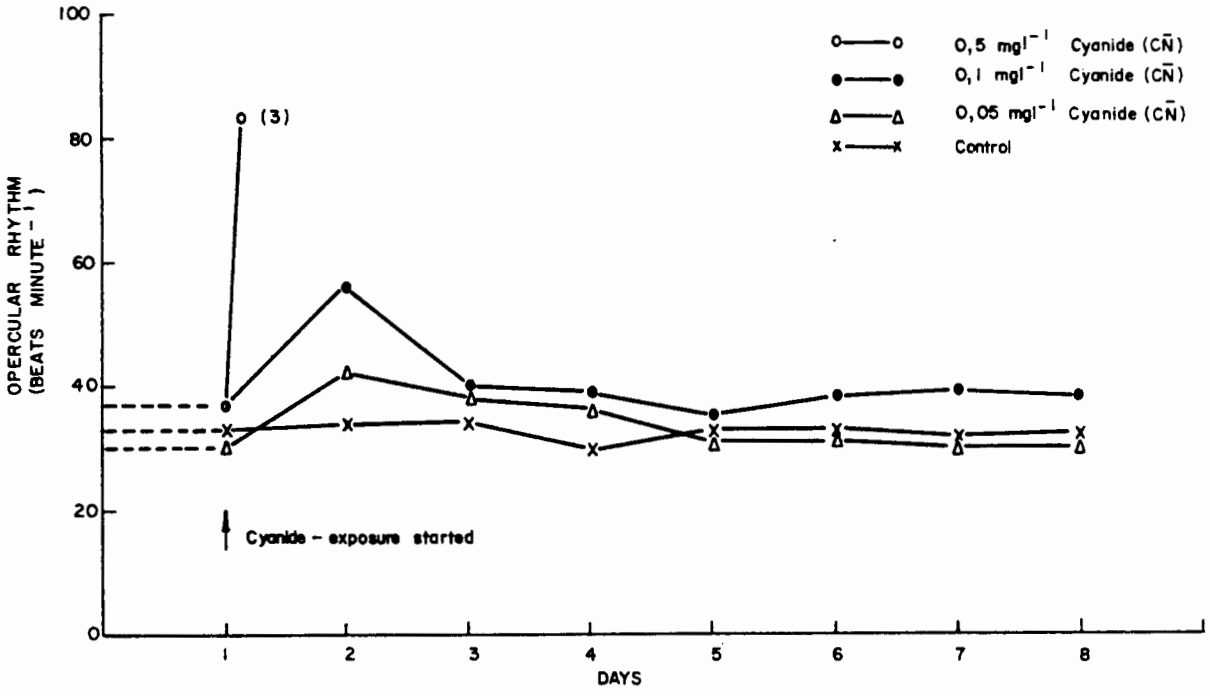

FIGURE 11. Daily average opercular frequencies of *Micropterus salmoides* exposed to different concentrations of cyanide. Each point represents the 24-h period preceding it. Numbers in brackets denote times to death (h) for individual fish.

Sensor fish responded to cyanide concentrations quite rapidly. The recovery periods, however, were much shorter than for equivalent concentrations of the other toxicants utilized in the analyses (Tables 18, 19 and 20, Figure 11). There is no evidence that cyanide causes any damage to the gill apparatus itself (Jones, 1964). However, the fact that gills of fish dying in cyanide were seen to be much brighter than the gills of normal fish was thought to prove that cyanide affected oxygen utilization by inactivating respiratory enzymes (Alexander, Southgate, and Bassindale 1935). The almost immediate increase in the breathing rate of sensor fish exposed to cyanide would indicate that the process of respiratory enzyme inhibition is very fast leading to a sudden oxygen debit. Recovery from cyanide poisoning is thought to be accomplished by the conversion of cyanide to thiocyanate under the influence of the enzyme rhodanase (Williams, 1949). The decline in opercular rate subsequent to the brief

TABLE 18. Hourly breathing rates for a sensor fish before and after exposure to 0,5 mg CN^-/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								42	39	36	35	39	41	43	43	45	46	40	34	34	34	33	33	32	38
2	37	37	37	37	38	38	40	42	39	37	36	39	41	43	43	44	47	39	35	35	34	34	33	33	38
3	37	37	38	38	38	39	40	47	43	37	33	42	45	46	49	49	45	44	35	35	34	33	32	30	40
4	35	36	37	40	41	41	53	47	41	36	35	40	45	47	47	49	52	43	38	36	34	34	33	32	39
5	35	35	32	31	39	40	44	39	40	35	34	38	40	41	41	42	44	39	35	35	34	33	33	32	37
	36	36	37	37	37	38	39	*71*	*78*	*83*															
a	36	36	36	37	39	39	43	43	40	36	35	40	42	44	45	46	47	41	35	35	34	33	33	32	
b	1,0	0,8	2,4	3,4	1,5	1,3	5,8	3,5	1,7	0,8	1,1	1,5	2,4	2,5	3,3	3,1	3,1	2,4	1,5	0,7	0	0,6	0,5	1,1	
c	38	38	41	43	42	42	55	50	44	38	37	43	47	49	51	52	53	46	38	36	34	35	34	34	
d	39	38	42	45	43	43	58	53	45	38	38	44	49	50	53	54	55	47	39	37	34	35	34	35	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 70.

TABLE 19. Hourly breathing rates for a sensor fish before and after exposure to 0,1 CN^-/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								43	41	35	38	33	42	44	45	46	45	42	32	31	31	29	28	26	37
2	30	35	37	38	38	38	42	36	32	32	35	38	40	41	42	37	36	32	32	31	31	30	30	29	35
3	32	34	34	34	35	46	39	41	37	32	28	37	40	41	44	44	40	37	28	28	27	27	26	24	35
4	26	28	30	40	40	41	45	43	38	33	32	37	42	44	44	46	49	40	35	33	31	31	30	29	36
5	32	32	29	28	36	37	41	44	40	34	30	39	42	43	46	46	42	41	33	32	31	30	29	27	37
6	30	34	35	37	38	38	47	*59*	*72*	*76*	*80*	*81*	*74*	*66*	*58*	*56*	*55*	*62*	*65*	*58*	*53*	*51*	*48*	*42*	*57*
7	*41*	*50*	*40*	*37*	*47*	*48*	*49*	*43*	*40*	*37*	*36*	*40*	*42*	*44*	*44*	*46*	*47*	*41*	*35*	*35*	*35*	*34*	*34*	*33*	*39*
8	*38*	*36*	*39*	*38*	*38*	*38*	*41*	*41*	*40*	*36*	*35*	*39*	*41*	*42*	*43*	*42*	*45*	*40*	*36*	*36*	*35*	*34*	*33*	*31*	*38*
9	*34*	*37*	*38*	*37*	*39*	*39*	*40*	*41*	*38*	*36*	*35*	*38*	*40*	*42*	*42*	*43*	*46*	*38*	*34*	*34*	*33*	*33*	*32*	*32*	*37*
10	*35*	*36*	*38*	*37*	*37*	*38*	*39*	*45*	*40*	*35*	*33*	*39*	*44*	*47*	*48*	*50*	*52*	*42*	*33*	*32*	*32*	*31*	*31*	*30*	*38*
11	*34*	*34*	*35*	*36*	*37*	*38*	*44*	*46*	*42*	*36*	*32*	*41*	*44*	*45*	*48*	*48*	*44*	*43*	*34*	*34*	*33*	*32*	*31*	*29*	*39*
12	*34*	*35*	*36*	*39*	*40*	*40*	*50*	*44*	*40*	*36*	*35*	*40*	*43*	*46*	*46*	*47*	*48*	*41*	*33*	*32*	*32*	*31*	*30*	*29*	*38*
	35	*36*	*37*	*38*	*39*	*40*	*42*																		
a	30	33	33	35	37	40	43	41	38	33	33	37	41	43	44	44	42	38	32	31	30	29	29	27	
b	2,5	2,8	3,4	4,7	2,0	3,7	3,2	3,2	3,5	1,3	4,0	2,3	1,1	1,5	1,5	3,9	4,9	4,0	2,6	1,9	1,8	1,5	1,7	2,1	
c	35	38	40	45	41	47	49	48	45	36	40	41	43	46	47	51	52	46	37	35	34	32	32	31	
d	36	40	42	48	42	50	51	50	47	37	43	43	44	47	48	54	55	49	39	36	35	33	33	33	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 68.

TABLE 20. Hourly breathing rates for a sensor fish before and after exposure to 0,05 mg CN^-/dm^3

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								34	31	29	27	30	32	34	35	36	38	31	29	29	28	26	26	25	30
2	25	25	27	27	31	31	32	38	36	30	33	28	37	39	40	41	40	37	27	26	26	24	23	21	32
3	28	29	31	32	33	33	37	34	33	29	28	32	34	35	35	36	38	33	29	29	28	27	27	26	31
4	30	30	31	31	31	32	33	37	33	27	23	32	35	36	39	39	35	34	25	25	24	23	22	20	29
5	25	26	27	30	31	31	41	36	33	30	29	33	36	37	37	39	40	34	28	28	28	27	27	26	32
6	31	31	31	31	32	32	34	*39*	*36*	*40*	*41*	*44*	*43*	*50*	*52*	*56*	*60*	*63*	*68*	*50*	*41*	*32*	*28*	*24*	*41*
7	*24*	*24*	*28*	*30*	*33*	*38*	*46*	*44*	*40*	*36*	*35*	*40*	*43*	*46*	*46*	*47*	*48*	*41*	*33*	*32*	*32*	*31*	*30*	*29*	*38*
8	*33*	*35*	*37*	*38*	*39*	*40*	*42*	*44*	*38*	*33*	*31*	*37*	*32*	*45*	*46*	*48*	*50*	*40*	*31*	*30*	*30*	*29*	*29*	*28*	*36*
9	*31*	*32*	*33*	*34*	*35*	*36*	*40*	*38*	*36*	*30*	*33*	*37*	*37*	*39*	*40*	*41*	*40*	*28*	*27*	*26*	*26*	*24*	*22*	*21*	*32*
10	*25*	*26*	*30*	*34*	*35*	*35*	*39*	*40*	*35*	*30*	*29*	*34*	*39*	*41*	*41*	*43*	*46*	*37*	*32*	*30*	*28*	*28*	*27*	*26*	*33*
11	*29*	*29*	*26*	*25*	*33*	*34*	*38*	*39*	*35*	*29*	*25*	*34*	*37*	*38*	*41*	*41*	*37*	*36*	*27*	*27*	*26*	*25*	*24*	*22*	*32*
12	*27*	*28*	*29*	*32*	*33*	*33*	*43*	*37*	*38*	*35*	*27*	*27*	*33*	*37*	*39*	*40*	*42*	*44*	*36*	*27*	*26*	*26*	*25*	*24*	*32*
	23	*28*	*29*	*30*	*32*	*32*	*33*																		
a	28	28	29	30	32	32	35	36	33	29	28	31	35	36	37	38	38	34	28	27	27	25	25	24	
b	2,8	2,6	2,2	1,9	0,9	0,8	3,7	1,8	1,8	1,2	3,6	2,0	1,9	1,9	2,3	2,2	2,1	2,2	1,7	1,8	1,8	1,8	2,4	2,9	
c	33	33	34	34	33	34	43	39	37	31	35	35	39	40	42	43	42	38	31	31	30	29	30	29	
d	35	35	35	35	34	34	45	40	38	32	37	36	40	41	43	44	44	39	32	32	31	30	31	31	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99% confidence limits.

Figures in italics represent the hourly breathing rates after toxicant addition.

The stress response for this fish was 66.

period of heightened respiratory activity may be due either to the efficiency of this detoxification enzyme system or to the property of cyanide as a respiratory depressant or both.

CONCLUSIONS

The fact that such a multiplicity of adverse toxic effects upon the physiological well-being of fish, as described, gave rise to recognizable alterations in a parameter which was easily measured, indicated that the utilization of fish respiratory rate to detect and monitor a wide range of toxicants was feasible. It was considered probable, therefore, that the technique could be adopted in a variety of situations where control of industrial effluent discharge, thus maintenance of receiving water quality, is necessary.

Changes in physiological processes do not, naturally, provide the type of absolute response which death does, and it is, therefore, necessary to select as the response a degree of change in function which, for monitoring purposes, may be regarded as significant. The more rapidly a biological monitoring system indicates a toxic hazard, the more useful it will be. The degree of response selected should, therefore, allow the required sensitivity and yet avoid frequent false alarms. Accordingly, more than one sensor fish should be employed in the system as a whole in order to reduce the probability that any outcome would be determined by an under-or over-responsive individual. A positive alarm situation should only be recognized when a majority of the sensors show aberrant behaviour.

All statistical techniques employed to establish critical response levels of intoxication were successful in providing advanced warning of hazardous increases in toxicity (Table 23). In this respect the stress response level of respiratory rate, i.e. the maximum rate recorded on transfer to the experimental chamber, was the least useful in that the time-lag between intoxication and sensor fish response, especially during exposure to sub-lethal conditions, did not occur timeously enough to prevent possible harm. The use of breathing rate variance for each hourly period of the day, in order to establish critical limits of

response based upon significant increases in breathing rate at two confidence levels (95 % and 99 %), was more than adequate to provide for timeous warning of impending danger. The difference in significance levels did not provide any advantage one over the other when hourly variances were considered, but maximum critical limits were attained sooner at a confidence level of 95 % than at 99 % . The use of hourly variances, rather than the maximum, would, therefore provide for a quicker recognition of response. However, it was found that the usefulness of this method was negated by the fact that the number of false responses occurring in control fish, in the absence of intoxication was much higher (Table 24). Further, the use of hourly variances would necessitate manual or automatic manipulation of numerical values in any control system, thus involving the employment of either full-time operative personnel or sophisticated, and expensive, microprocessing.

TABLE 24. Degree of false response (responses occurring in the absence of intoxication) obtained utilizing different statistical criteria*

Statistic	Number of false responses
95 % Confidence level - hourly variance	11
- maximum variance	2 in 576 h
99 % Confidence level - hourly variance	6
- maximum variance	NIL

* Figures extracted from Tables 21 an 22.

The effect of photoperiod upon respiratory rate demonstrated potential problems in the use of maximum critical limits. Certain water quality changes which, in water management terms, would not be regarded as significant could provide a positive response. The need to control such physical chemical parameters as temperature, oxygen concentration and pH, is absolute. Further, the time-lag between intoxication and sensor fish response could be artificially extended during dark periods because of the difference between the maximum critical level, invariably calculated from a day time variance, and the low level night time respiratory rate. Whether use of the maximum variance would provide less

TABLE 21. Hourly breathing rates for a sensor fish utilized as control during experimental investigation of intoxication by copper

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								40	38	32	30	35	39	41	42	42	43	39	30	28	28	26	25	23	34
2	29	31	33	34	35	35	39	38	37	33	32	37	39	40	39	40	42	37	34	34	33	32	32	30	35
3	32	33	33	34	35	36	37	37	36	32	31	35	37	38	38	39	41	36	32	32	31	30	30	29	34
4	31	32	36	34	36	35	37	39	35	29	25	35	36	37	41	41	37	36	28	28	26	25	24	22	33
5	21	26	29	37	38	41	43	37	36	31	30	34	36	37	37	40	41	35	29	29	29	28	28	27	33
6	30	32	34	34	34	35	37	38	35	33	32	35	37	39	39	40	43	35	31	31	30	30	29	29	34
7	33	33	34	34	35	34	36	36	32	32	36	38	40	41	42	37	36	32	32	31	31	30	30	29	35
8	33	33	34	34	35	46	39	38	35	31	30	35	37	39	39	40	41	35	30	30	30	29	29	28	34
9	33	33	33	33	34	34	36	36	33	31	29	32	34	36	39	40	42	33	31	31	30	28	28	27	32
10	30	30	27	27	32	32	34	35	34	30	29	33	35	36	36	37	39	34	30	30	29	28	28	27	32
11	31	31	32	32	32	33	34	36	32	30	31	28	34	37	38	45	39	34	28	27	27	26	26	26	32
12	29	30	30	30	31	31	33	41	38	30	30	36	40	42	43	45	47	39	30	29	29	28	27	26	35
	31	32	33	35	35	36	40																		
a	29	31	31	35	36	36	39	38	36	31	30	35	37	39	39	40	41	37	31	30	29	28	28	26	
b	4,4	2,8	3,0	1,3	1,5	2,6	2,6	1,3	1,1	1,5	2,7	1,1	1,5	1,8	2,1	1,1	2,3	1,5	2,4	2,7	2,7	2,9	3,4	3,6	
c	37	36	37	37	39	42	44	41	39	34	35	37	40	42	44	43	45	40	35	36	35	34	34	33	
d	40	38	39	38	40	43	45	42	39	35	37	38	41	43	45	43	47	41	37	37	36	36	36	35	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 % and 99 % cofindence limits.

The stress response for this fish was 68.

TABLE 22. Hourly breathing rates for a sensor fish utilized as control during experimental investigation of intoxication by cyanide

Day	Time of day																								Daily average rate
	am												pm												
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1								36	35	31	30	34	36	37	37	38	40	35	31	31	30	29	29	28	33
2	31	32	33	34	33	34	35	36	33	31	30	32	34	37	38	39	41	33	29	29	28	27	26	27	32
3	30	31	32	32	32	33	37	34	30	30	33	36	38	39	40	35	34	30	30	29	29	28	28	27	33
4	31	31	32	32	33	44	37	41	37	33	32	37	40	43	43	44	45	38	30	29	29	28	27	26	35
5	32	33	34	35	36	37	39	38	33	33	31	34	36	38	39	40	42	35	33	33	32	30	30	29	34
6	32	32	29	29	34	34	36	42	38	32	28	37	40	41	44	44	40	39	30	30	29	28	27	25	35
7	30	31	32	35	36	36	46	37	37	32	31	36	41	43	43	45	48	39	34	32	30	30	29	28	35
8	31	31	28	27	35	36	40	42	35	29	32	27	36	38	39	40	39	36	26	25	25	23	22	20	31
9	27	28	30	31	32	32	36	41	37	32	30	36	41	44	45	47	49	39	30	29	29	28	28	27	35
10	31	31	32	33	34	35	40	42	37	33	32	37	40	43	43	44	45	38	30	29	29	28	27	26	35
11	32	33	34	35	36	37	39	37	36	32	31	35	37	38	38	39	41	36	32	32	31	30	30	29	34
12	33	33	34	34	34	35	36	39	36	33	32	36	38	40	40	42	43	37	31	31	31	30	30	29	35
	34	34	34	34	35	35	37																		
a	31	32	32	32	34	36	37	37	34	32	31	35	37	39	39	39	40	34	31	30	30	28	28	27	
b	0,8	0,8	1,9	2,3	1,5	4,5	1,5	2,7	2,6	1,3	1,3	2,0	2,3	2,5	2,3	3,3	4,0	3,0	1,5	1,8	1,5	1,1	1,6	1,1	
c	33	34	36	37	37	45	40	42	39	34	34	38	41	44	44	46	48	40	34	34	33	31	31	30	
d	33	34	37	38	38	48	41	44	40	35	35	40	43	45	45	48	51	42	35	35	34	31	32	30	

a, b, c and d are, respectively, the mean, standard deviation, maximum 95 and 99 % confidence limits.

The stress response for this fish was 72.

TABLE 23. The relationships between time to opercular rhythm response, utilizing various statistical parameters, and time to death for *Micropterus salmoides* subjected to different toxicant regimes

Nominal concentration of toxicant (mg/dm³)		Times to response (h) utilizing 95 % confidence limits hourly	95 % confidence limits maximum	99 % confidence limits hourly	99 % confidence limits maximum	Stress response	Time to death (h)
Copper	5,0	1	2	1	2	2	6
	1,0	1	2	1	3	3	18
	0,1	2	4	3	7	22	-
Cadmium	1,0	4	4	4	9	15	46
	0,5	5	10	5	15	23	55
	0,1	6	13	6	27	34	84
	0,05	12	16	12	17	-	-
Phenol	10,0	1	1	1	1	1	7
	5,0	2	6	3	9	23	-
	1,0	2	18	3	25	53	-
	0,5	5	11	5	26	58	-
Ammonia	10,0	2	7	2	10	11	23
	5,0	18	19	18	19	22	-
	1,0	11	33	11	33	44	-
	0,5	35	-	35	-	-	-
Cyanide	0,5	1	1	1	1	1	3
	0,1	1	1	1	1	2	-
	0,05	3	5	3	7	12	-

efficient monitoring was, therefore, a factor necessitating further investigation. Nevertheless, from the results obtained during preliminary experimentation, it seemed that the utilization of the maximum 95 % confidence limit to provide the sensor fish response level would best suit the requirements.

THE CONSTRUCTION OF AN ELECTRONIC UNIT DESIGNED TO MONITOR EFFECTS OF CHANGES IN WATER QUALITY UPON FISH OPERCULAR RHYTHMS·

Preliminary investigations into the effects of intoxication upon fish have shown increase in opercular rhythm to be a viable pre-mortal indication of toxic stress. The response, processed as a change in electric potential, was suitable for automatic and continuous measurement. Thus it may be used, in conjunction with appropriate technical apparatus, to operate an early warning device which indicates the appearence of toxic conditions.

The electronic monitoring system was designed bearing in mind two major aspects: simplicity and cost effectiveness. As well as being constructed to operate automatically and continuously, the monitoring unit was built in such a way as to minimize the need for maintenance and operation by highly trained personnel. The use of a single critical limit setting, for each sensor channel, eliminated the necessity for sophisticated computerization technology. It was decided to utilize ten fish sensor channels to preclude alarm situations being established due to over-responsive behaviour on the part of sensitive individuals. Two control channels were also included to guard against alarms being initiated during experimental monitoring due to any outside interference.

GENERAL DESCRIPTION

No alterations were deemed necessary in the design of the electrode chambers and they were constructed according to the design previously described (Figure 1). The input signals to the system arose as a result of a change in potential between two stainless-steel electrodes, the change being produced by the opercular movements of the sensor fish.

The electronic system (Plates 4 and 5, Figure 12) provided for simultaneous monitoring of the opercular rhythms of twelve independent channels. A permanent record of variations in breathing rate with time was obtained via two 6-channel multipoint chart recorders (ARUCOMP 4902).

Signals from the electrode chambers were fed, via coaxial connectors, to preamplifiers, which boosted the low level breathing signal (of the order of microvolts) about 2 000 times. The amplified signal was fed to a two-

stage high-pass filter, which exhibited an extremely sharp cut-off characteristic below 0,4 Hz. This unit filtered out the slow potential variations produced by other muscular activities of the sensor fish. The pulses were summed over 60-s periods, summing being achieved by an integrating circuit, controlled by a 30-s clock. The integrating circuit also converted the pulses to a d.c voltage which was directly proportional to the breathing rate, as opercular beats per minute. The outputs from the twelve integrators were passed to the two 6-channel multipoint recorders, as well as to the alarm facility.

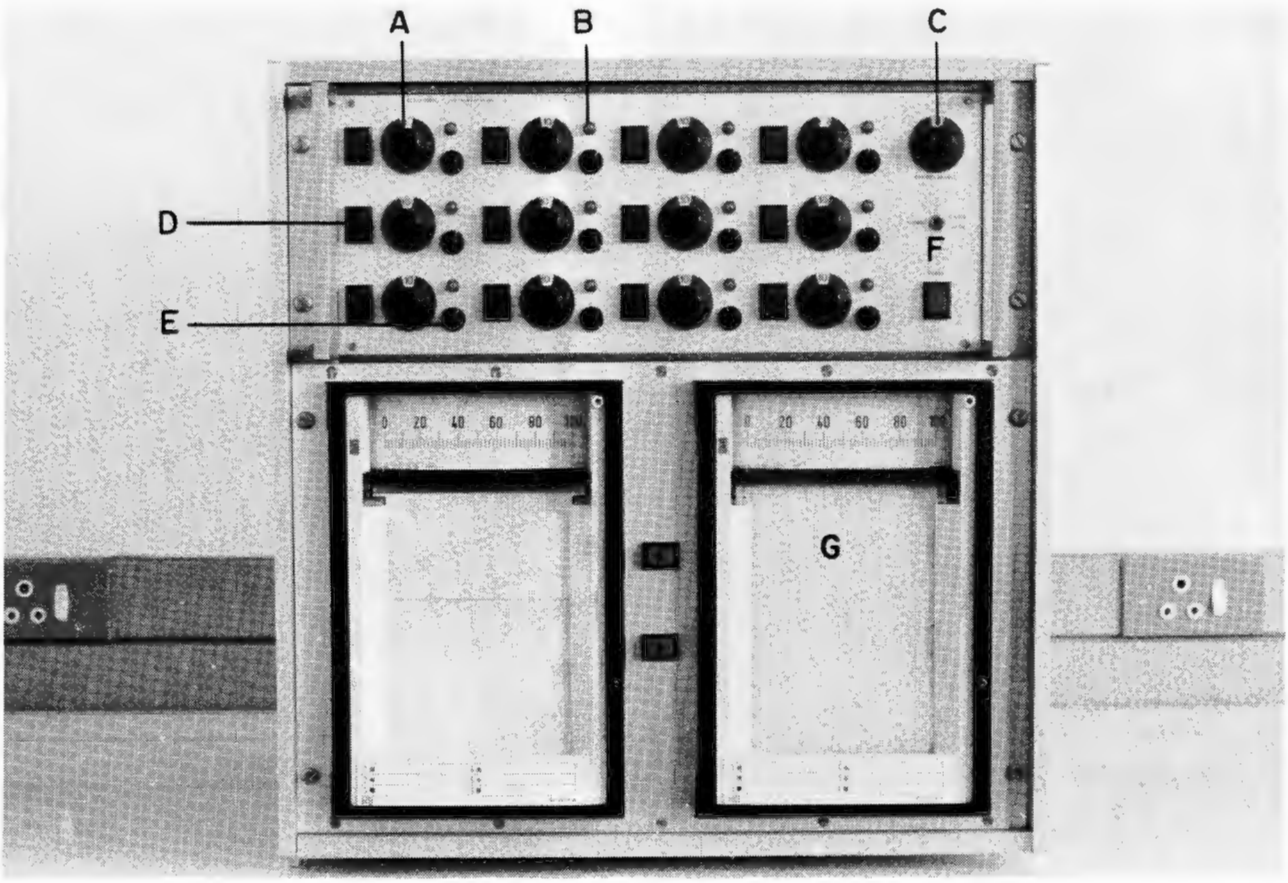

PLATE 4. Front view of electronic monitoring system including processing unit and recorders.

A - alarm threshold control
B - trigger level indicator
C - channel selector
D - channel alarm indicator
E - trigger level control
F - mode selector
G - recorder.

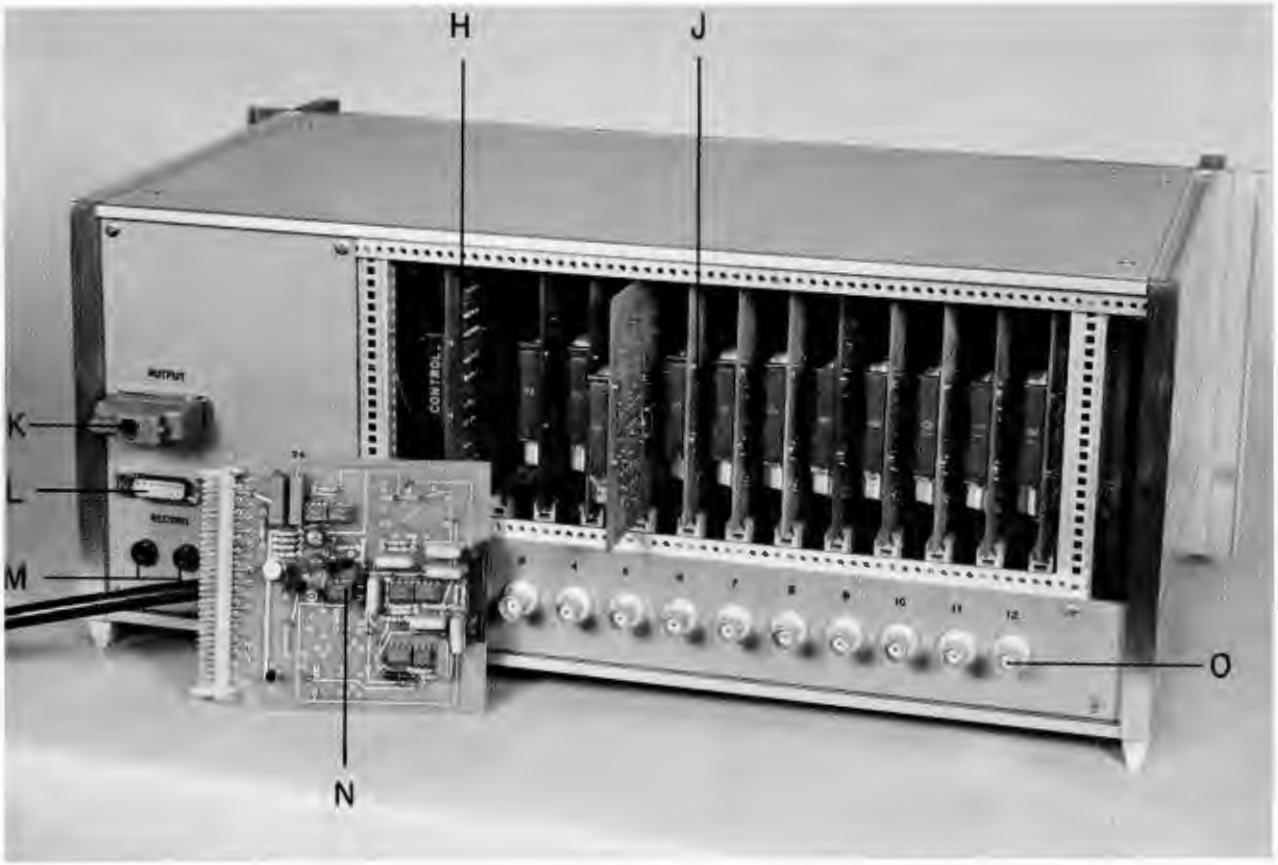

PLATE 5. Rear view of processing unit with panel removed.

H - control logic card
J - one of twelve identical channel printed circuit cards
K - alarm outputs
L - twelve channel recorder output
M - single channel recorder output (signal check facility)
N - overall view of one of the channel printed circuit cards
O - signal input connectors.

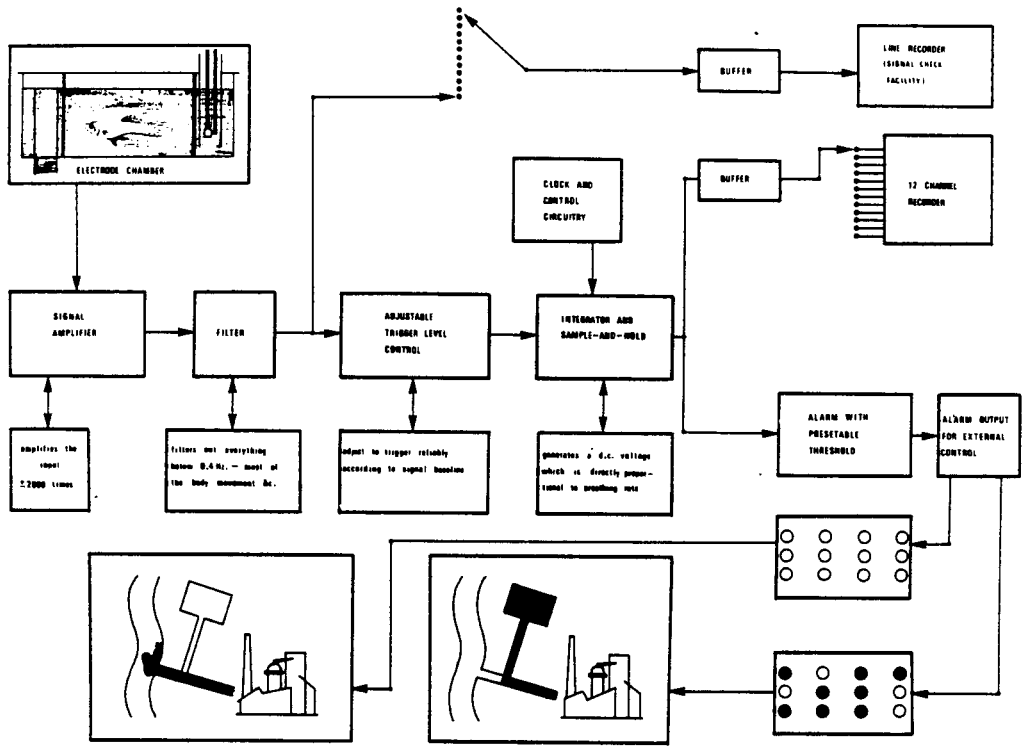

FIGURE 12. Schematic diagram of the automatic monitoring system including a possible industrial application.

Each channel had a presetable alarm facility. Should the breathing rate of any fish sensor exceed a predetermined limit, indicative of a toxic condition, the alarm circuit was triggered and remained in that state until manually reset. The triggered condition was indicated by a red warning light provided for each channel. At the same time, a d.c voltage was initiated at the rear panel output of the appropriate channel. This output may be utilized to afford control over the water being monitored. A signal from the rear panel output may, by means of electrically operated valves, be used to divert the water to a holding facility where the nature of the toxicant may be determined. This output signal would be initiated only after a majority of the alarm circuits had been activated.

The outputs of the high-pass filters were also taken to a 12-position selector switch. The selected signal was passed through a buffer to an output at the rear panel. The breathing pulses were monitored at this

output by a separate single-channel potentiometric recorder. Thus, the opercular rhythm of each sensor fish could be studied independently and in detail.

TECHNICAL DESCRIPTION

Signals from the electrode chambers were fed to the inputs of 12 identical channels (Figure 13). Each channel consisted of a preamplifier which boosted the low level breathing signal (of the order of microvolts) by a factor of 2 000. It also provided a high impedance to match the electrodes. A high-pass filter filtered out variations in potential due to body movements of sensors, such variations being somewhat slower than those caused by the lowest breathing rate of interest. An integrator converted the pulses to a d.c voltage which was proportional to the breathing rate. Reset and transfer pulses for all 12 integrators were obtained from a single control board. The outputs from the 12 integrators were passed to the two 6-channel multipoint recorders.

Each channel had a presetable alarm facility. Whenever the breathing rate of that input exceeded the preset value, an alarm for the channel was triggered and remained so until manually reset. The rear panel was provided with 12 alarm outputs, each rising to +6V when the appropriate alarm was triggered.

The outputs of the high-pass filters were all taken to a twelve-position selector switch. The selected signal was passed through a buffer to an output at the rear panel. The breathing pulses could be monitored at this output by a separate, single-channel recorder. When the front panel switch was moved from NORMAL to SIGNAL CHECK, the selected signal was routed through channel 1 of the 12-channel multipoint recorder system and could be monitored without using a separate recorder unit. However, a line pen needed to be attached to the multipoint recorder for this purpose, enabling a continuous line to be drawn rather than a series of dots.

A single power supply unit provided the various operating voltages for the circuitry. These consisted of

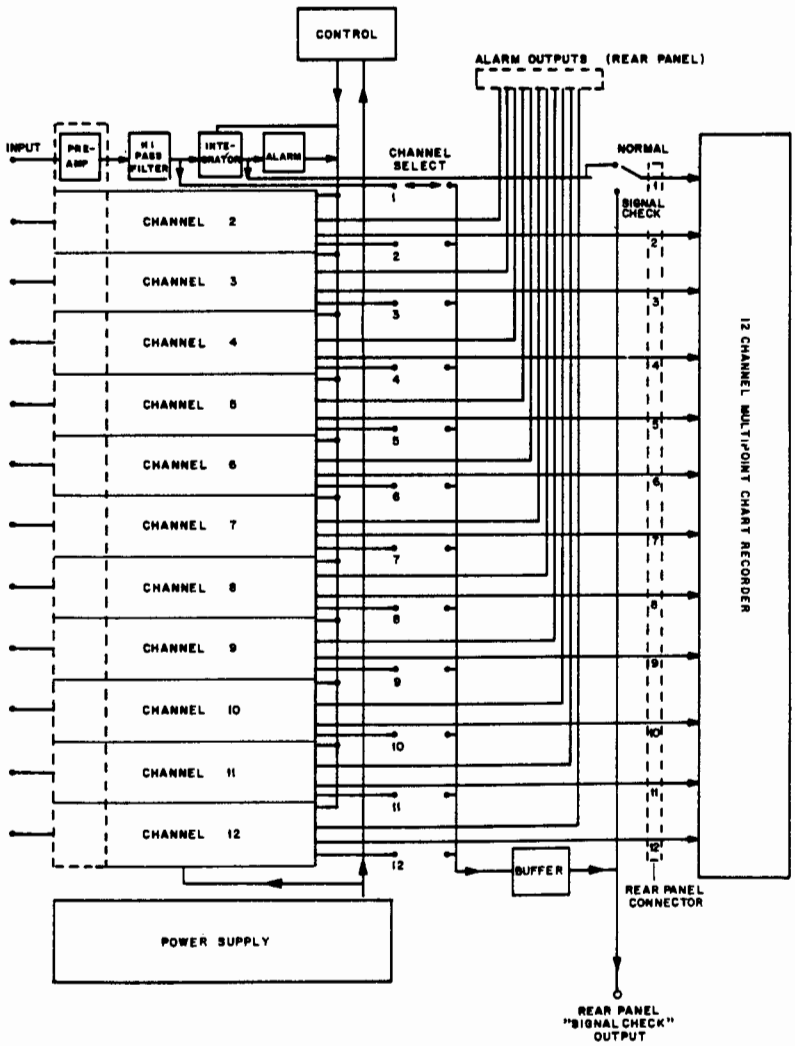

FIGURE 13. Block diagram of the electronic processing system.

(a) +15 and -15 V regulated, for all operational amplifiers and associated circuitry,

(b) +10 and -10 V regulated, derived from the 15 V supplied for the preamplifiers,

(c) +5 V regulated, for the logic circuitry on the control board, and

(d) +6 V (approximately) unregulated, for all indicator lamps.

CIRCUIT DESCRIPTION

The input signals were fed, via 12 coaxial connectors on the rear panel, to the preamplifiers which were mounted in a screened compartment (Figure 14). These preamplifiers were provided with +10 and -10 V supplies. Adequate care was taken to ensure correct grounding of these stages. Each preamplifier consisted of a high impedance voltage follower input stage with a voltage gain of 16. The output of this stage was capacitor coupled to remove the d.c component which was normally present as a result of electrolyte action within the electrode chambers. The second stage consisted of a normal inverting amplifier with a gain of 50. High frequency compensation was applied to this stage to ensure stability.

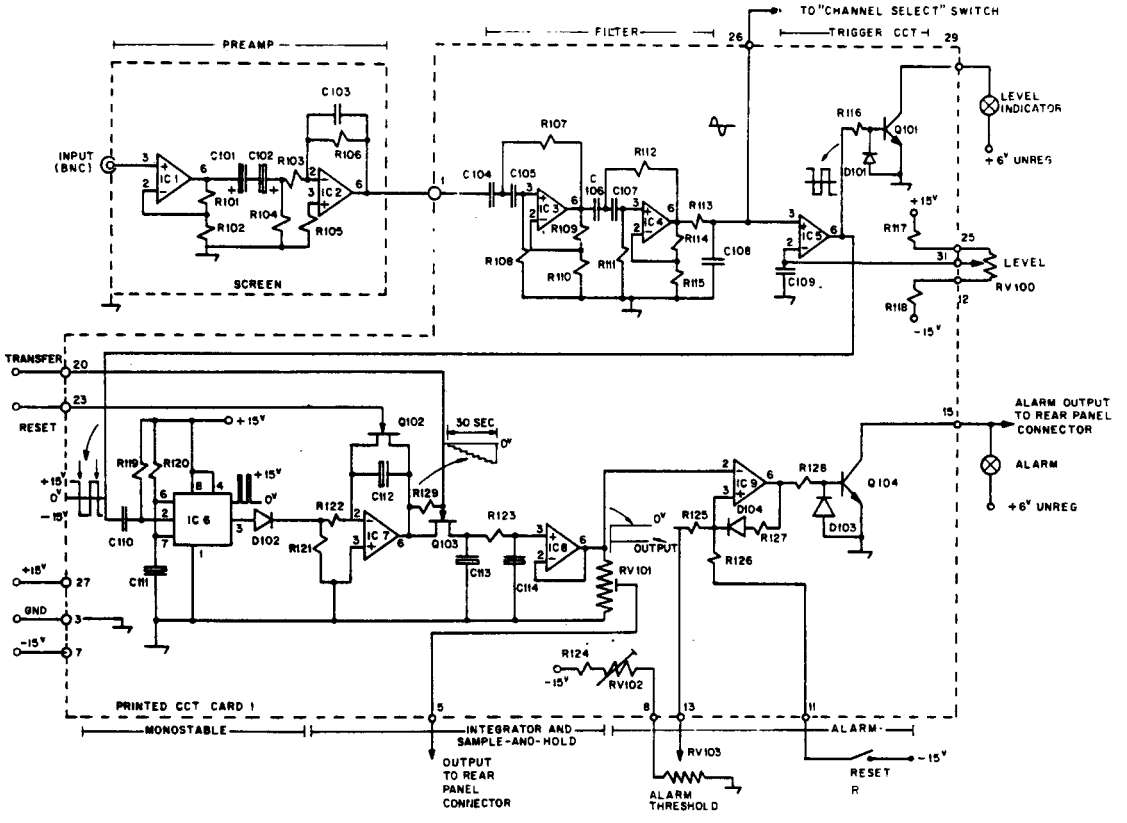

FIGURE 14. Preamplifier and printed circuit card 1.

PRINTED CIRCUIT CARD 1

Twelve of these boards were used, each carrying the signal processing and alarm circuitry for one channel (Figure 14).

The amplified signal from the preamplifier was fed to a two-stage high-pass filter. This network exhibited an extremely sharp cut-off characteristic below 0,4 Hz. The filter unit filtered out the slow potential variations produced by body movement of the sensor fish. It had a gain of about four. R113 and C108 (see component list) at the output filtered out any 50 Hz interference. A take off was provided at this point to the channel select switch for the SIGNAL CHECK facility.

The signal also passed to a trigger circuit comprising IC5 and associated circuitry. As the signal at Pin 3 crossed the preset level at Pin 2, the output changed state from +15 to -15 V, or vice-versa. The sensitivity of the trigger circuit could be adjusted by altering the level. Q101 drove the level indicator which flashed on and off as the signal at Pin 3 crossed the preset level, providing an indication of correct triggering.

The sharp negative-going transition of the trigger circuit output was used to drive a monostable circuit formed by IC6. Thus a pulse of 15 ms width was obtained at the output for each breath taken by the sensor fish in the electrode chamber.

The pulses were summed over 30-s periods, the value after 30 s being transferred to a hold circuit. The summing was achieved by the integrating circuit comprising IC7. The output was incremented linearly by the 15-ms pulses at the input. After 30 s, the peak value of the staircase waveform was transferred by the gate Q103. Immediately after this transfer the integrator was reset via gate Q102. The transfer and reset pulses were derived from a 30-s clock on the control board. The transferred potential was held by C113. R123 and C114 smoothed out the sudden changes which appeared on C113. The voltage follower circuit IC8 provided an extremely high impedence to the voltage across C114. Potentiometer RV101 allowed adjustment of the full scale sensitivity of each channel. The output was taken to the multipin connector on the rear panel, and from there to the 12-channel recording system.

The output of IC8 was also passed to the alarm circuit. This consisted of IC9 and its circuitry. The signal was applied to the inverting input of IC9. The non-inverting input was connected to an adjustable potential representing the preset threshold above which the circuit must trigger.

This potential was adjusted by means of a potentiometer with a 270-deg scale. The scale was graduated in breaths per minute to a maximum of 100. A preset potentiometer RV102 was included so that the circuit could be calibrated. When the signal at the non-inverting input became more negative than the preset potential at the inverting input, the output of IC9 immediately changed from -15 to +15 V . A diode in the feedback circuit pulled the non-inverting input further positive, and the circuit was locked in the ON position. Whenever the negative potential at the inverting input decreased below the threshold, the circuit remained in the ON position until reset by the manual reset button. The drive transistor Q104 activated a warning lamp and provided an output via the 12-pin connector at the rear panel.

POWER SUPPLY

The power supply circuitry (Figure 15) was straightforward and requires little comment. The +15 and -15 V supplies for the 741 operational amplifiers and their associated circuitry were regulated using LM309 integrated circuit regulators. These voltages were able to be adjusted by means of the ten-turn helitrim preset potentiometers. An extra diode was included between the bridge and the smoothing capacitor of the +15 V supply, so that the 100 Hz reference could be taken off for the clock on the control board. The +10 and -10 V supplies were derived from the 15 V supplies and were used for the preamplifiers. Owing to the extremely high gain of the system, these supplies were separated from the 15 V supplies to prevent interference between channels and channel oscillation.

The supply for the various indicator lamps was an unregulated 6 V . The regulated 5 V were obtained from this unregulated supply, a LM209 integrated circuit regulator was used for this purpose.

CONTROL PRINTED CIRCUIT CARD

The control printed circuit card contained the circuitry for controlling the integrator and sample-and-hold circuits on printed circuit card 1 (Figure 16).

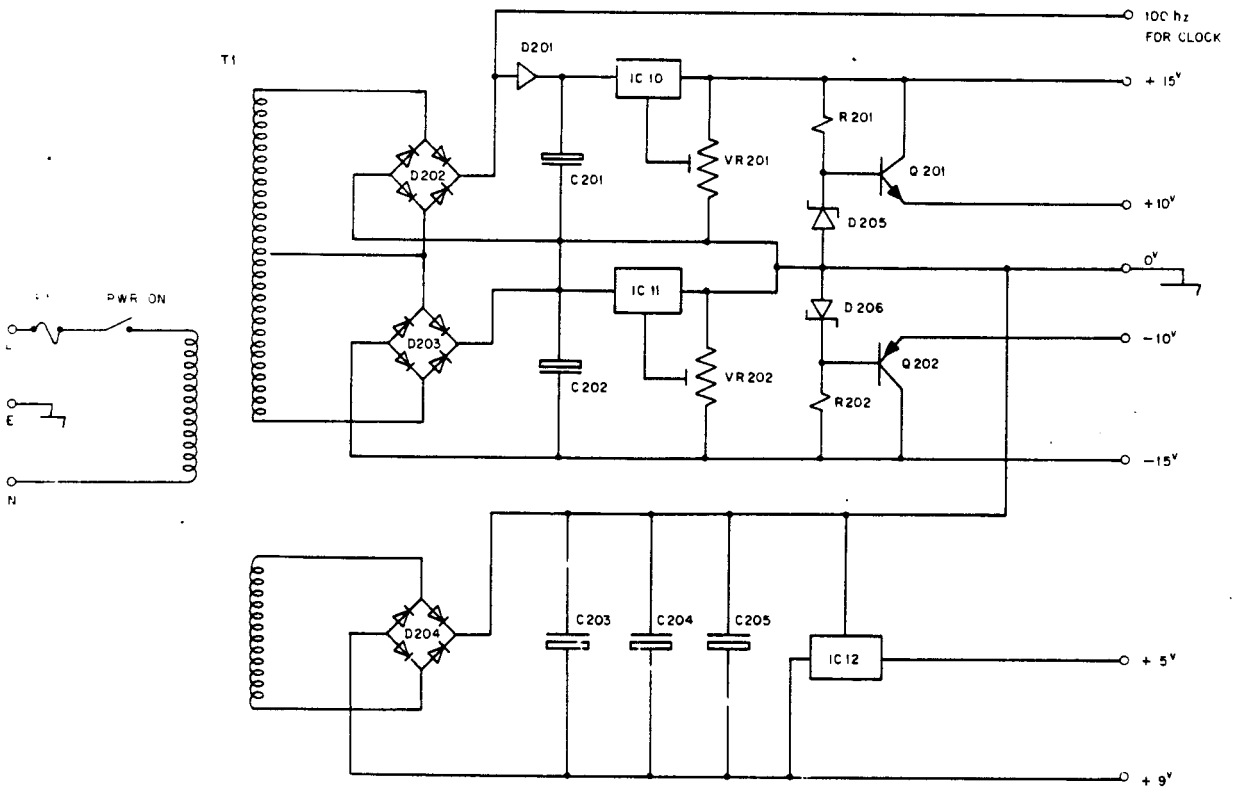

FIGURE 15. Power supply circuitry.

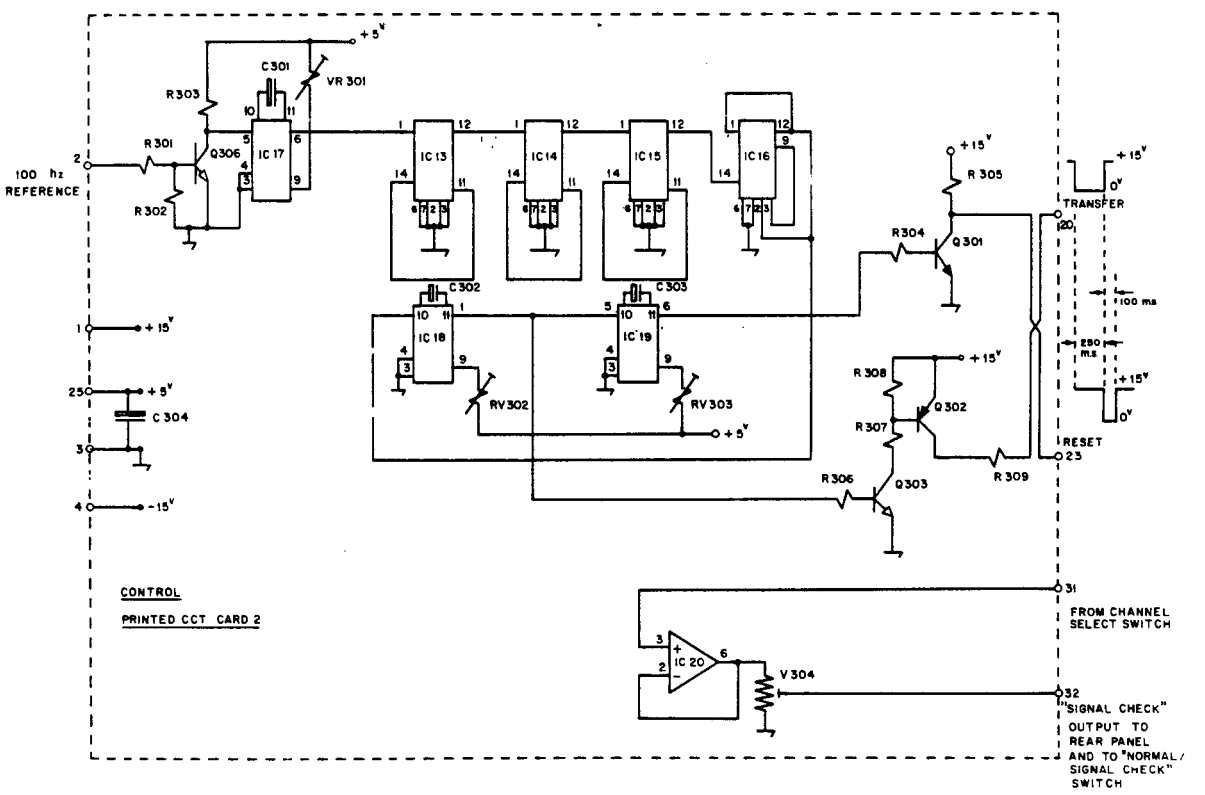

FIGURE 16. Control circuitry for integrator and sample and hold printed circuit cards.

A 100 Hz reference signal which was derived from the mains frequency was applied at Pin 2. After squaring, by Q304, the signal was applied to the trigger input of the monostable multivibrator IC17. The constant width pulse at the output of IC17 was adjusted to 8,5 ms by RV301, thus the circuit was sensitive to transients and mains borne interference during a small portion of each cycle.

The positive going edge of the pulse triggered monostable IC18, producing a ±100 ms pulse at Pin 1, adjustable by means of RV302, which was negative going. This pulse was converted to a 15 V transfer pulse, via Q302 and Q303, which was available at Pin 23 and was fed to all 12 channels. A second monostable, IC19, was triggered at the end of the transfer pulse and produced a ±250 ms pulse, which was adjustable by means of RV303, which was positive going. This pulse was converted, via Q301, to a 15 V reset pulse which was available at Pin 20 and was also fed to all 12 channels.

The buffer amplifier for the signal check facility was also situated on this board. The required signal, selected by the 12-position channel select rotary switch, was fed to IC20 which was a 741 package operating in voltage follower mode. The output of this buffer was then routed to the output banana jacks on the rear panel and also to the NORMAL/SIGNAL CHECK toggle switch on the front panel. In the SIGNAL CHECK position the output of the buffer IC20 was connected to the channel 1 output to the 12-channel recording system.

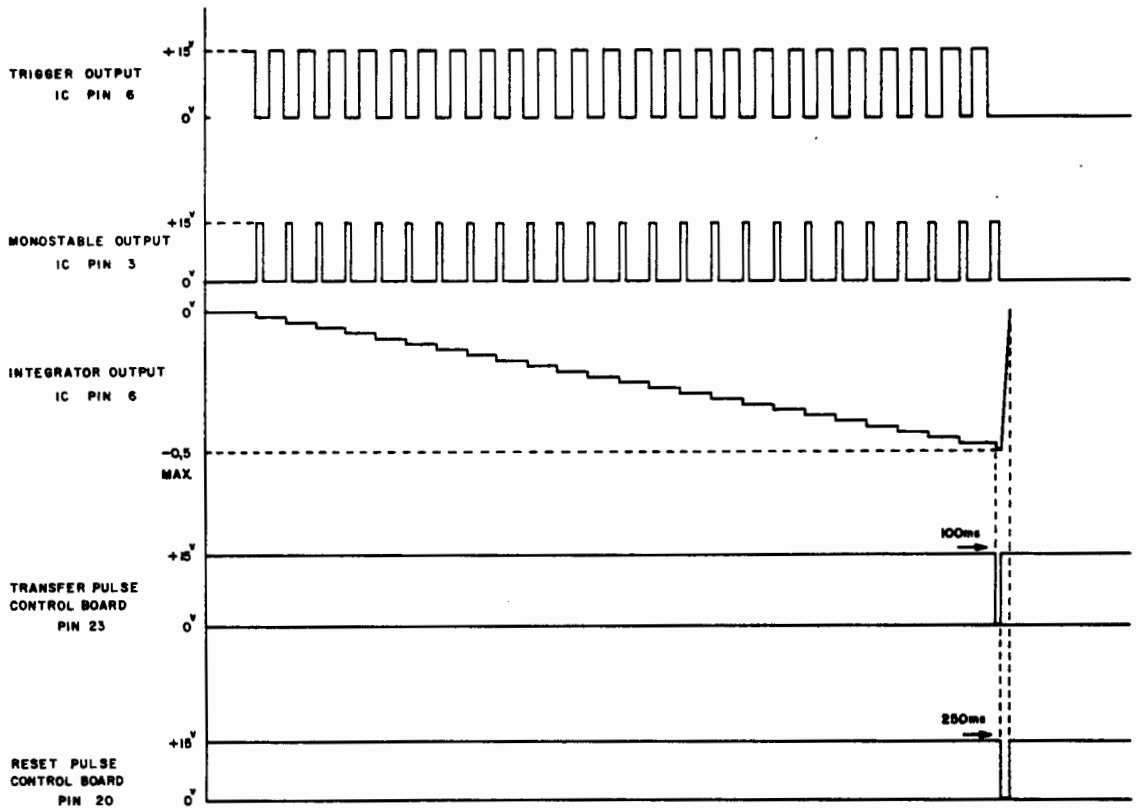

FIGURE 17. Timing diagram.

COMPONENTS LIST

PREAMP AND PRINTED CIRCUIT CARD 1

The preamp and printed circuit card 1 are shown in Figure 14.

Resistors

R101	82	kΩ
R102, R125, R128	4,7	kΩ
R103, R104, R105, R122	270	kΩ
R106	15	MΩ
R107, R108, R111, R112	820	kΩ
R109, R110, R114, R115	68	kΩ
R113	100	kΩ
R116, R121, R124, R126	12	kΩ
R117, R118	22	kΩ
R119, R127	27	kΩ
R120	150	kΩ
R123, R129	560	kΩ
RV100, RV103	2,2	kΩ
RV101, RV102	5	kΩ

Capacitors

C101, C102	33	μf	30 *v* tantalum
C103	2,2	n*f*	100 *v* ceramic
C104 to C109	0,47	μf	250 *v* polyester
C111	0,1	μf	30 *v* tantalum
C112	47	μf	6 *v* tantalum
C113, C114	120	μf	6 *v* tantalum

Semiconductors

Q101, Q102	2N1402	transistor
Q103, Q104	2N3820	field effect transistor
D101 to D104	34P4	diode

Integrated Circuit Packages

IC1 to IC5	SN72741C	operational amplifier
IC6	NE555 V	timer
IC7 to IC9	SN72741 C	operational amplifier

POWER SUPPLY

For the power supply, see Figure 15.

Semiconductors

D201	1N4007 diode
D202, D203	1-A full wave rectifier bridge
D204	3-A full wave rectifier bridge
D205, D206	10-V Zener diode 400 mw
Q201	BD139 transistor
Q202	BD140 transistor

Integrated circuits

IC10 to IC12	LM309 k regulator

Resistors

R201, R202	560 Ω
VR201, VR202	5 kΩ, 10-turn helitrim preset potentiometer

Capacitors

C201, C202	2200 μf	electrolytic 25 v
C203 to C205	5000 μf	electrolytic 15 v

T_1 power transformer:

secondary windings	15 v - 0 - 15 v at 1 A
	0 - 6 v at 3 A

F_1 fuse: ξ10 - blo 2 A 230 v

CONTROL PRINTED CIRCUIT CARD

See Figure 16 for an example of the control printed circuit card.

Resistors

R301	22 kΩ
R302, R303, R309	4,7 kΩ
R304 - R308	12 kΩ
RV301, RV302	22 kΩ
RV303	47 kΩ
RV304	10 kΩ

Capacitors

C301	1 μf	tantalum	35 v
C302, C303	10 μf	tantalum	35 v
C304	1 μf	electrolytic	16 v

Semiconductors

Q301, Q303, Q304	transistor BC317
Q302	transistor BC320

Integrated Circuits

IC13 to IC16	SN7490	decade counter
IC17 to IC19	SN74121	monostable multivibrator
IC20	SN74121	operational amplifier

PRACTICAL APPLICATION OF THE CONTINUOUS AUTOMATIC BIOLOGICAL MONITORING SYSTEM

A biological monitoring system using fish should not only provide continuous information on the effects of intoxication upon fish, but also have the ability of preventing industrial effluents from damaging fish populations in streams. The use of such a system may, in some cases, obviate the need for over-treatment of trade waste effluent when a lesser degree of treatment would be sufficient to protect fish.

The electronic system, designed to monitor continously the respiratory activity of fish, compared their breathing responses to normal rates obtained before the introduction of any toxic influence. Preliminary research has indicated that abnormal responses appear in the breathing of fish exposed to acute and sub-lethal concentrations of a number of toxicants, and that abnormal responses may well appear in advance of irriversible damage should fish be exposed to lethal amounts. In other words, symptoms of ill-health probably occur in the sensor fish early enough to prevent immediate damage to fish, and in sufficient time to preclude disasterous effects on the receiving ecosystem.

The intent of the research described in this section was to challenge the biomonitoring system in the laboratory, in order to determine whether it would fail to provide protection to a freshwater ecosystem under certain conditions. Emphasis was placed on the following questions:

(i) Would the monitoring system distinguish harmful toxicant concentrations in sufficient time?
(ii) What is the threshold level of detection?
(iii) Can different species of fish be programmed for different situations dependent upon detection level requirements?
(iv) Will fish, exposed to low toxicant concentrations over the long-term, continue to exhibit a response to higher concentrations?

EXPERIMENTAL DESIGN

Some 84 experimental series were analyzed in order to determine the efficacy of the system. Three species of sensor fish were used, each

having different sensitivities to acute intoxication. The fish species employed were *Micropterus salmoides* (500 individuals), *Sarotherodon mossambicus* (120) and *Barbus holubi* (120).

For each experimental series sensor fish were established individually in 12 electrode chambers, two of which were used as controls. The chambers were supplied with continuously flowing dechlorinated municipal tap water (approximate hardness 60 mg/dm^3 as $CaCO_3$), the pH being adjusted to 7,0 and the temperature to 23 ±1 °C. All experiments were carried out under conditions of natural photoperiod. The sensor fish were allowed to acclimate to enclosure within the chambers for 48 h before any respiratory records were charted. Normal opercular rhythm levels, for each sensor fish, were recorded for five days after the sensor fish had recovered from the initial stress of being enclosed within the chambers. The multipoint recorders were adjusted to give a measure of the opercular rhythm, in beats per minute, for every minute of the day. Preliminary experimentation had indicated that for any individual the maximum breathing rate variance occurred when there was rapid change in respiratory activity, viz. just after the dawn period or before dusk. The maximum upper 95 % confidence limits for these periods were, therefore, calculated from the five-day record to provide the critical response level for each fish. Hourly averages were assessed from 60 minute periods spanning each hour. Thus, for example, the breathing rate for 18h00 was calculated as an average of all points recorded between 17h30 and 18h30. The response levels were set, independently for each channel using the alarm threshold controls.

Each sensor fish was deemed to have responded as soon as the channel alarm circuit triggered, indicated by a red warning light on a particular channel. The toxicant was defined as being detected when a majority of the sensor fish (60 %) had elicited a response. It was adjudged, even if somewhat arbitrarily, that, to be effective, in an industrial situation, detection should occur within a day of initial exposure. Therefore, all experiments were curtailed after an exposure period of 24 h .

DEFINING THRESHOLD DETECTION LEVELS

The response of three species of sensor fish to increasing concentrations of a number of toxicants was utilized to determine the threshold levels

of detection. The initial toxicant concentrations established in the electrode chambers were based upon results obtained in preliminary experiments and were set up by the addition of predetermined volumes of 1 g/dm^3 solutions. Thereafter toxicant levels were maintained using the dosing units previously described. Each toxicant concentration was maintained in the chambers for a period of 24 h . After this period of exposure, sensor fish were allowed to resume a normal breathing rate in freshwater before being exposed to successively higher concentrations. Each experimental series was concluded when 60 % or more of the sensor fish had elicited a response to a particular toxicant concentration within 24 h of initial exposure. For all determinations a separate group of fish were directly exposed to the final toxicant concentration in order to confirm the defined threshold.

THE EFFECT OF DIURNAL RESPIRATORY PATTERNS ON FISH SENSOR RESPONSE TO INTOXICATION

It has been suggested that the use of critical response limits based on maximum variance, calculated from hourly breathing rates, would result in a delayed response, on the part of sensor fish, to intoxication, during periods of the day when the breathing rate is naturally low. In order to establish whether this would have any effect upon system performance, sensor fish were exposed to selected acute and sub-lethal concentrations of a number of toxicants where exposure was initiated at different times of the day. Toxicant concentrations were chosen to provide for responses by all sensor fish within 24 h of initial exposure. For each concentration four independent groups of five sensor fish were utilized, exposure being initiated at either 06h00, 12h00, 18h00 or 24h00. The average response times for the groups were compared, to determine whether any significant differences existed between them, using Student's 't' tests at two significance levels ($P < 0,05$; $P < 0,01$). Toxicant addition, to the individual chambers, was discontinued as soon as the particular sensor fish had elicited a response. On the completion of the experiment each group of 20 fish were maintained separately in compartmented stock tanks, supplied with continuously flowing freshwater, for a period of three months.

EFFECTS OF ACCLIMATION TO INTOXICATION UPON SENSOR FISH RESPONSE

Twelve experiments were designed to establish whether sensor fish, exposed for long periods to "safe" levels of toxicants, would provide adequate advance warning of harmful conditions. In each case, groups of five sensor fish were maintained in the maximum toxicant concentrations which elicited no responses in previous experiments for a period of six months. On transference to the electrode chambers the sensors were exposed to acute and sub-lethal toxicant concentrations, and their average response times to intoxication compared with results obtained previously for similar conditions. On completion of the experiment, the independent groups were maintained separately in freshwater in order to establish survival potential.

DISCUSSION OF RESULTS

In most cases *Micropterus salmoides* and *Barbus holubi* were more sensitive to intoxication than was *Sarotherodon mossambicus*, and, although in many respects the smallmouth yellow fish would provide a more timeous alarm of hazardous conditions, the extreme care required to maintain this species under laboratory conditions could be disadvantageous to its utilization as a sensor.

Responses were obtained from the more sensitive fish in concentrations as low as 10 µg Cu^{2+}/dm^3, 50 µg Cd^{2+}/dm^3, 5 µg Hg^{2+}/dm^3 and 1 µg CN^-/dm^3 (Tables 25, 26 and 27). However, the levels of intoxication producing alarm conditions, as defined for industrial situations, were, generally, higher by a factor of five. *Barbus holubi* was much more successful, as a sensor animal, in detecting phenol and ammonia (Table 17), and it is questionable whether the threshold levels defined by *Micropterus salmoides*, 1,0 and 5,0 mg/dm^3 respectively, would be adequate to provide for freshwater ecosystem protection over the long-term.

Sensor fish exposed directly to the established threshold levels confirmed in all cases, except one, that the procedure of exposing the experimental animals to successively higher toxicant concentrations had no influence on the final result. The exception was cadmium. Sensor fish exposed directly to cadmium exhibited an average response time somewhat less than that of fish which had experienced conditions of exposure to increasing

TABLE 25. Elapsed times to response by *Micropterus salmoides* exposed to various degrees of intoxication

Toxicant	Concentration (mg/dm³	Times to response (h) for sensor fish									
		1	2	3	4	5	6	7	8	9	10
Copper	0,01	-	-	-	-	-	-	-	22,40	-	-
	0,025	-	19,55	-	-	17,08	-	-	8,90	-	-
	0,05	12,35	4,93	15,12	-	3,82	-	17,85	1,42	17,28	11,58
	0,05	*2,90*	*8,87*	-	*10,35*	*3,78*	-	*16,68*	*19,32*	*13,13*	-
Cadmium	0,05	21,90	-	-	-	-	-	19,85	-	-	-
	0,1	11,20	20,47	-	14,23	-	-	12,87	-	-	16,27
	0,25	4,68	6,38	12,20	10,17	15,87	17,32	4,03	13,63	13,40	2,92
	0,25	*2,40*	*3,55*	*6,97*	*9,20*	*7,28*	*12,87*	*9,57*	*8,30*	*3,62*	*5,83*
Mercury	0,005	-	-	23,70	-	-	-	-	-	-	-
	0,01	12,93	10,17	4,70	8,30	8,85	8,47	4,53	7,28	9,23	14,13
	0,01	*8,63*	*9,42*	*4,45*	*5,28*	*7,32*	*9,90*	*11,18*	*6,92*	*6,27*	*4,07*
Phenol	0,5	-	-	-	21,93	-	-	-	-	-	-
	1,0	22,27	-	-	4,93	7,45	17,20	20,08	9,32	11,18	-
	1,0	-	*14,70*	*16,85*	-	*14,78*	-	*23,80*	*8,30*	*21,35*	-
Ammonia	1,0	-	-	17,70	-	-	-	-	-	-	-
	2,5	-	-	7,28	-	-	-	21,47	-	-	-
	5,0	13,27	17,12	4,63	-	22,45	-	11,15	-	17,93	21,03
	5,0	*9,13*	*14,67*	-	-	*9,72*	-	*19,32*	*16,85*	*18,52*	-
Cyanide	0,001	17,40	-	-	-	20,40	-	21,43	-	-	-
	0,005	7,28	-	21,47	-	11,18	-	19,87	-	-	-
	0,01	2,20	9,32	3,50	14,70	1,95	18,90	11,70	13,22	7,10	6,27
	0,01	*14,70*	*14,80*	*5,25*	*14,23*	*7,93*	*11,22*	*12,75*	*8,30*	*11,55*	*10,17*

Only those toxicant concentrations in which any sensor fish response was observed, within 24 h of exposure, are represented.

Figures in italics represent responses by separate groups of sensor fish, in each case, utilized for confirmation of threshold levels.

TABLE 26. Elapsed times to response by *Sarotherodon mossambicus* exposed to various degrees of intoxication

Toxicant	Concentration (mg/dm³)	Times to response (h) for sensor fish									
		1	2	3	4	5	6	7	8	9	10
Copper	0,05	-	-	23,38	-	-	-	-	-	-	-
	0,1	19,32	16,88	13,52	19,93	-	18,30	14,68	-	-	-
	0,1	-	-	*21,70*	-	*23,42*	*20,78*	*19,85*	-	*18,15*	-
Copper	0,1	-	21,40	-	-	-	19,63	-	-	19,70	-
	0,15	13,28	11,72	16,10	19,05	-	14,78	-	10,20	13,88	10,62
	0,15	*11,52*	*14,28*	*15,38*	-	*15,75*	-	*18,30*	*18,38*	*14,12*	-
Cadmium	0,1	-	-	-	21,57	-	-	-	-	22,97	-
	0,15	9,32	11,23	8,22	7,90	14,45	17,32	16,78	10,72	9,32	-
	0,15	*12,78*	*7,70*	*9,32*	*7,28*	*11,07*	*13,57*	*11,48*	*14,68*	*7,28*	*9,48*
Phenol	1,0	-	-	-	-	-	-	19,95	-	-	-
	1,5	14,28	22,95	-	10,17	-	-	7,28	-	-	13,87
	2,0	8,13	7,25	10,78	5,27	13,53	12,70	3,40	11,73	10,55	6,90
	2,0	*10,50*	*11,53*	*5,98*	*7,82*	*8,27*	*10,17*	*13,30*	*14,72*	*7,28*	*5,85*
Ammonia	2,5	-	-	-	17,70	-	-	-	-	-	-
	5,0	18,47	15,23	-	14,62	-	12,95	-	19,32	22,03	-
	5,0	-	*14,88*	*13,40*	*21,37*	*20,10*	*17,20*	*11,73*	*14,08*	-	-
Cyanide	0,01	-	17,78	-	-	-	21,20	-	-	-	-
	0,05	8,30	4,73	9,88	11,30	-	6,27	5,45	3,22	12,48	-
	0,05	*6,27*	*14,68*	-	*12,38*	*17,85*	-	*11,68*	*9,15*	*10,40*	-

Only those toxicant concentrations in which any sensor fish response was observed, within 24 h exposure, are represented.

Figures in italics represent responses by separate groups of sensor fish, in each case, utilized for confirmation of threshold levels.

TABLE 27. Elapsed times to response by *Barbus holubi* exposed to various degrees of intoxication

Toxicant	Concentration (mg/dm³)	Times to response (h) for sensor fish									
		1	2	3	4	5	6	7	8	9	10
Copper	0,01	-	23,38	-	-	-	21,70	-	-	-	-
	0,025	-	12,35	-	-	-	11,87	-	-	17,32	19,57
	0,05	8,30	6,95	10,45	7,28	11,70	4,57	13,52	15,32	7,08	5,28
	0,05	*8,97*	*7,70*	*16,27*	*8,30*	*8,75*	*5,92*	*17,85*	*13,57*	*11,18*	*14,68*
Cadmium	0,05	-	19,68	-	-	18,23	-	-	20,28	-	-
	0,1	-	10,70	-	19,32	11,68	-	-	13,50	-	16,53
	0,25	17,85	3,97	11,22	10,90	5,25	11,28	13,22	11,68	15,88	10,17
	0,25	*6,87*	*5,37*	*3,52*	*7,28*	*8,23*	*3,05*	*5,90*	*9,92*	*2,80*	*4,78*
Mercury	0,001	19,87	-	15,70	-	-	-	22,98	-	-	-
	0,005	4,87	7,23	3,90	9,32	11,28	7,75	5,88	6,27	8,68	12,35
	0,005	*8,27*	*7,68*	*5,25*	*10,17*	*7,72*	*7,85*	*8,95*	*9,78*	*8,72*	*9,62*
Phenol	0,25	-	19,52	-	-	-	-	-	-	-	-
	0,5	14,30	9,27	-	17,75	-	17,85	-	-	-	-
	1,0	7,23	3,23	9,52	9,70	9,38	11,40	8,98	14,18	11,57	11,03
	1,0	*11,68*	*9,92*	*7,87*	*11,90*	*10,10*	*8,28*	*6,57*	*9,32*	*12,95*	*10,72*
Ammonia	0,25	-	-	-	21,75	-	-	-	-	21,85	-
	0,5	-	17,37	-	14,70	-	-	-	17,90	11,80	-
	1,0	13,38	9,45	12,28	7,75	8,52	-	10,92	9,50	11,78	10,92
	1,0	*12,30*	*12,23*	*10,22*	*9,70*	*7,57*	*14,78*	*13,92*	*11,05*	*8,23*	*10,03*
Cyanide	0,001	19,98	-	-	-	-	17,57	-	-	-	-
	0,005	8,23	-	-	11,72	-	5,03	-	-	-	-
	0,01	1,40	4,80	3,23	5,43	3,50	0,75	3,22	7,70	3,87	4,85
	0,01	*2,55*	*5,27*	*3,93*	*5,45*	*5,28*	*5,93*	*4,53*	*3,27*	*7,07*	*3,40*

Only those toxicant concentrations in which any sensor fish response was observed, within 24 h exposure, are represented.

Figures in italics represent responses by separate groups of sensor fish, in each case, utilized for confirmation of threshold levels.

TABLE 28. Threshold opercular rhythm responses, of three species of sensor fish, to intoxication

Toxicant	Lowest concentration (mg/dm³) eliciting response in 60 % of sensor fish within 24 h exposure		
	Micropterus salmoides	*Sarotherodon mossambicus*	*Barbus holubi*
Copper	0,05	0,1 - 0,15	0,025 - 0,05
Cadmium	0,1 - 0,25	0,15	0,1 - 0,25
Mercury	0,01		0,005
Phenol	1,0	1,5 - 2,0	0,5 - 1,0
Ammonia	5,0	5,0	0,5 - 1,0
Cyanide	0,005 - 0,01	0,05	0,01

concentrations. The average response times for *Micropterus salmoides* and *Barbus holubi* exposed to increasing concentrations of cadmium were 10,06 and 11,14 h respectively, at the threshold detection level, whereas sensor fish exposed directly to that concentration exhibited average response times of 6,95 and 5,77 h, respectively. The significant differences ($P < 0,05$) between these response times indicated that the sensor fish concerned acclimated to cadmium over the short term. However, in both cases, all fish responded to the threshold level. It is, therefore, probable that, over the short term, previous exposure to cadmium would not adversely influence the function of the system in providing advance warning of hazardous conditions.

The response of sensor fish to acutely toxic effects was not influenced by the time of day a toxicant entered the system (Table 29). In lethal concentrations, the response by sensor fish was, in any case, quite rapid. On the other hand, the increase in opercular rhythm, in response to sub-lethal toxicant concentrations, was gradual and, therefore, more amenable to influence by the diurnal respiratory pattern. The sensitivity of fish exposed to 0,05 mg Cu^{2+}/dm³ was apparently decreased when toxicant exposure was initiated at 12h00 when compared with the 06h00, 18h00 and 24h00 initiations. Sensor fish initially exposed to 0,05 mg Cu^{2+}/dm³ at 12h00 would have been expected to elicit responses at or about 01h00. In fact the response was delayed, on average, by four h . Similar results were obtained when sensor fish were exposed to sub-lethal concentrations of ammonia and cyanide. In both cases, where responses were expected to occur during the dark interval of the photoperiod, a

TABLE 29. The effect of time of initiation of intoxication upon response for *Micropterus salmoides* exposed to different toxicants

Nominal toxicant concentration (mg/dm^3)	Exposure initiation time	Times to response (h) for sensor fish 1	2	3	4	5	Average response time (h)
0,1 Cu^{2+}	06h00	3,23	4,68	6,07	5,72	5,90	5,12
	12h00	2,85	4,93	3,27	6,18	6,27	4,70
	18h00	4,18	3,85	6,35	5,07	4,52	4,79
	24h00	3,13	3,90	3,93	6,03	5,72	4,54
0,05 Cu^{2+}	06h00	15,42	13,80	10,52	17,08	11,72	13,71
	12h00	18,63	20,20	17,85	12,82	15,67	17,03
	18h00	13,70	12,15	15,27	17,03	10,63	13,76
	24h00	11,78	7,32	13,90	17,52	15,40	13,18
0,1 Hg^{2+}	06h00	2,45	3,85	2,32	1,63	4,27	2,90
	12h00	1,28	1,87	3,27	2,27	3,20	2,38
	18h00	2,85	2,08	2,18	2,12	4,38	2,72
	24h00	1,93	2,78	2,35	4,67	4,55	3,26
0,01 Hg^{2+}	06h00	8,63	7,67	9,87	11,47	9,32	9,39
	12h00	5,42	7,63	10,55	9,42	8,85	8,37
	18h00	11,90	8,93	7,68	6,63	9,03	8,83
	24h00	8,78	5,17	6,40	6,35	9,70	7,28
10,0 Ammonia	06h00	5,87	6,63	11,72	9,27	8,03	8,30
	12h00	7,40	7,47	8,20	12,12	10,72	9,18
	18h00	6,93	6,55	9,03	12,78	9,68	8,99
	24h00	9,17	9,27	7,70	8,93	6,23	8,26
5,0 Ammonia	06h00	20,63	16,08	17,38	21,27	14,55	17,98
	12h00	18,78	16,82	15,03	17,67	20,35	17,73
	18h00	14,20	15,40	17,23	17,20	19,63	16,73
	24h00	21,52	22,72	19,90	-	-	21,38*
0,05 Cyanide	06h00	3,32	4,93	2,85	5,70	3,93	4,15
	12h00	5,27	5,35	2,87	2,08	3,70	3,85
	18h00	4,90	3,52	3,40	2,70	3,90	3,68
	24h00	5,15	3,72	3,55	3,40	3,52	3,87
0,01 Cyanide	06h00	9,20	14,13	11,28	13,23	7,28	11,02
	12h00	13,70	15,50	12,52	17,57	15,52	14,96†
	18h00	8,48	6,23	12,88	10,20	10,38	9,63
	24h00	13,28	7,70	14,98	8,38	7,45	10,36

* Average of three sensor fish.

† $P < 0,05$.

delay of up to four h was experienced, and in ammonia two of the five fish did not elicit any response.

There is no doubt, therefore, that the utilization of response levels based upon the maximum upper 95 % confidence limit was adversely affected by the wide variation in diurnal breathing rate. Nevertheless, responses

were obtained in sufficient time to protect the fish, since not one of the individuals used died when removed to freshwater. This fact, together with the advantages of cost and simplicity in the use of a definite rather than variable delimitation value, validates the utilization of the confidence limit statistic, despite the disadvantage of apparent loss of sensitivity during dark periods.

Long term exposure to low toxicant concentrations did not have any effect upon the sensitivity of response, by sensor fish, to acute toxicant concentrations, except in the case of cyanide (Table 30) where fish were more sensitive to intoxication when compared with sensor fish used in previous experiments ($P < 0,01$). Sensor fish also displayed heightened sensitivity to a sub-lethal concentration of cyanide subsequent to long term exposure to a low concentration.

TABLE 30. Elapsed times to response by *Microterus salmoides* exposed to acute and sub-lethal toxicant concentrations subsequent to long term exposure to low toxicant levels.

Nominal toxicant concentration (mg/dm³)	Times to response (h) for sensor fish					Average response time
	1	2	3	4	5	
0,05 Cu^{2+}	15,27	20,93	7,32	17,27	12,13	14,58
0,1 Cu^{2+}	6,63	4,55	4,17	5,20	7,90	5,69
0,25 Cd^{2+}	12,12	15,78	13,32	19,13	11,58	14,39†
1,00 Cd^{2+}	4,45	5,85	5,93	3,03	6,47	5,15
0,01 Hg^{2+}	13,78	15,70	17,45	15,85	11,03	14,76††
0,1 Hg^{2+}	5,17	7,87	3,38	3,63	4,32	4,87
1,0 Phenol	10,45	13,28	11,82	19,72	17,52	14,56
10,0 Phenol	0,90	1,17	0,93	0,35	0,70	0,81
5,0 Ammonia	13,20	21,15	15,85	14,40	17,55	16,43
10,0 Ammonia	4,70	7,48	8,70	11,38	9,03	8,26
0,01 Cyanide	4,48	6,70	3,90	4,63	4,23	4,79††
0,05 Cyanide	2,27	1,32	0,93	2,03	2,55	1,82††

† and †† indicate that average response times were significantly different from those obtained in previous experiments at significance levels of $P < 0,05$ and $P < 0,01$ respectively.

Long term exposure to cyanide possibly influenced the enzyme detoxification system to a degree where the process of recovery from cyanide intoxication was deleteriously affected. Two of the sensor fish exposed to a lethal cyanide concentration did not recover when transferred to freshwater, on receipt of response, and died within three days.

The average times to response for sensor fish exposed to sub-lethal concentrations of mercury and cadmium, after long term exposure to "safe" concentrations of these elements, were significantly greater ($P < 0,01$, $P < 0,05$ respectively) than fish exposed directly to similar concentrations. Pre-exposure to cadmium and mercury, therefore. induced a certain amount of resistance to intoxication by these metals. Response to intoxication, nevertheless, occurred swiftly enough to provide for adequate protection. No fish died when transferred to freshwater from sub-lethal concentrations of mercury and cadmium, on receipt of responses.

CONCLUSIONS

The respiratory response, i.e. increased opercular rhythm, of fish to both lethal and sub-lethal concentrations of toxicants has been found to be a reliable parameter for use in biological monitoring systems.

Although the monitoring system, when compared to some physico-chemical systems, has the inherent disadvantage of not being able to identify the toxicant, the severity of its effect can be judged by a consideration of the percentage of sensor fish which exhibit a response as well as the time lag between initial and final sensor responses, all individuals responding. For example, a sub-lethal concentration of copper produced a time lag of 13,61 h, in the final experiment described above (Table 29), whereas a lethal concentration resulted in the much shorter time lag of 3,73 h .

Results of exposure of three species of fish to a number of toxicants have suggested that the response level of sensor fish lies between 5 and 10 % of a lethal concentration, indicating a generalized premortal stress response, at threshold levels, rather than a single physiological one. It is difficult to conceive of any alteration in the design of the electronic system which will result in increased sensitivity. However, the fact that opercular signals have been successfully recorded from a number of fish

species, including the pumpkinseed sunfish (Roberts, 1964), bluegill sunfish (Spoor, Neiheisel and Drummond, 1971), largemouth bass, blue kurper and smallmouth yellowfish, suggests that many fish species may be employed, each with differing degrees of sensitivity to various toxicants. Moreover, the electrode chamber is more suitable for fish species too small for use with other techniques which depend upon the surgical attachment of electrodes. A study of the particular situation in which a monitoring system is to be used would indicate which species of sensor fish will give optimum sensitivity.

The criterion for toxicant detection, in an industrial situation, was based upon responses by a majority of sensor fish within a definite time span. A consideration of the experimental determinations indicated two further methods, other than using fish of different sensitivity, which may be utilized to provide enhanced system sensitivity. Firstly, the critical level for opercular rhythm response could be lowered by using 85 or 90 % maximum upper confidence limits. Secondly, the detection level could be regulated by the more sensitive individuals, rather than the majority, in a group of sensor fish. The sensitivity of the system may be more than doubled by utilizing responses by three fish, rather than six, of a group of ten sensors, to provide for toxicant detection. In both cases, however, one would have to accept a greater degree of 'false' response together with enhanced sensitivity.

The monitoring system has proved more than adequate in practice to provide protection against fish kills in waters receiving industrial effluents. The monitor would enable an industry to take almost immediate remedial action should the toxicity of its waste increase, thus preventing accidental discharge, which, in the past has caused many ecological crises. However, the degree of protection may not be sufficient to provide safeguards against long term low level toxic effects upon such parameters as reproduction and growth.

Another important negative factor is the lag that exists between the onset and detection of toxic conditions. This is partly due to the delay inherent in biological processes. The rate of uptake of a toxic substance often depends upon the concentration of the substance in the water. The larger the test chamber and the slower the flow of in-coming water, the

longer it would take to attain a threshold concentration in the test chamber. The time-lag inherent in the biological part of the biomonitoring system could be reduced by adjusting the flow rate and size of the test chamber as much as possible without affecting the breathing rate of the fish. The time-lag could also be reduced by supplying proportionately more of the effluent to the test system than is delivered to the receiving system. This could also serve to introduce a safety factor based upon effluent concentrations known to affect reproduction and growth. The degree of protection afforded by the monitoring unit should also be evaluated by regular examinations of biological structural and functional parameters in the receiving system.

Should industries comply with water quality standards and also guard against accidental discharge using a biological monitoring system, then the fullest use could be made of the ecosystem without damaging fish life.

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SECTION III

FISH LOCOMOTOR BEHAVIOUR PATTERNS AS A MONITORING TOOL

INTRODUCTION

Public anxiety regarding the impact of chemical pollution upon the biological quality of freshwater ecosystems has been largely responsible for stimulating research into the biological effects of potentially dangerous substances which may be discharged as industrial wastes. This concern for the environment has led to a vast amount of information being accumulated on acutely morbid and lethal effects of toxicants upon aquatic organisms, emphasis having been laid upon the relationships between these effects and the concentration of, and exposure time to, the toxicants concerned.

The massive quantity of information, in the literature, pertaining to acute and chronic toxic effects upon aquatic organisms, indicates the importance placed on this problem. Such effects have a dramatic impact upon the general public. However, the occurrence of such effects possibly represents only a small part of the total damage inflicted upon aquatic ecosystems by pollution. Although, in many cases, a highly concentrated slug of pollution may be carried some way along a water course without significant dilution, acutely lethal or chronic levels of a pollutant are more likely restricted to the vicinity of an industrial effluent discharge point. Further from the impact point, more or less rapid dilution and dispersion of the toxic substances occur, depending upon various physical and chemical factors. Therefore, only a relatively small volume of water, close to the pollution source, will contain a high concentration. It may be assumed that a much larger biomass will be exposed to low, sub-lethal concentrations of pollution than that confronted by lethal levels.

Accordingly, in recent years researchers have tended to deal with sub-lethal effects of intoxication upon physiological, developmental and behavioural parameters of biological entities. Behavioural aspects have received the least attention being the most difficult to assess quantitatively. Whether behaviour patterns are based on genetic or acquired information, they are adaptive activities in response to environmental variables of physical, chemical or biological nature. Toxicological modification of any aspects of a behavioural pattern is not likely to be

overt in its manifestation but may, and probably will, have subtle effects on the animal's capabilities to deal with the challanges of its environment. The very subtleness of such behavioural modification over the course of time creates additional difficulties for their quantification and, therefore, the establishment of relationships between them and various toxic effects.

Among the many behavioural patterns of fish, locomotion is of particular significance, enabling the animal to move adaptively and in a non-random fashion in relation to physical, chemical and biological conditions in the environment. Sensory information with respect to the chemical properties of the environment is acquired, processed and integrated in the nervous system and relayed to control mechanisms which determine the nature of the orientated locomotion required in response to the prevailing environmental system.

A complex biological system of this kind should be highly susceptible to stress by toxic substances in general, particularly in view of evidence that swimming performance can be affected by exposure to sub-lethal concentrations of pollutants (Sprague, 1971; Bull and McInerney, 1974). Substances that affect basic neural functions would be of special interest in this context; therefore, locomotion might present a suitable parameter for testing toxicological effects of pollutants on behaviour in fish.

Many techniques have been employed to measure the activity or movement patterns of fish under laboratory conditions. Much of the data presently available has resulted from experiments which examined the extent to which fish could detect and avoid toxic substances. The apparatus used for much of this research has been based on that designed by Shelford and Allee (1913), which they employed to investigate the reactions of fish to varying concentrations of atmospheric gases. Principally the apparatus consisted of either a rectangular or cylindrical tank to which water was delivered at both ends and drained from the centre, precise metering yielding distinct separation of the two bodies of water at the centre. Toxicants were injected to either side of the tank utilizing dosing units. The behaviour of fish,

liberated in the center of the tank, was recorded from visual observations.

Recording has been a major problem for researchers when analyzing avoidance reactions. From Shelford and Allee (1913) to Jones (1947) charts were drawn from notes written intermittently during the experiments. Continuous records were first obtained by Sprague (1964) who recorded fish behaviour patterns on a vertical kymograph chart. Equipped with chart and stop watch the observer watched the fish and copied its movements by drawing a line on the chart; thus a rapid movement from one end of the tank to the other was represented by a horizontal line. Should the fish remain motionless the trace became a vertical line in the area of the chart corresponding with that part of the tank in which the fish was stationary.

Another apparatus which has been used to test the reactions of fish to intoxication is the 'channelled avoidance trough'. This was devised by Jones *et al.* (1956) to test the reactions of salmon and trout to pulp-mill effluents. Its design was fairly simple, consisting of a trough with a large main compartment into which opened four channels formed by the insertion of three parallel glass partitions. Water flowed separately to the main compartment and to each of the four channels, the flow being adjusted so that a distinct separation occurred between them at the drainage area situated at the dividing line between the main area and the channel apertures. Of the four channels, two were provided with freshwater and two with polluted water. Avoidance was computed by comparing the number of times fish entered the non-toxic channels with entries into the polluted channels.

Few fish species become habituated to the presence of an observer, and, therefore, many techniques that rely on direct observation, employed in the behavioural studies outlined above, were considered unsuitable for long term monitoring. Scherer and Nowak (1973) described an apparatus for continuous measurement of movement of fish exposed to chemical substances where the rectangular avoidance tank was viewed through a one-way mirror. The observer, using an open sight viewer connected to the shaft of a power supplied potentiometer, could track the movements

of a fish in the test chamber. Varying the position of the viewing device, in sympathy with the animal's movement, changed the output voltage from the potentiometer to a strip chart recorder, giving an instantaneous permanent record of fish movement and position. Although the system provided greater accuracy than those previously described, it would be difficult, if not impossible, to monitor behavioural activity throughout a 24 h period.

Considerable effort has been expended by various researchers in an attempt to overcome the limitations imposed by visual observations. Szymanski (1914) developed an apparatus whereby a small thread was attached to the dorsal or caudal fin of a fish. The thread was connected to a light lever fixed on an axis, movement of the fish was recorded on a kymograph by means of a pointer attached to the long arm of the lever. Spoor (1941) designed an 'activity detector' which consisted of a light-weight paddle which was freely suspended in the water of an experimental chamber. When the paddle was moved by the water currents generated by the movements of a fish, an electric circuit was completed which activated a signal magnet or counter which recorded the fish's movement. A similar apparatus was used by Jones (1955) to study photokinesis in ammocoete larvae of the brook lamprey. Pilot experiments conducted by Chaston (1968) showed, however, that the paddle was rather insensitive, and together with similar apparatus (Davies, 1963), based on fish activity records, may be criticised on the grounds that the paddle presented an obstacle to fish movements.

Of the variety of methods that have been devised to study locomotor activity patterns of aquatic organisms, photoelectric systems have certain advantages in that they require no physical restraint of the animal, are very sensitive and can provide for continuous recording. Shirer *et al.* (1968) employed common fish aquaria as test tanks and arranged light beams to traverse the length of the tanks near the bottom, center and just below the water surface. Interruption of the light beams, falling on photoresistors, advanced counters and deflected pens of an event recorder. The apparatus functioned adequately in providing a quantitative appreciation of fish movement patterns.

Accordingly, in order to examine whether the use of fish movement patterns would be a viable technique for the continious monitoring of fish response to intoxication, measurement by light beam interruption was considered to be the most effective available technique.

PRELIMINARY INVESTIGATIONS INTO THE USE OF FISH ACTIVITY ABERRATIONS AS AN INDICATOR OF TOXIC STRESS

With our present state of knowledge it is difficult to say, with any degree of certainty, that a given form of energy will have no effect on fish behaviour. Whilst many types of apparatus may be devised using acoustical, thermal or electrical fields to sense swimming movements of unrestrained fish, each is equally suspect of exerting some biological influence. The design philosophy of the system, described below, was to determine the change in patterns of chemically induced behaviour as compared to control fish in the same system. Therefore the choice of light beam interruption as the sensing means was based upon technical simplicity and reliability rather than biological innocuousness.

THE FISH ACTIVITY MONITORING SYSTEM

GENERAL DESCRIPTION

A matrix of 32 photoresistors was mounted along the side of a rectangular perspex tank (Figure 18). The matrix was made up of eight groups, each containing four photoresistors. Each group was arranged vertically and was so connected that an output from that group was obtained whenever the light from any one of the four was interrupted. The groups of photoresistors divided the tank into eight compartments so that the position of a fish along the length of the tank would be indicated by the presence of an output from the corresponding group of photoresistors. The vertical groups of photoresistors were so spaced that the light to at least one of them would be interrupted by a fish passing in front, no matter at what depth it may swim by.

Each photoresistor was illuminated by a six volt panel lamp mounted on the opposite side of the tank. Adequate intensity was obtained by simply forming the beams with 3 mm holes in baffles 55 mm apart between the lamps and the side of the tank.

The signals from the photoresistor matrix were processed so as to continually monitor the position of the fish along the length of the

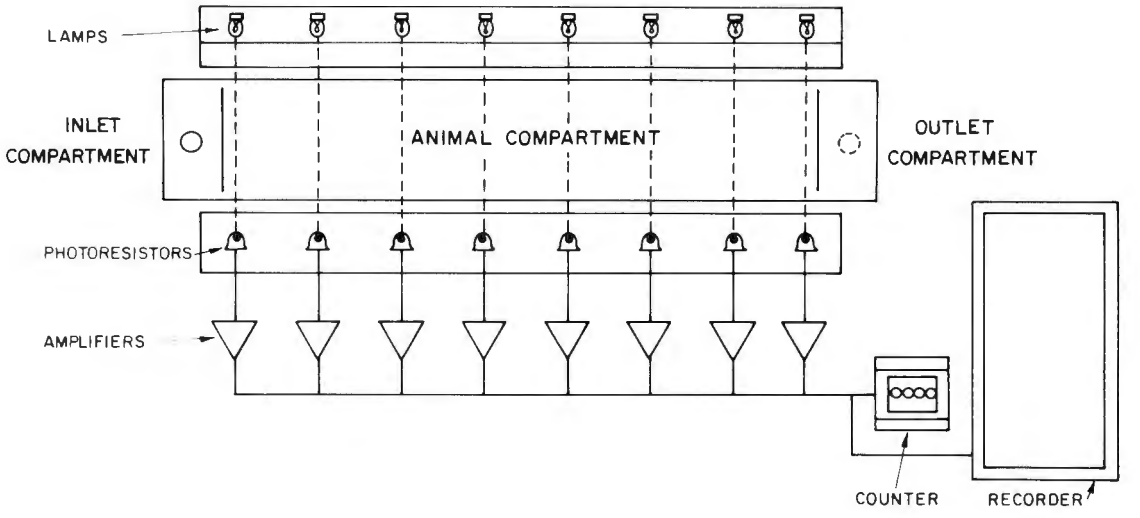

FIGURE 18. Block diagram of the activity monitoring system.

tank. The fish's position at any one moment was indicated by a row of eight lamps on the front panel of the monitor (Plate 6). The lamp corresponding to the fish's position being illuminated. In addition, a chart recorder was linked to the monitor in such a way as to provide a permanent record of the movement of the fish within the tank. This was achieved by having the chart width correspond to the length of the tank.

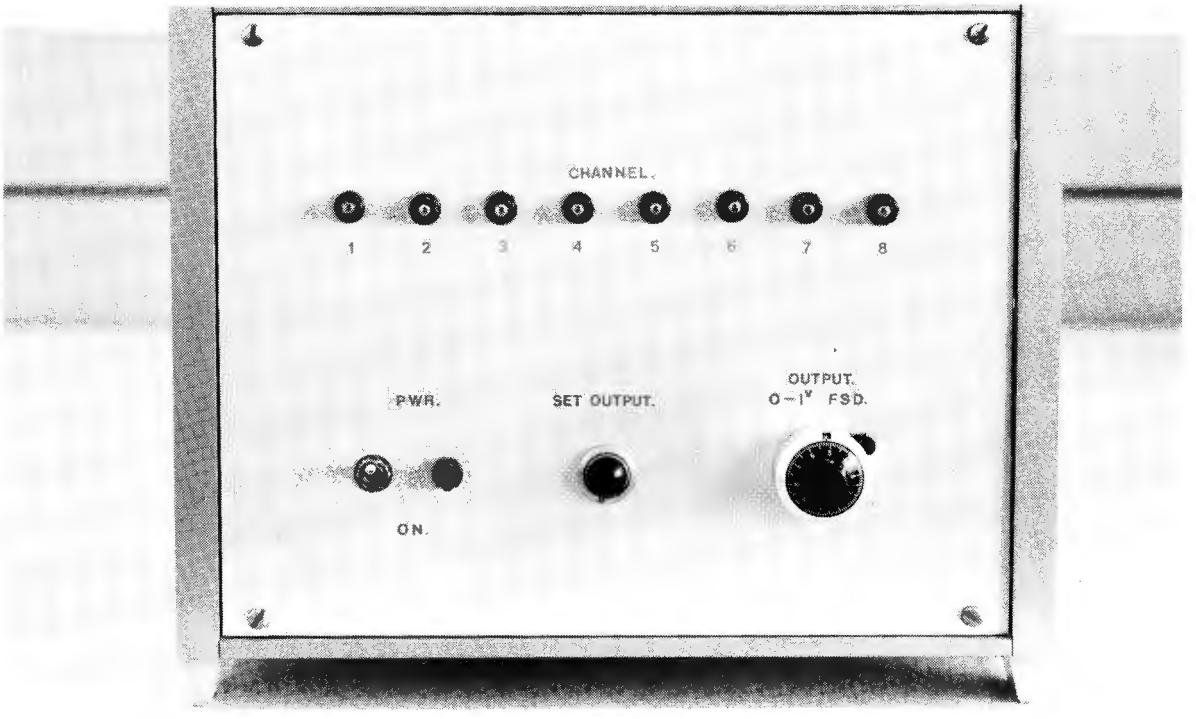

PLATE 6. Electronic monitoring module showing fish position indicator lamps and signal output control.

CIRCUIT DESCRIPTION

Figure 19 shows the wiring of the photoresistors. Each group of four was simply connected in series so that whenever the light to any of the four was interrupted, the resistance of the group increased sharply. The resistance of the eight groups caused the transistors Q1-Q8 (Figure 20) to switch. Individual sensitivity adjustment was provided by R1-R8, while C1-C8 removed the 50Hz component caused by the panel lamps.

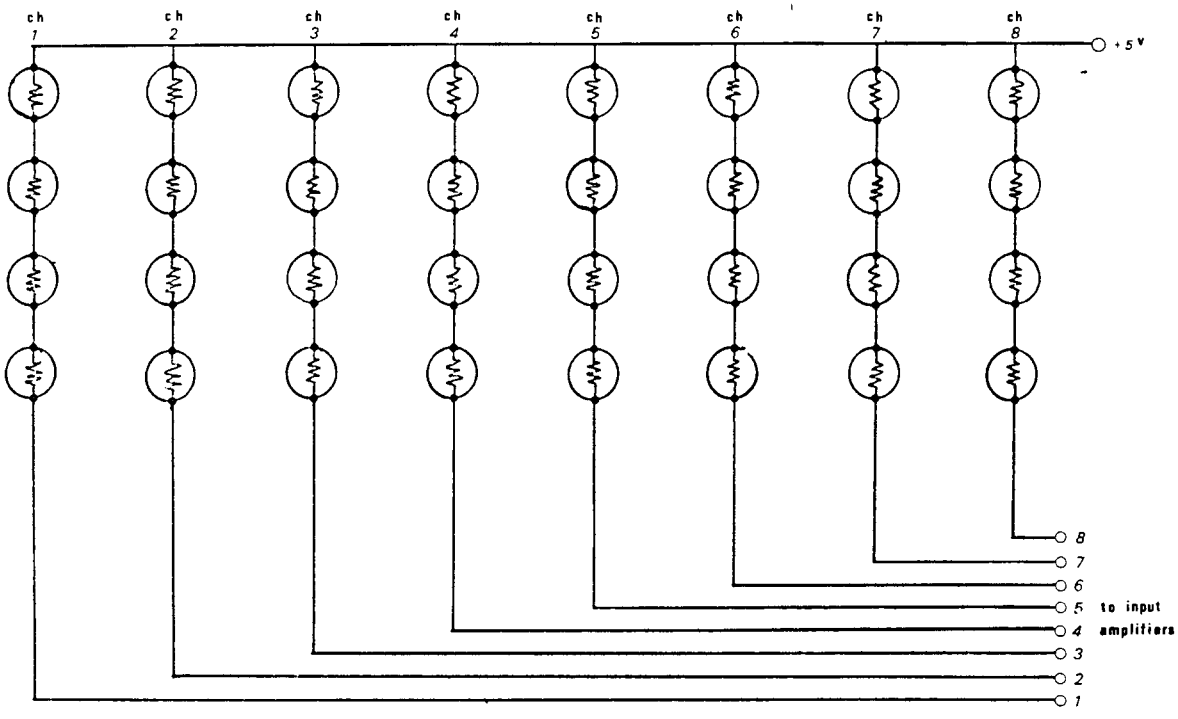

FIGURE 19. Photoresistor wiring.

The 5 volt level changes at the collectors of Q1-Q8 were converted to fast-rise pulses of uniform duration by being applied to the Schmidt trigger inputs of the SN74121 monostables IC5-IC12. These pulses were gated by the inhibiting circuitry comprising IC1-IC4. This ensured that no two channels were activated simultaneously, the input being removed from an activated channel before an adjacent channel could react to an input.

The pulses which passed through the inhibiting circut set flip-flops IC13-IC16. Thus the flip-flop which was set at any moment indicated the position of the fish in the tank. Whenever a pulse set one of the flip-flops it also generated a reset pulse via IC21-IC25 and IC26, IC3D

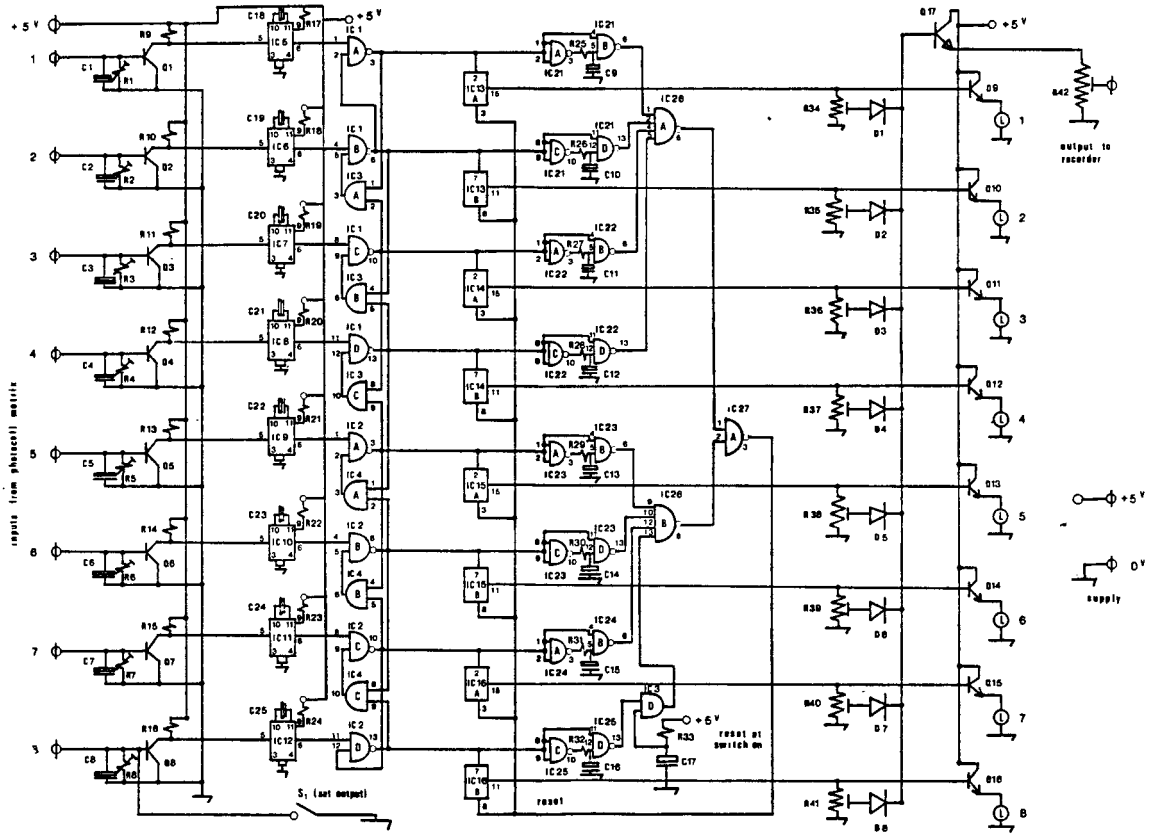

FIGURE 20. Circuit diagram.

and IC27. This pulse reset all other flip-flops. The outputs of the flip-flops illuminated lamps L1-L8 via drivers Q9-Q16. The lamps indicated the precise position of the fish. The flip-flop outputs were also scaled by potentiometers R34-R41 and fed via diodes D1-D8 and buffer Q17 to the variable attenuated R42 and thence to a millivolt chart recorder.

The scaling potentiometers R34-R41 were so arranged that the voltage at the emitter of Q17 increased in 250 millivolt steps as the instrument switched from channel one through channel eight. The output voltage was then adjusted by means of the ten-turn potentiometer R42 to suit the sensitivity of the recorder in use. Switch S1 (set output) provided a convenient means of setting up amplitude control R42. When depressed this switch caused channel eight to be activated. R42 was then adjusted for the required deflection of the recorder.

The regulated power supply (Figure 21) provided +5 volts with a current capability of 1 Amp. An LM309 integrator circuit was employed for regulation.

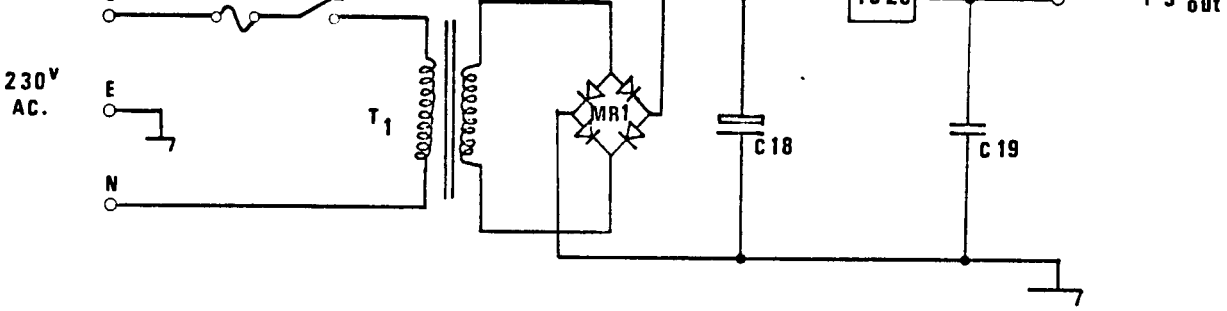

FIGURE 21. Regulated power supply.

COUNTER

A counting circuit was attached to the monitor. A four-decade electro-mechanical counter was incremented every time one of the eight channels was activated thus providing a simple method for analyzing the fish's activity quantitatively. A mechanical reset button was provided so that the accumulated count could be cleared after each period of monitoring.

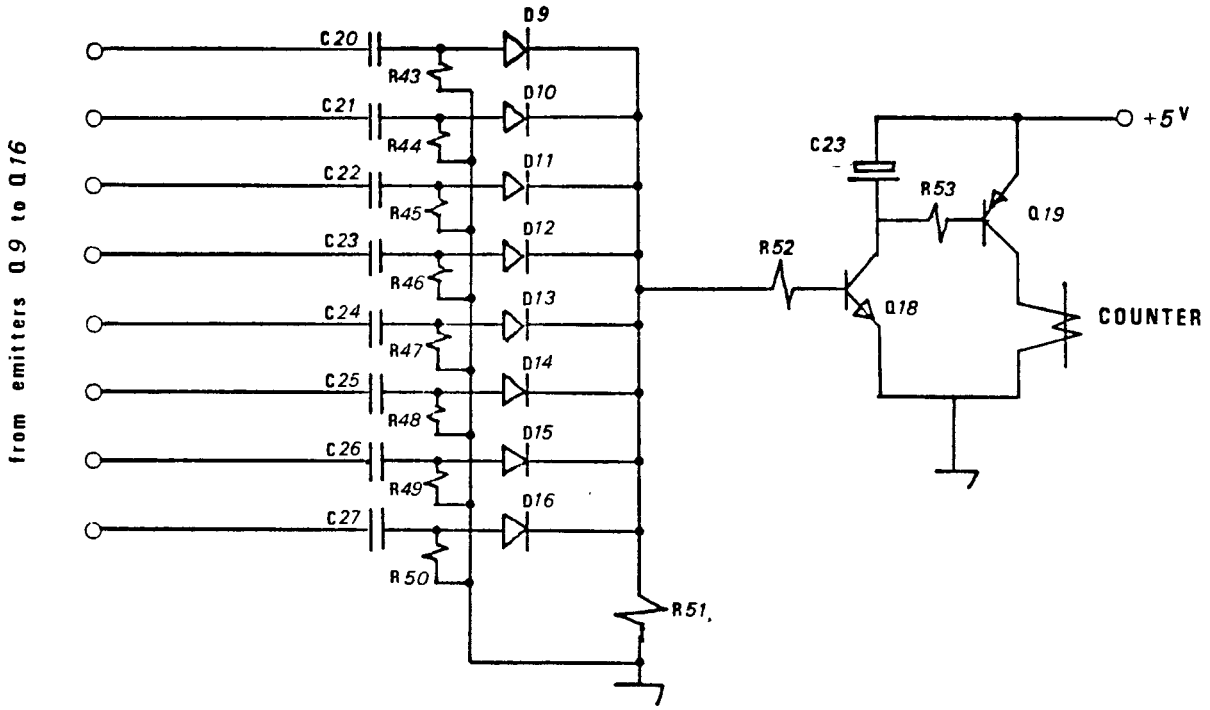

FIGURE 22. Counter circuit.

The counter circuit is shown in Figure 22. The inputs were obtained from the emitters of drive transistors Q9-Q16. The inputs were differentiated and applied to OR gate D9-D16. A pulse at the output of this

gate incremented the counter via Q18 and Q19. C28 ensured that the drive pulse was of sufficient duration to provide reliable operation of the counter.

COMPONENTS LIST

MAIN CIRCUIT BOARD

The main circuit board is shown in Figure 20

Resistors

R 9 - R16	2,2 KΩ	1/4 W	5%
R17 - R24	6,8 KΩ	1/4 W	5%
R25 - R33	1,5 KΩ	1/4 W	5%

Potentiometers

R 1 - R 8	47 KΩ	Miniature preset
R34 - R41	4,7 KΩ	Miniature preset
R42	1 KΩ	Ten turn "Helipot"

Capacitors

C 1 - C 8	10 µf	Tantalum	35 V
C 9 - C16	1,5 µf	Electrolytic	64 V
C17	100 µf	Electrolytic	16 V
C18 - C25	10 µf	Tantalum	35 V

Integrated circuits

IC 1	SN7400	Quad NAND gate
IC 2	SN7400	Quad NAND gate
IC 3	SN7408	Quad NAND gate
IC 4	SN7408	Quad NAND gate
IC 5 - IC12	SN74121	Monostable
IC13 - IC16	SN7476	Dual flip-flop
IC21 - IC25	SN7400	Quad NAND gate
IC26	SN7440	Quad NAND gate
IC27	SN7402	Quad NOR gate

Transistors

Q 1 - Q 8	BC317
Q 9 - Q17	BC317

Diodes

D 1 - D 8	34P4

Switch

S 1	Push button type

Lamps

L 1 - L 8	6 V 1 A "Pea" lamps with holders and coloured lenses

Photoresistors

Pillips resin encapsulated - Dark value >10 M;
light value 75 - 300 at 1 000 lux

POWER SUPPLY

For the power supply see Figure 21.

T 1 - 6 V 1 A transformer
MR1 - 1 A rectifier bridge
C18 - 5 000 µf 12 V Electrolytic capacitor
C19 - 0,1 µf Polyester capacitor
IC28 - LM309 Solid state regulator
F1 - Fuse 0,5 Amp
S2 - On/Off toggle switch 230 V 1 A

COUNTER CIRCUIT

The counter circuitry is shown in Figure 22.

R43 - R50	2,2 KΩ	1/4 W	5%	resistors
R51	6,8 KΩ	1/4 W	5%	resistors
R52	3,9 KΩ	1/4 W	5%	resistors

R53	1 KΩ	1/4 W	5%	resistors
C20 - C27	10 µf	Tantalum	35 V	capacitors
C28	5 µf	Tantalum	35 V	capacitors
Q18	BC317	Transistor		
Q19	BC320	Transistor		
Counter	4 decade 12 V electromechanical with manual reset			

THE EFFECT OF INTOXICATION UPON FISH ACTIVITY PATTERNS

EXPERIMENTAL PROCEDURE

Fish movement patterns were monitored and quantified using the apparatus described. However, it quickly became apparent that the light beam matrix was inducing an abnormal duirnal pattern upon the fish's locomotor behaviour. A comparison of activity levels obtained from a sensor fish used, in the present study, to investigate the effect of 1,0 mg/dm^3 of phenol with activity rhythms normally displayed by *Micropterus salmoides* (Reynolds and Casterlin, 1976) indicated that the depressed activity, which the experimental animals usually elicit during dark periods, was eliminated (Figure 23). Nevertheless, it was decided to continue experimentation, albeit somewhat reduced in scope, in order that some insight may be obtained into the degree of locomotor aberration to be expected under the influence of acute and sub-lethal intoxication.

The fish used as sensors were largemouth bass (*Micropterus salmoides*) obtained from the Transvaal Provincial Administration Fish Hatchery at Lydenburg. The fish were transported in sealed, oxygenated, plastic containers to a fish stock holding laboratory where they were maintained under fairly constant temperature conditions (22 - 24°) in continuously flowing municipal tap water (Table 1, Section II) for at least three months before being used for experimentation. The mean weight of the eight fish used in the experimental determinations was 61,4 g (49,7 - 108,6 g) and the mean length, 136 mm (123 - 156 mm). The critical factor in choice of sensor fish was length, in that it, of necessity, had to be less than 160 mm, i.e. the distance between two successive vertical groups of photoresistors.

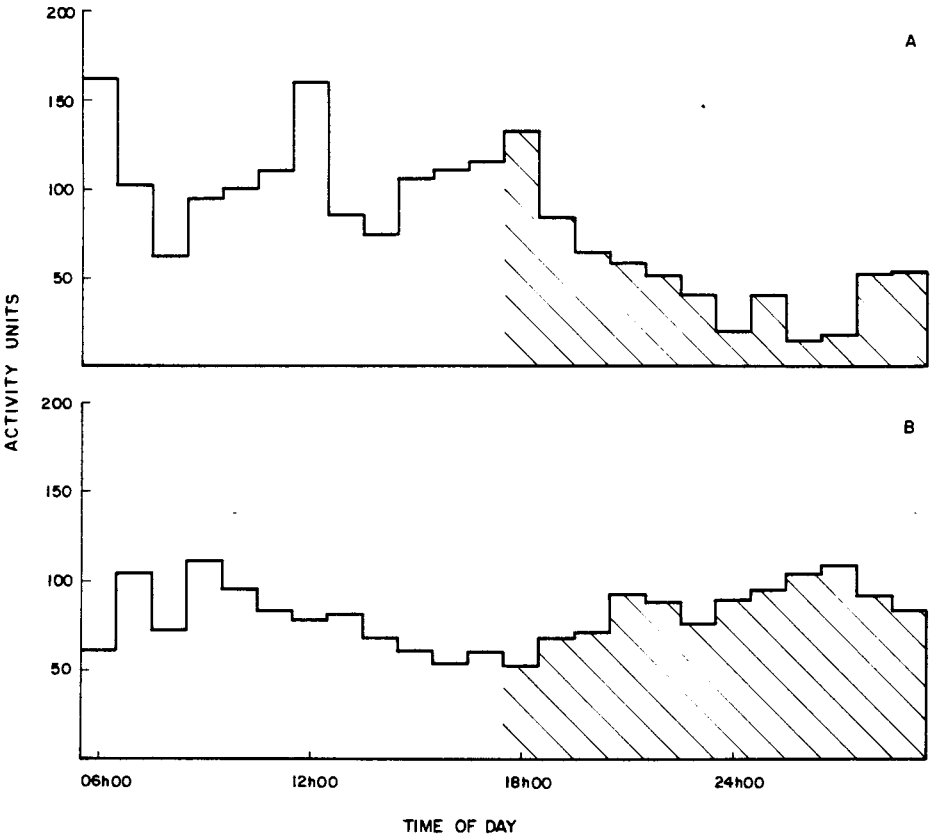

FIGURE 23. Activity records for largemouth bass exposed to normal diurnal photoperiod (A) compared to activity elicited when exposed to constant light conditions (B). Shaded bars represent hours of darkness. The results for A were extracted from Reynolds and Casterlin (1976).

Investigations into the effects of intoxication upon locomotor behaviour did not proceed until such time as the activity of the sensor fish had settled to a fairly regular pattern, taking into account the elimination of alternating light and dark periods due to the influence of the artificial light sources. The acclimation process did not, generally, exceed a period of four days subsequent to sensor fish transfer from a stock tank to the experimental chamber.

Cumulative light beam interruptions were recorded for each hour of the day during the 24 h period immediately preceding toxicant addition, in order to provide information upon which to formulate a quantitative appreciation of any toxic effect on activity levels. It was considered

that a sensor fish should not, under the pertaining circumstances, provide, in any one 60-minute period, a cumulative count exceeding the upper 99% confidence limit, based upon the mean and standard deviation of all the hourly counts which it had produced during the 24 h period preceding toxicant addition. This figure was utilized, in each individual case, to provide a quantitative critical response limit.

Nominal concentrations of mercury, cadmium, copper and phenol, chosen arbitrarily to provide acute and sub-lethal conditions, were established in the experimental tank by the addition of a predetermined volume of a 1 g/dm^3 solution of toxicant. Thereafter the nominal concentrations were maintained using a dosing unit similar to that of Stark (1967) wherein solenoid valves operated by a microswitch activated by a perspex float replaced the suggested pneumatic valve arrangement. A continuous flow rate of approximately 5 dm^3/h was maintained throughout the experimental period.

Subsequent to toxicant addition, the counts registered on the electromechanical counter were abstracted at each hour of the day and night, for the following 48 h, the counter being reset manually after each hourly period. Total length and wet weight of the sensor fish was recorded on completion of each test period.

DISCUSSION OF RESULTS

The results indicated that exposure of sensor fish to both acute and sub-lethal toxicant concentrations caused increases in swimming activity. The locomotor response to intoxication was almost immediate and pronounced. Acutely toxic concentrations of mercury (0,2 mg/dm^3), cadmium (20,0 mg/dm^3), copper (5,0 mg/dm^3) and phenol (1,0 mg/dm^3) induced, in sensor fish, activity levels exceeding the designated response limits within two hours of exposure. Increased sensor fish activity also resulted from exposure to sub-lethal levels of mercury (0,02 mg/dm^3), cadmium (2,0 mg/dm^3), copper (0,1 mg/dm^3) and phenol (0,1 mg/dm^3), response limits being attained after 15, 13, 9 and 3 h exposure respectively (Tables 31, 32 and 33).

TABLE 31. The effect of acute intoxication upon activity levels, measured as cumulative light beam interruptions, of *Micropterus salmoides*

Time of day	Toxicant concentration: 0,2 mg Hg^{2+}/dm^3			20,0 mg Cd^{2+}/dm^3			5,0 mg Cu^{2+}/dm^3			1,0 mg C_6H_5OH/dm^3		
06h00	40	*54*	*238*	6	*63*	*11*	32	*57*	*74*	61	*84*	*90*
07h00	40	*301*	*199*	12	*260*	*17*	41	*111*	*78*	104	*144*	*86*
08h00	149	*792*	*91*	18	*195*	*17*	52	*131*	*88*	72	*148*	*64*
09h00	12	*1193*	*64*	37	*48*	*11*	36	*124*	*79*	111	*131*	*79*
10h00	39	*1789*	*65*	25	*47*	*7*	39	*108*	*94*	95	*138*	*112*
11h00	90	*1815*	*47*	9	*112*	*11*	61	*114*	*101*	83	*154*	*117*
12h00	26	*1577*	*78*	15	*63*	*23*	72	*134*	*108*	78	*164*	*168*
13h00	136	*751*	*131*	32	*201*	*7*	90	*110*	*124*	81	*161*	*174*
14h00	3	*457*	*43*	56	*66*	*20*	74	*94*	*114*	68	*114*	*181*
15h00	142	*375*	*39*	29	*89*	*19*	81	*84*	*138*	61	*74*	*134*
16h00	76	*461*	*39*	40	*88*	*23*	56	*56*	*144*	54	*78*	*112*
17h00	45	*425*	*42*	47	*102*	*16*	32	*68*	*158*	60	*82*	*164*
18h00	33	*424*	*71*	92	*153*	*20*	35	*69*	*196*	53	*91*	*154*
19h00	49	*232*	*36*	181	*106*	*12*	51	*74*	*201*	68	*90*	*141*
20h00	41	*478*	*71*	54	*121*	*15*	64	*81*	*189*	71	*68*	*134*
21h00	26	*200*	*34*	204	*119*	*70*	81	*80*	*134*	93	*61*	
22h00	102	*265*	*20*	6	*91*	*84*	76	*91*	*184*	88	*73*	
23h00	126	*681*	*44*	104	*51*	*92*	86	*87*		76	*77*	
24h00	85	*515*	*85*	69	*49*	*645*	54	*77*		89	*81*	
01h00	145	*595*	*108*	16	*19*	*291*	34	*56*		94	*68*	
02h00	7	*114*	*83*	30	*15*		47	*64*		104	*71*	
03h00	192	*110*	*190*	56	*6*		51	*67*		108	*64*	
04h00	47	*131*	*276*	48	*11*		37	*71*		91	*62*	
05h00	74	*247*	*308*	6	*22*		42	*74*		83	*75*	
a	72			50			55			81		
b	53			51			19			17		
c	207			182			104			125		

a, b and c are, respectively, the mean, standard deviation and upper 99 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 32. The effect of sub-lethal intoxication upon activity levels, measured as cumulative light beam interruption, of *Micropterus salmoides*

Time of day	Toxicant concentration											
	0,02 mg H^{2+}/dm^3			2,0 mg Cd^{2+}/dm^3			0,1 mg Cu^{2+}/dm^3			0,1 mg C_6H_5OH/dm^3		
06h00	13	*13*	*12*	52	*46*	*34*	71	*90*	*104*	38	*54*	*36*
07h00	18	*19*	*17*	43	*53*	*41*	82	*87*	*56*	46	*48*	*27*
08h00	23	*31*	*18*	41	*51*	*45*	104	*96*	*57*	53	*78*	*27*
09h00	19	*26*	*17*	61	*62*	*61*	91	*54*	*51*	36	*103*	*21*
10h00	26	*27*	*16*	44	*61*	*67*	78	*63*	*62*	29	*105*	*29*
11h00	31	*17*	*23*	39	*38*	*54*	84	*81*	*66*	48	*96*	*35*
12h00	17	*31*	*26*	38	*68*	*51*	63	*101*	*81*	46	*98*	*36*
13h00	15	*18*	*34*	47	*45*	*48*	58	*51*	*93*	56	*108*	*42*
14h00	18	*24*	*28*	51	*32*	*37*	59	*124*	*54*	53	*113*	*41*
15h00	27	*24*	*31*	68	*41*	*36*	104	*138*	*51*	48	*101*	*52*
16h00	33	*34*	*29*	66	*64*	*29*	73	*154*	*76*	44	*96*	*60*
17h00	36	*58*	*28*	32	*52*	*30*	81	*142*	*72*	21	*91*	*37*
18h00	27	*31*	*31*	41	*76*	*31*	87	*144*	*68*	36	*82*	*47*
19h00	27	*34*	*30*	41	*80*	*28*	88	*161*	*64*	38	*78*	*52*
20h00	31	*48*	*13*	47	*104*	*26*	98	*158*	*53*	31	*84*	*44*
21h00	25	*54*	*15*	37	*110*	*42*	103	*131*	*56*	27	*76*	*43*
22h00	17	*61*	*29*	55	*101*	*51*	94	*120*	*74*	47	*69*	*61*
23h00	14	*71*	*19*	62	*94*	*56*	83	*98*	*86*	44	*53*	*58*
24h00	34	*74*	*30*	57	*91*	*61*	76	*84*	*90*	58	*56*	*51*
01h00	19	*60*	*17*	32	*77*	*44*	56	*90*	*91*	38	*48*	*50*
02h00	26	*58*	*18*	35	*40*	*36*	58	*81*	*82*	24	*44*	*48*
03h00	27	*56*	*15*	47	*31*	*40*	68	*86*	*74*	48	*19*	*30*
04h00	24	*31*	*31*	41	*28*	*44*	78	*78*	*71*	34	*26*	*19*
05h00	30	*28*	*19*	45	*36*	*48*	81	*91*	*52*	27	*31*	*27*
a	24			47			80			40		
b	7			10			15			10		
c	41			73			118			67		

a, b and c are, respectively, the mean, standard deviation and upper 99% confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 33. The relationship between time to behavioural response and time to death for *Micropterus salmoides* exposed to various toxicant regimes

Toxicant	Concentration (mg/dm^3)	Time to response (h)	Time to death (h)
Mercury	0,02	12	-
	0,2	2	53
Cadmium	2,0	13	-
	20,0	2	44
Copper	0,1	9	-
	5,0	2	41
Phenol	0,1	3	-
	1,0	2	39

The decisive increase in locomotor activity, on the part of sensor fish, in response to acutely lethal conditions (the toxicant concentrations utilized approximated the 48-h LC50) occurred in sufficient time to have enabled the animal, in nature, to escape the immediate harmful effects of intoxication. However, within the completely enclosed environment of the experimental chamber, locomotor activity continued to increase to the time of death (Figure 24). On the other hand, sensor fish exposed to sub-lethal toxicant concentrations resumed a regular activity pattern, similar to that of the day preceding exposure, within 24 hours. Whether the results, therefore, would indicate that fish have the ability to differentiate between lethal and sub-lethal intoxication and consciously accommodate to the latter is, at present, conjectural.

Nevertheless, the results indicated the increase in locomotor activity to have been primarilly due to an avoidance reaction in response to harmful conditions. The sensor fish possibly reacted to a gradual physiological impairment or utilized its highly developed olfactory sense.

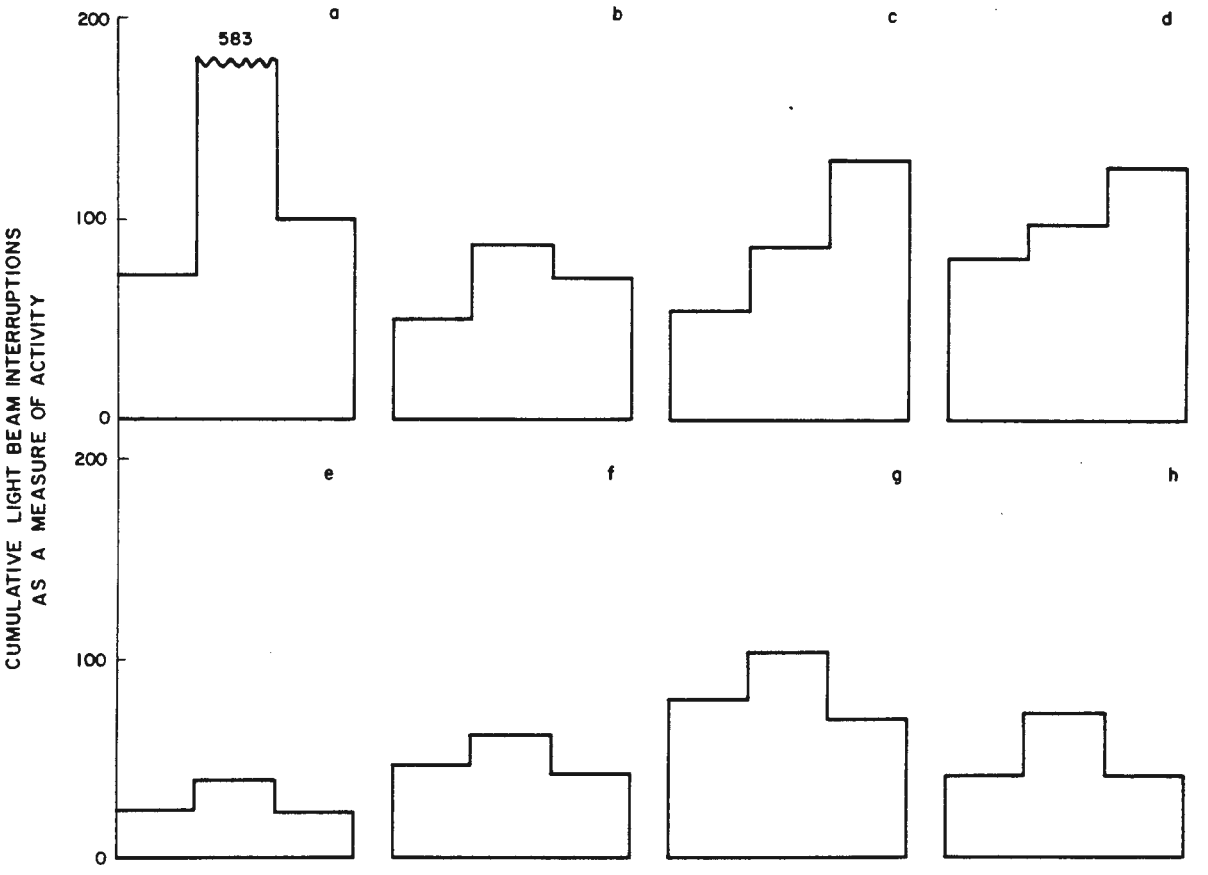

FIGURE 24. Daily average activity levels for sensor fish exposed to (a) 0,2 mg Hg^{2+}/dm^3 (b) 20,0 mg Cd^{2+}/dm^3 (c) 5,0 mg Cu^{2+}/dm^3 (d) 1,0 mg Phenol/dm^3 (e) 0,02 mg Hg^{2+}/dm^3 (f) 2,0 mg Cd^{2+}/dm^3 (g) 0,1 mg Cu^{2+}/dm^3 and (h) 0,1 mg Phenol/dm^3 for one day before and two days after toxicant exposure.

CONCLUSIONS

The results obtained from the preliminary investigations indicated that

(1) the apparatus described functioned adequately and served the purpose for which it was designed, and

(2) acute and sub-lethal toxicant concentrations did alter the movement patterns of largemouth bass.

However, certain major modifications in the design of the test chamber and monitoring unit were required in order to enable the system to perform automatically and continuously.

THE CONSTRUCTION OF AN ELECTONIC SYSTEM DESIGNED TO MONITOR EFFECTS OF CHANGES IN WATER QUALITY ON FISH LOCOMOTOR BEHAVIOUR PATTERNS

Preliminary investigation has indicated that fish movement patterns, monitored by light beam interruptions, gave a reliable index of pre-mortal intoxication. The apparatus utilized did not in any way interfere with fish movement within the test chamber and allowed for the maintenance of fish for long time periods. Further, it was considered that the process of quantitatively appreciating movement by light beam interruption would be suitable for automatic and continuous measurement.

Certain modifications in system design, with respect to both the test chamber and the electronic surveillance module, were, however, deemed necessary in order to provide for continuous and automatic measurement. The second generation system was constructed in such a way as to allow the sensor fish to adopt a normal diurnal behaviour pattern. The initial system, using visible light beams, proved to have had an adverse effect upon fish behaviour in this respect. Accordingly, it was decided to employ an infra-red beam matrix in place of the original visible light beam system. Also, in order to eliminate the necessity for constant personal surveillance an automatic print out facility was incorporated into the electronic monitoring unit.

Financial considerations and the size of the activity test chamber precluded the use of more than one channel for the investigation of system efficacy.

GENERAL DESCRIPTION

A specially designed tank (Figure 18) was fitted with a set of infra-red emitters and photoelectric sensors that divided the tank vertically into eight compartments. The use of infra-red emitters was deemed advisable because the use of visible light beams was found to have had an effect upon exogenous rhythms during darkness. The placement of filters over incandescent lights may not adequately limit the emission to the infra-red range (Cripe, Cripe and Livingstone, 1975).

Whenever a sensor fish intruded into one of the compartments, a signal was generated that, after suitable processing, was fed to the counter of the electronic monitor (Plate 7). Thus each time the sensor fish entered a different compartment, the activity counter was incremented. The contents of the counter were shown on a four-decade digital display and were printed out by a digital printer once every hour.

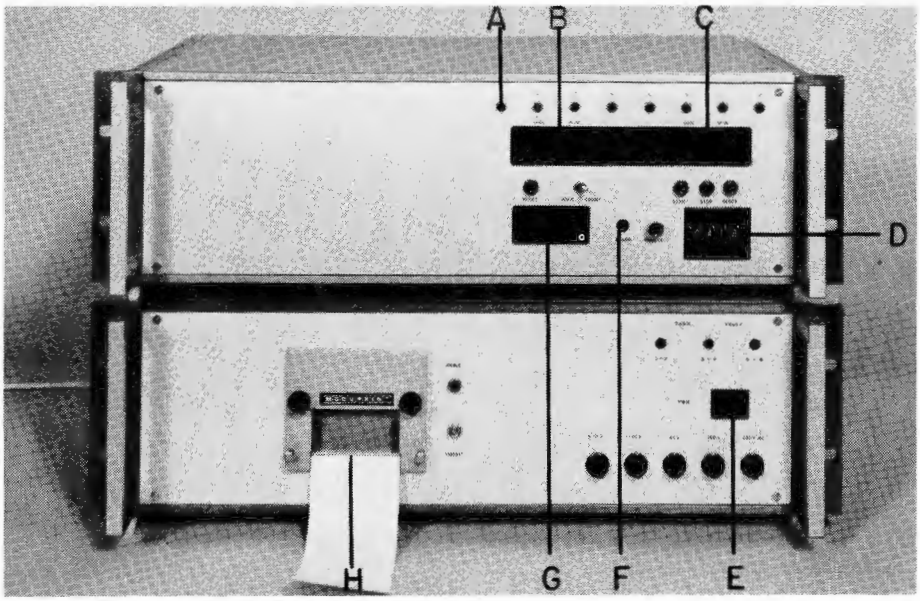

PLATE 7. Front view of monitor/alarm system. A - one of eight fish position indicator lamps, B and C - electronic clocks, D - alarm threshold control, E - on/off switch, F - alarm signal lamp, G - activity counter, H - digital print out.

A four-decade thumbwheel switch on the front panel of the electronic monitor was used to set the activity threshold. Whenever the content of the activity counter exceeded the preset threshold, an alarm signal was initiated that was indicated on the print out and (by means of a flashing red lamp) also shown on the front panel.

The actual position of the fish in the tank was displayed by means of a row of eight lamps, one of which was activated corresponding to the appropriate compartment that the fish had entered. The actual position of the fish was also converted to an analogue signal which could be displayed on a chart recorder to provide a permanent record of the fish's changes of position (Figure 25).

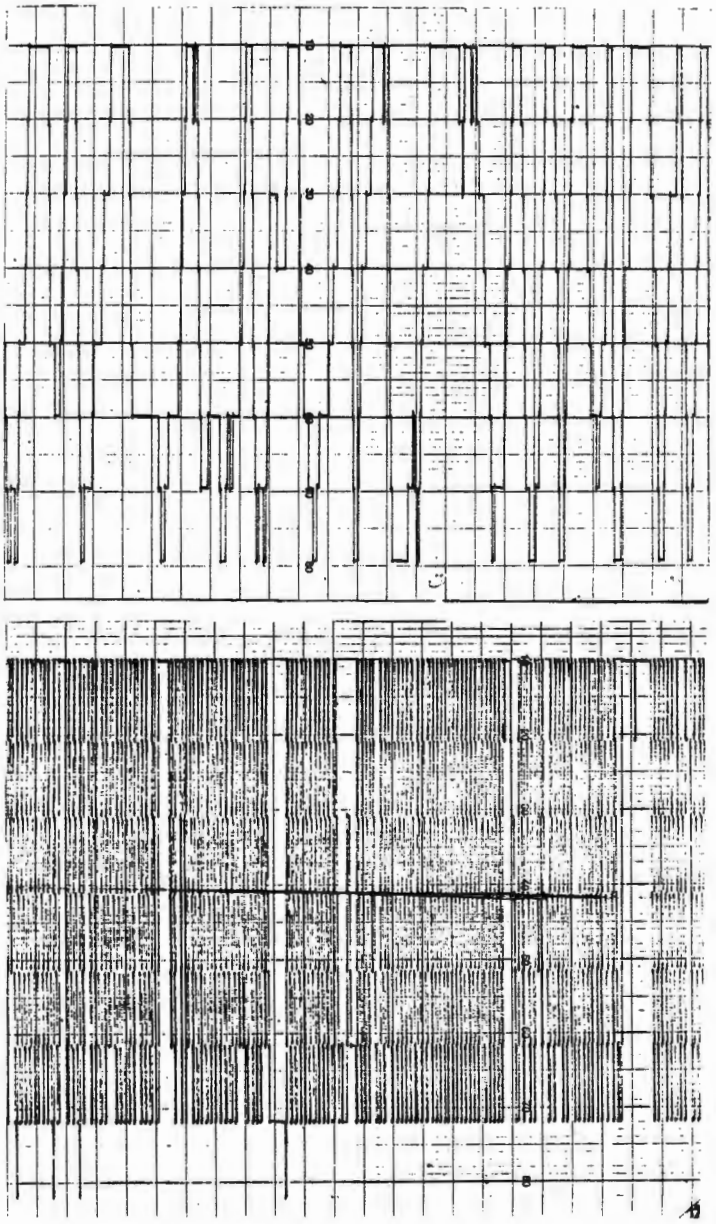

FIGURE 25. The effect of 1,0 mg Hg^{2+}/dm^3 (lower record) on the normal movement pattern of *Micropterus salmoides* (upper record). Exposure time one h .

Two clocks were provided: one was activated by signals from the left half of the tank, the other by signals from the right half. The time spent by the sensor fish in each half of the tank was displayed on the front panel by these two clocks, thus providing for an analysis of avoidance behaviour when the monitoring unit was used in conjunction with a suitable avoidance test chamber.

CIRCUIT DESCRIPTION

Thirty-two infra-red emitting diodes were positioned on one side of the tank. These were arranged in eight banks of four, thus dividing the tank into eight compartments. The four emitters of each group were spaced vertically to prevent a sensor fish from passing any group without interrupting at least one of the infra-red beams.

The current to the emitters was chopped at a rate of 2 000 cycle/s . This eliminated the effect of changes in the ambient light level, which contained a considerable level of infra-red radiation. Each of the four rows of eight emitters was driven in sequence with 10^{-3}/s bursts of 2 000 cycle/s . This reduced the total current necessary to drive the emitters. It also gave a duty cycle for any one emitter of one to eight This meant that the emitter could be pulsed at a much higher peak current than if it were to have been driven continuously.

The signals from each group of four phototransistors were fed to a differential imput amplifier (CCT 421, Figures 26 and 27). Because of the sequential drive to each group of four emitters, it may be seen that while there was nothing interrupting any of the four beams of any one group, there would be a continuous 2 000 cycle/s signal at the output of the corresponding amplifier. When any one of the beams was broken, a gap appeared in this signal. The signal was demodulated, and any change in direct current level, caused by an interrupted beam, was used to set a latch, which indicated that a particular channel was activated. The latch remained set until reset by another channel being activated.

Amplifier CCT 422 (Figure 28) contained eight monostable multivibrators, one of which was triggered every time a latch was set. The output pulse of any one of these on passing through the gates in CCT 422 activated the signal ANY CHANNEL. The pulse reset all latches except the one that generated it. Also contained in CCT 422 was a series of field effect transistor (FET) switches, and two operational amplifiers that converted the output of each latch to a corresponding voltage. This was fed to a chart recorder to provide a permanent record of the fish's position.

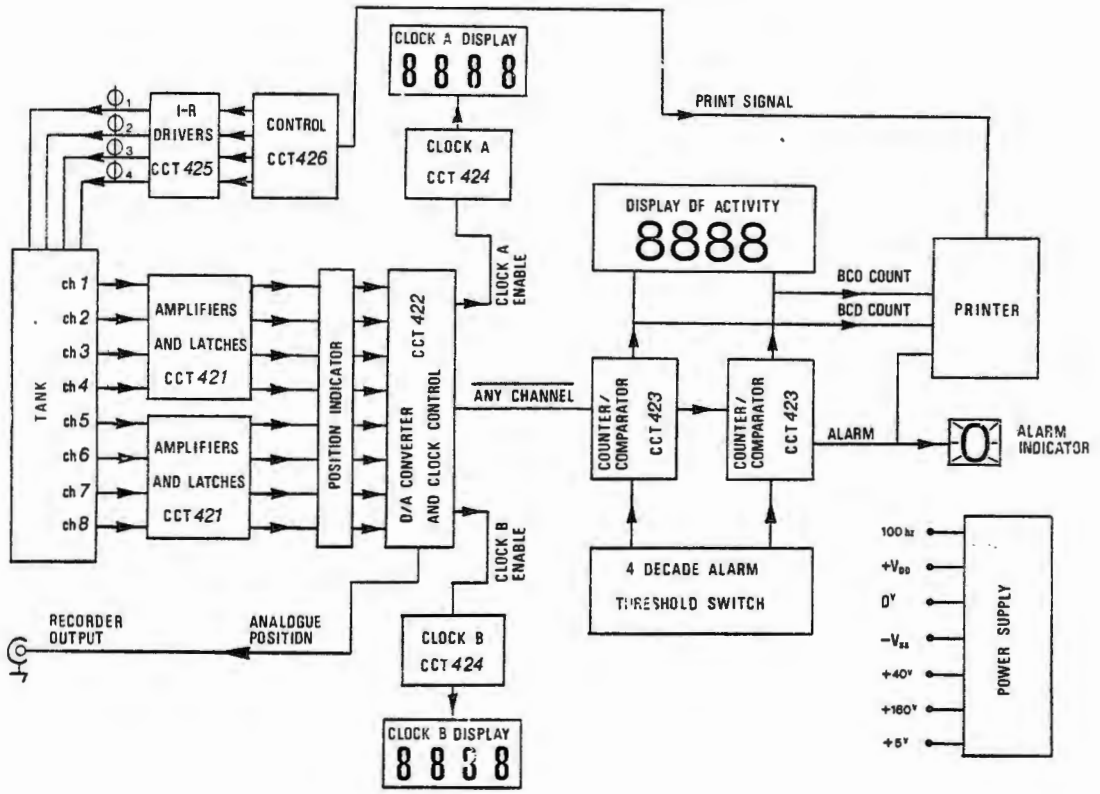

FIGURE 26. Block diagram.

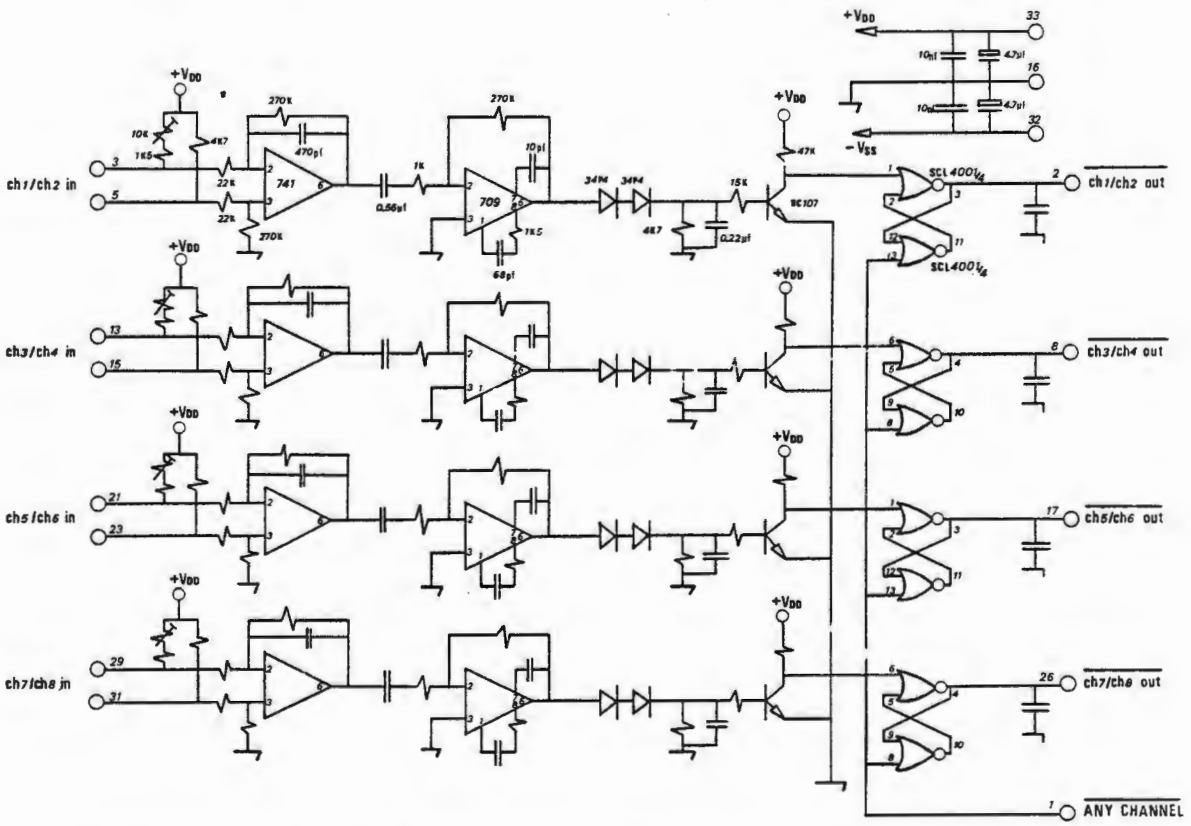

FIGURE 27. Amplifiers and latches CCT 421.

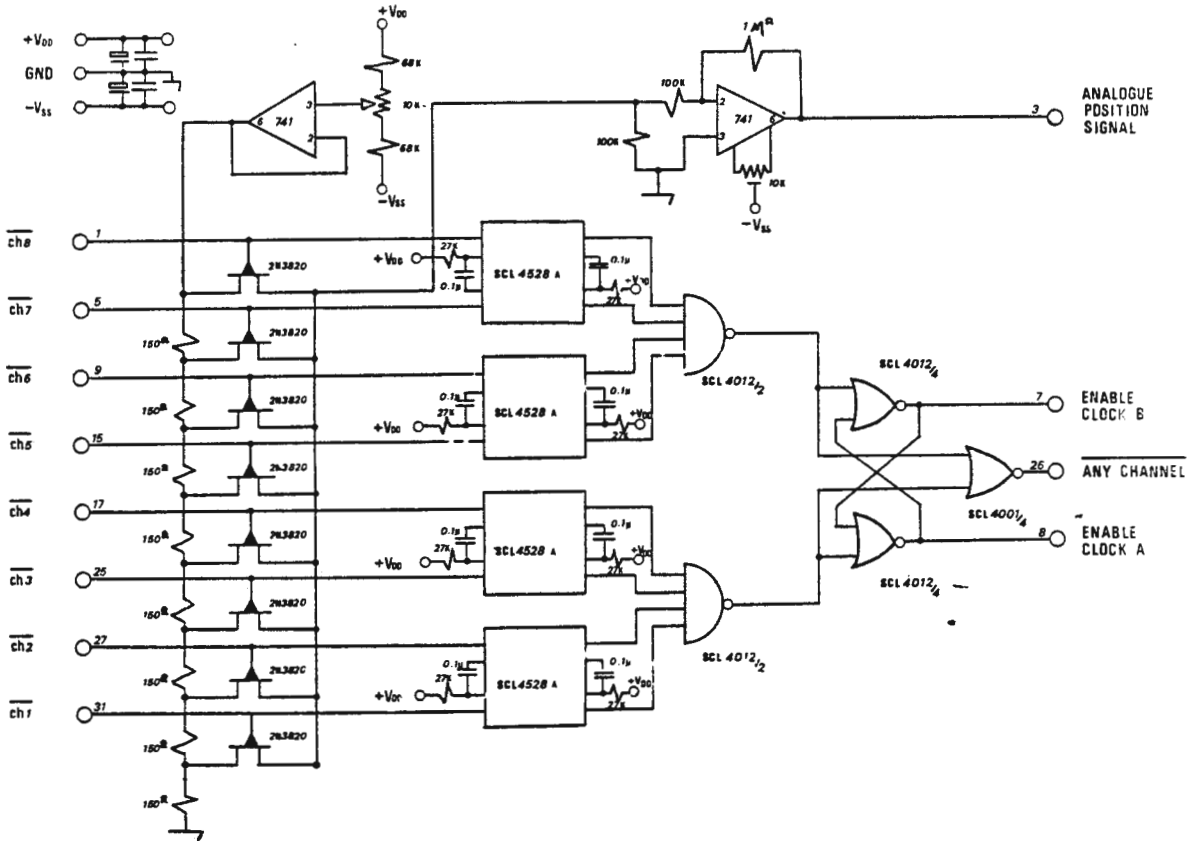

FIGURE 28. Channel switches CCT 422.

The ANY CHANNEL signal was also used to increment the activity counter (the two CCTs 423, see Figures 29 and 30). The first of these circuits contained the two least significant decades of the activity counter. It also contained the two four-bit magnitude comparators that compared the count of these two decades with the preset value of the corresponding decades on the alarm threshold thumbwheel switch. The second circuit contained the two most significant decades of the counter and the remaining two four-bit magnitude comparators. It also contained the alarm latch and alarm lamp driver. The activity counter was displayed by means of a four-digit planar gas discharge seven-segment display. This was mounted behind the front panel on a printed circuit board. The board also contained the necessary decoders and drivers for the display. The binary coded decimal (BCD) output from the activity counter was connected to those decoders and drivers. The counter could be reset from the front panel, and by means of a COUNT/HOLD switch the count could be held at any one stage instead of being further incremented. The BCD data from the counter were also connected to the digital printer together with an indication of alarm status.

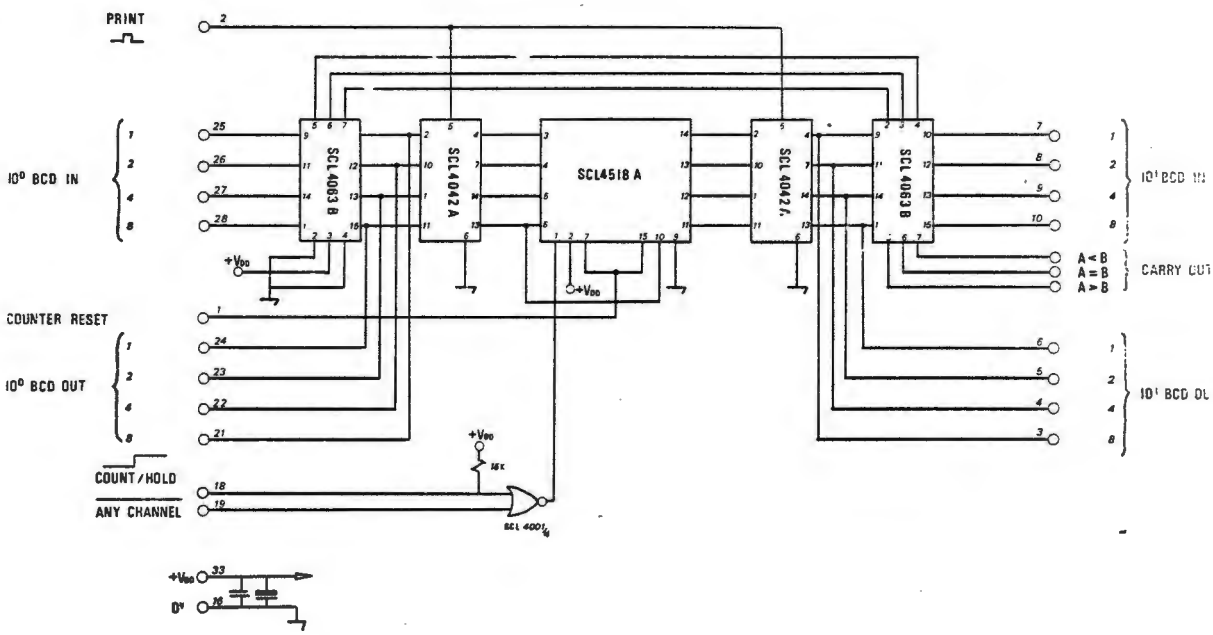

FIGURE 29. Counter alarm circuit CCT 423 - least significant board.

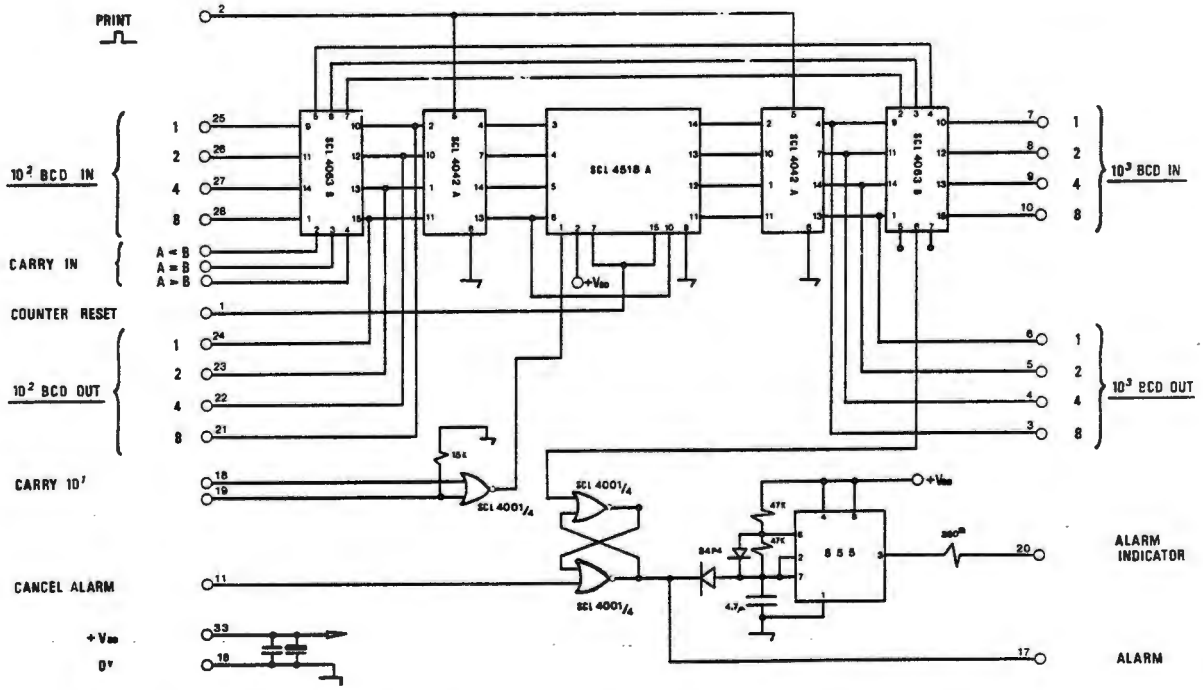

FIGURE 30. Counter alarm CCT 423 - most significant board.

The two clocks (CCT 424, Figure 31) displayed the elapsed time spent by the fish in each half of the tank. Clock A was enabled by any one of the signals from channels one to four, whilst clock B was enabled by any one of the signals from channels five to eight. the signals ENABLE CLOCK A and ENABLE CLOCK B were generated in circuit 422. This was done by passing the signals from the appropriate monostables through two four-input

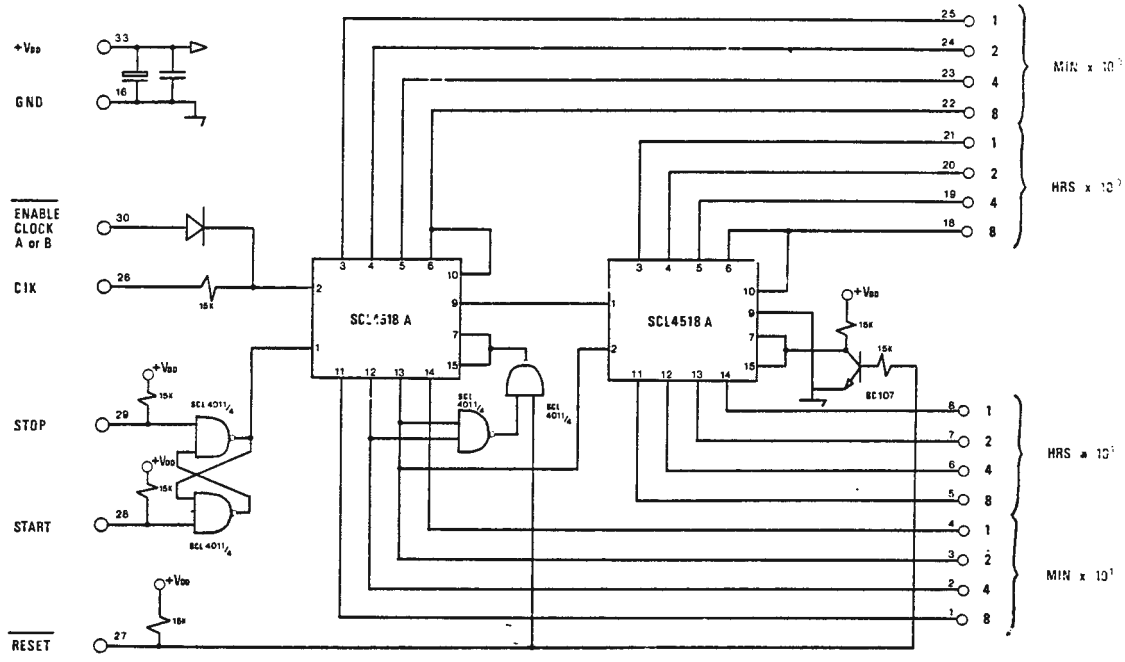

FIGURE 31. Clock CCT 424.

logic function gates. The one-minute pulses for incrementing the clocks were obtained from the CONTROL circuit (CCT 426, Figure 32). Each clock could display up to 99 hours 59 minutes. The displays for the clocks were two four-digit planar gas discharge seven-segment units similar to those used for the activity counter. They were also mounted with their associated decoder/driver circuits on a printed board behind the front panel. There were facilities for stopping, starting and resetting the clocks from the front panel.

The control circuit (CCT 426) generated all the timing signals necessary to control the system. A 100 cycle/s reference signal was derived from the 50 cycle/s main frequency. This was divided down to one-sec pulses, and were further divided to provide one-minute pulses to increment the clocks. Ten millisecond pulses were fed to a four-stage shift register. The four-phase signal thus generated gated together with a 2 000 cycle/s square wave provided Ø1, Ø2, Ø3 and Ø4, a four-phase 2 000 cycle/s signal which drove the infra-red emitters. The 2 000 cycle/s square-wave oscillator was also part of CCT 426 (Figure 32) and consisted of a Signetics NE 555 integrated circuit and associated components. The one-minute pulses were further divided to provide a pulse every hour.

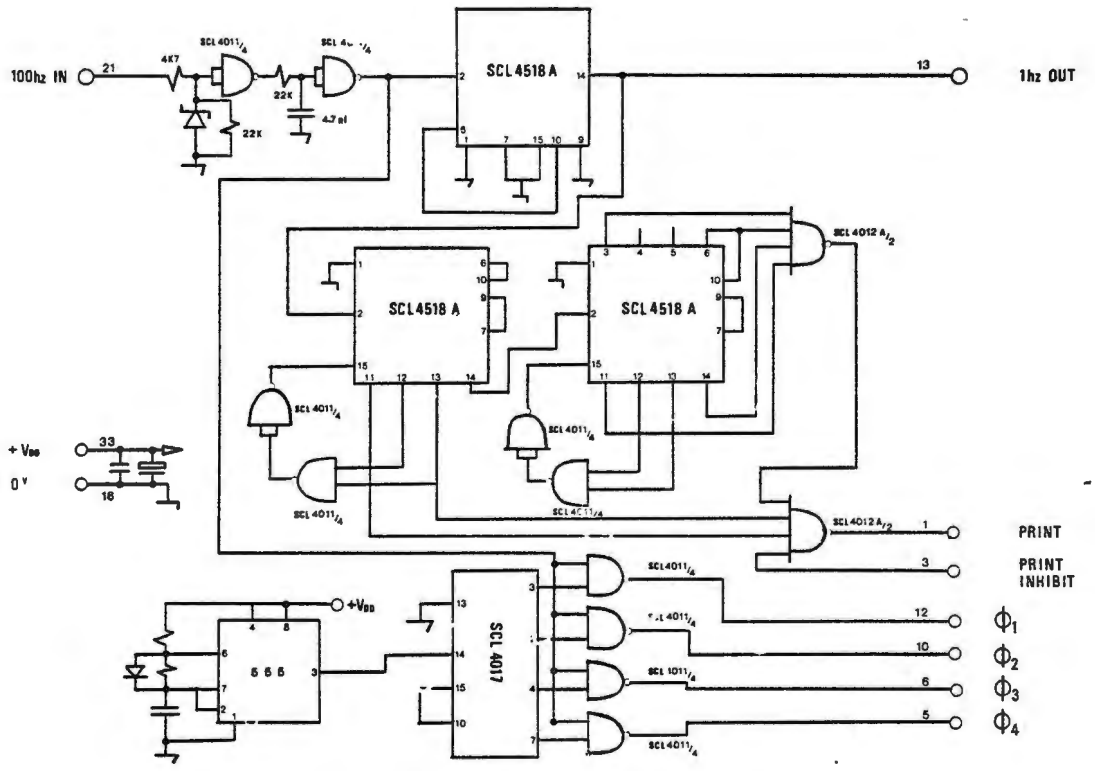

FIGURE 32. I - R Drivers CCT 425.

This pulse was used to cause the printer to print the content of the activity counter and the alarm status once an hour. After printing, the activity counter was reset in preparation for the next hour.

CCT 426 (Figure 33) amplified the four-phase signals to provide constant current pulses for the infra-red emitters. The pulses had a peak amplitude of 500 mA. However, because of the eight to one duty cycle, the average current was about 50 mA, well within the emitters capabilities.

The power supply unit provided the following voltages:

- + 7 V and 7 V regulated, for amplifiers and complementary metal oxide semi-conductor (CMOS) logic,
- + 160 V unregulated for the gas discharge displays
- + 40 V unregulated for the infra-red emitters
- + 5 V regulated for the printer logic
- + 30 V unregulated for the printer, and
- 24 V root mean square (rms) 50 cycle/s for the printer solenoids.

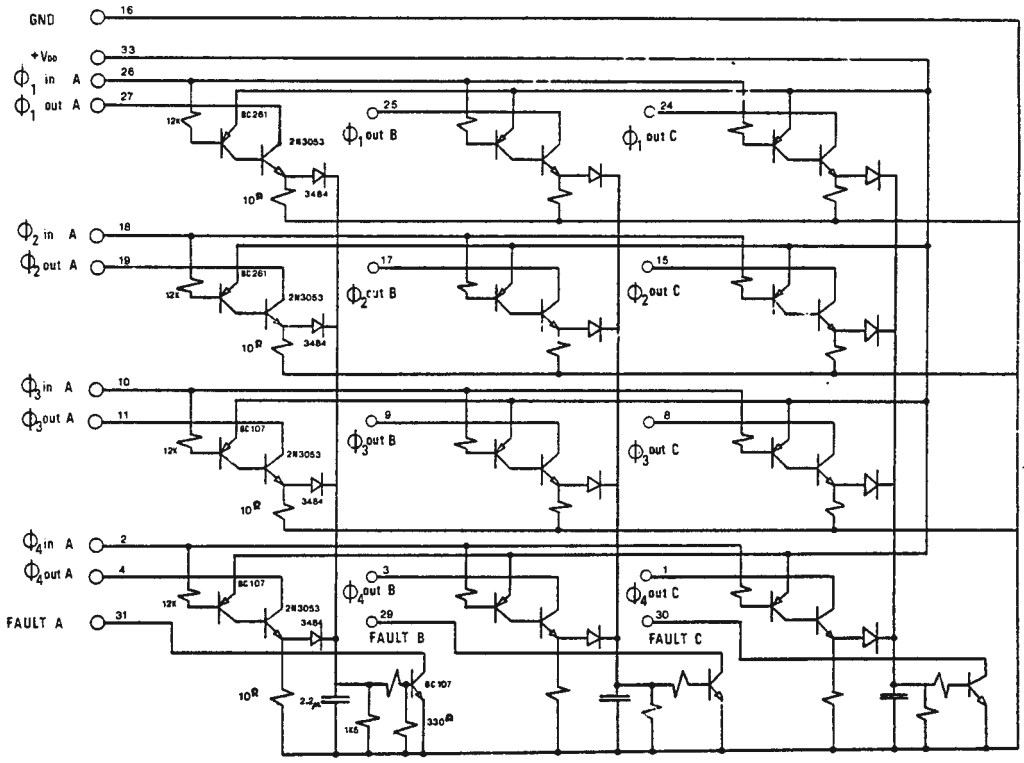

FIGURE 33. Control CCT 426.

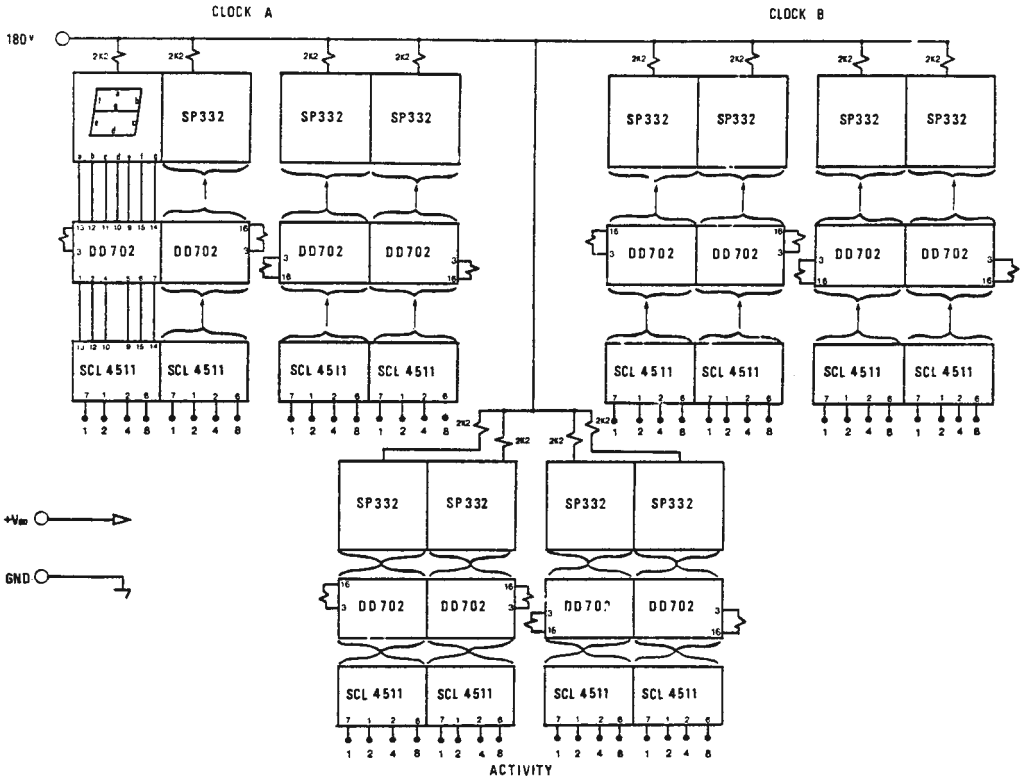

FIGURE 34. Display logic CCT 427.

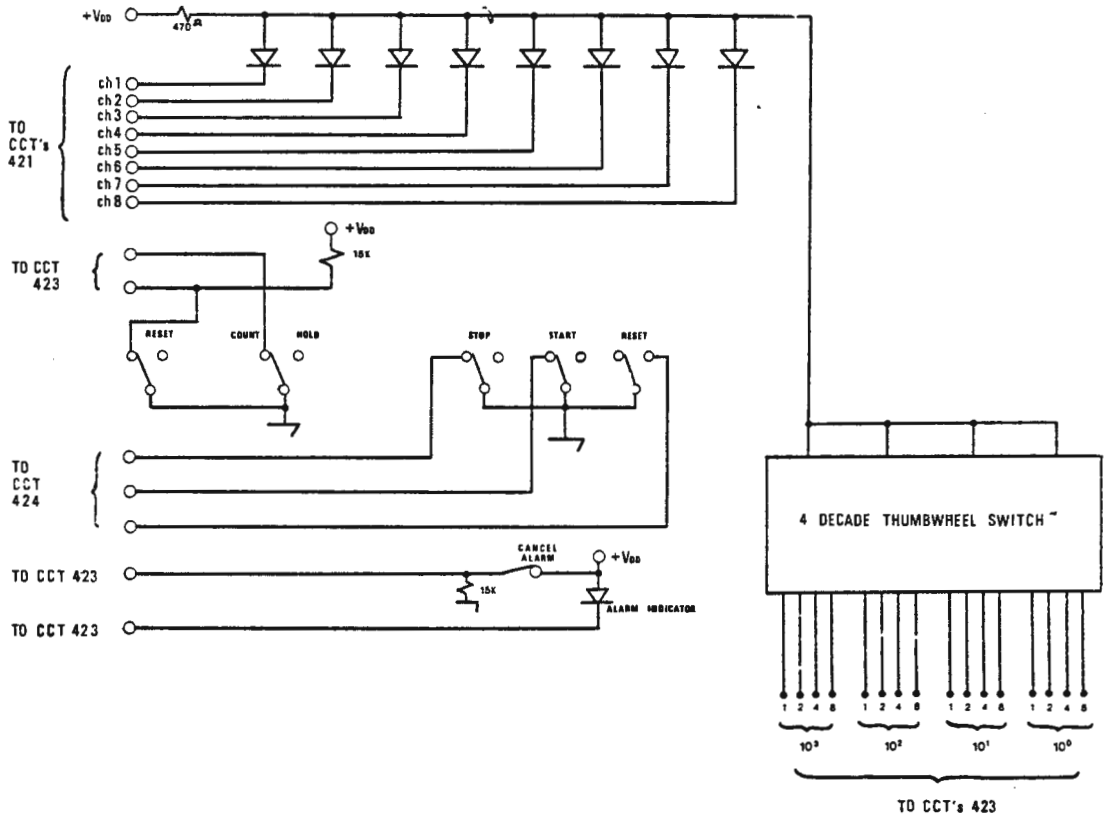

FIGURE 35. Front panel counter and clocks.

A 100 cycle/s reference signal for the control circuit was also obtained from the power supply unit.

PRACTICAL APPLICATION OF A CONTINUOUS AUTOMATIC BIOLOGICAL MONITORING SYSTEM BASED UPON FISH LOCOMOTORY RESPONSE TO INTOXICATION

In the natural, unpolluted freshwater environment, fish have the ability to detect and respond to altered water quality and avoid detrimental chemical conditions. Locomotory response to adverse effects may be more important in determining their distribution than the power of the fish to sustain a stable habitude and fish may, therefore, avoid a harmful situation before it becomes toxic (Wells, 1913; 1915). Thus, the locomotory behaviour of fish in response to pollution may be an important factor determining the toxicity of pollutants in nature.

Chemicals added to water containing fish may cause the fish to exhibit alarm, to display an avoidance response, to be repelled, or to be attracted. The alarm response involves restless behaviour and non-directional movements while the avoidance response involves a refusal of the fish to enter freely an area of contaminated water. When a chemical acts as a repellant or attractant, the fish purposefully move out of or into the area of treated water. In all cases the normal locomotory behaviour pattern is disturbed.

The electronic system, constructed to continuously monitor the locomotory activity of fish, compared their behavioural patterns in response to intoxication with those obtained in the absence of any toxic influence. Preliminary research indicated that aberrant behaviour patterns appear in locomotory activity of fish exposed to both acute and sub-lethal concentrations of a number of toxicants. The purpose of the research, described below, was to confirm the use of the technique, based upon fish movement pattern response, for a wider range of toxicants, as well as attempting to delineate the limits of response to various acute and sub-lethal concentrations of toxicants. The experimental procedures were designed in order to provide for a direct comparison of this technique with that which used respiratory responses to intoxication.

EXPERIMENTAL DESIGN

Fifty-one sensor fish (*Micropterus salmoides* Lacépède) were employed to establish the efficacy of the system. The sensor fish were transferred individually to the activity chamber which was supplied with continuously flowing dechlorinated tap water; the pH was adjusted to 7,0 and the temperature to 23±1°C. Each sensor was allowed to acclimate to enclosure within the chamber for 48 h before any records were made. Normal activity levels were recorded for seven days subsequent to sensor fish recovery from the initial stress brought about by transfer to the activity chamber. Hourly records were extracted from the print-out and used to calculate mean and standard deviation values for each hour of the day and night, and these were used to establish maximum 95% confidence limits for activity. These values were regarded as being the response levels for the sensor fish concerned. The response levels were set using the alarm threshold control.

During experimental toxicant addition, each sensor was deemed to have responded to the changed environment as soon as the alarm circuit triggered, indicated by a flashing red light situated on the control panel of the electronic monitor. An alarm status was also indicated on the print out, thus automatically establishing the period during which sensor fish elicited a response.

ACUTE AND SUB-LETHAL TOXIC EFFECTS

Sensor fish were exposed to nominal concentrations of copper, cadmium, cyanide, mercury, phenol and ammonia; the concentrations of the toxicants were chosen arbitrarily to provide acute and sub-lethal levels equivalent to those used in previous determinations which investigated the effect of acute and sub-lethal intoxication upon sensor fish opercular rhythms.

Toxicant concentrations were established in the experimental chamber by the addition of a predetermined volume of a 1 g/dm^3 solution of toxicant. Thereafter the nominal toxicant concentrations were maintained using a dosing unit similar to that of Stark (1967), wherein solenoid valves operated through a microswitch activated by a perspex

float replaced the suggested pneumatic valve arrangement. Toxicant addition was maintained for five successive days or to mortality of the sensor fish.

DEFINING THRESHOLD DETECTION LEVELS

Behavioural responses of sensor fish to increasing concentrations of a number of toxicants were utilized to establish the threshold levels of detection. The initial toxicant concentrations set up in the test chamber were based upon results obtained from previous experimentation and were established by the addition of predetermined volumes of 1 g/dm^3 solutions. Thereafter toxicant concentrations were maintained using the dosing units previously described. Each toxicant concentration was maintained in the chamber for a period of 24 h . After this period sensor fish were allowed to resume a normal locomotory behaviour pattern before being exposed to successively higher concentrations. Because the responses of only one sensor fish could be investigated at a time, each series was curtailed when the toxicant concentration, established in the chamber, was equivalent to the sub-lethal concentrations employed in previous determinations. Three sensor fish were exposed to each toxicant and the threshold limit of detection was defined as that concentration which elicited a response from any two sensors within 24 h of initial exposure. For all determinations a separate group of three sensor fish were exposed directly to that concentration defined as the threshold in order that it may be confirmed.

DISCUSSION OF RESULTS

The instrumentation worked adequately, without being entirely flawless, throughout the experimental determinations. The problem of major concern arose from the high degree of variability obtained in the locomotory patterns of a single sensor fish at different times of the day as well as between a number of sensor fish at the same time.

The magnitude of variation in the total counts accumulated every hour was particularly pronounced during the pretreatment days. This high degree of variation in movement patterns made it extremely difficult to compare responses of fish subjected to the same experimental conditions, and it was large enough to preclude any prediction on an established

movement pattern. This was, in fact, the prime reason for recording movements of each fish individually for seven days prior to the introduction of the toxicant. Recording movement patterns before and after toxicant introduction allowed each fish to serve as its own control.

The activity patterns presented in Figures 36 to 41 may be taken as typical examples for the species of sensor fish used. A low level of activity was maintained throughout the night, rising sharply during the dawn period. A further peak of increased activity occurred towards dusk. The responses to lethal levels of intoxication were again fairly typical reactions. One or two increases in activity, followed by partial recovery to normal levels, were noted. Subsequently the activity of the sensor fish rose to levels well above designated response limits, continuing at a very high level until the rapid decrease to mortality. Responses were also obtained from all sensor fish exposed to sub-lethal toxicant concentrations.

A lethal concentration of *copper* (1,0 mg/dm^3) elicited a response from the sensor fish within one h of exposure. Activity rose to a level well above designated response limits throughout the first three h of exposure before decreasing gradually to the normal level (Figure 36, Table 34). Locomotory activity increased again after an exposure period of approximately 12 h remaining at a high level until the gradual decrease to mortality which occurred between 28 h and 29 h after the introduction of toxicant. A sub-lethal amount of copper (0,1 mg/dm^3) elicited a response within six h of exposure (Table 35). The sensor fish resumed a diurnal activity pattern after 24 h and normal behaviour within 48 h .

Increase in activity of fish exposed to sub-lethal copper solutions is well known; for example, brook trout (*Salvelinus fontinalis*) exposed to copper solutions of 6-115 µg/dm^3 (Drummond, Spoor and Olsen, 1973) increased their activity to four to six times that of the controls during the first eight h of exposure; activity patterns returned to normal after three days in those fish exposed up to 12 µg/dm^3. Similarly brook trout exposed to copper levels greater than 17 µg/dm^3 stopped feeding and at 12 µg/dm^3 an initial reduced feeding rate returned to normal (albeit sluggishly) after four days; these levels were similar to those at which survival and growth of brook trout began to be affected (McKim and Benoit, 1971).

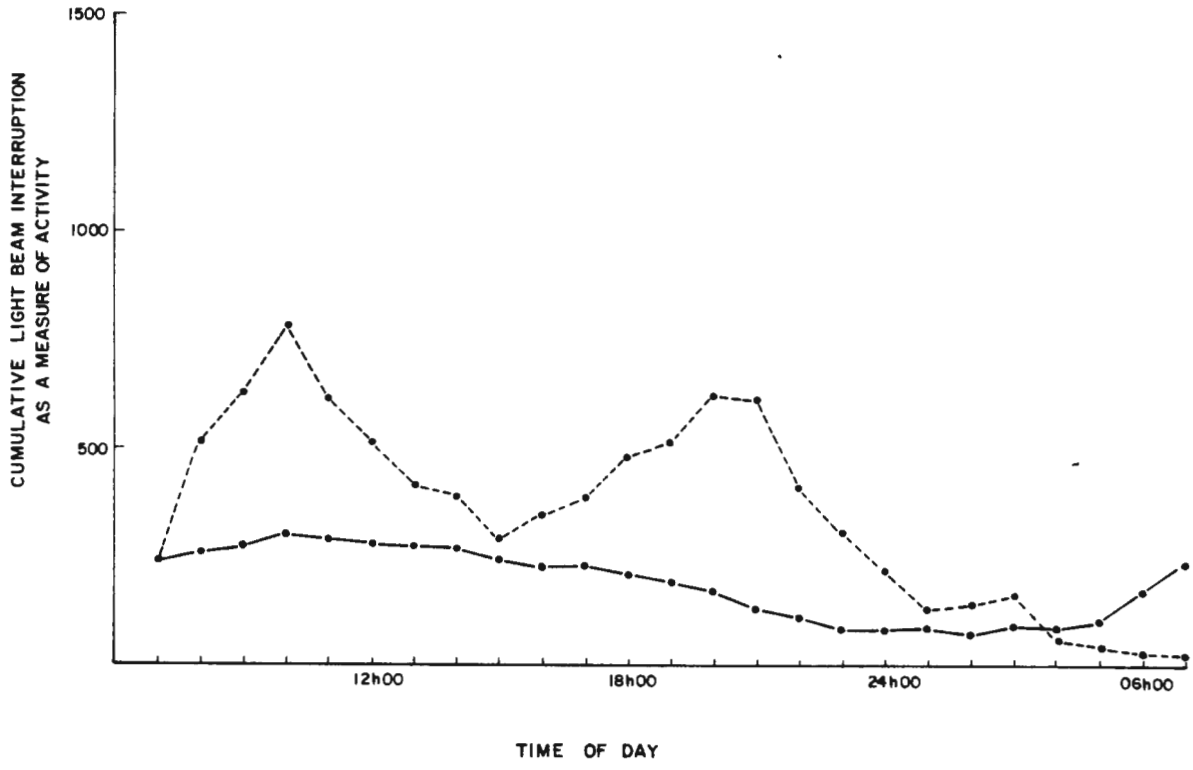

FIGURE 36. The effect of 1,0 mg Cu^{2+}/dm^3 (broken line) on the normal activity pattern of *Micropterus salmoides* (continuous line).

Sprague showed that, in the absence of other stimuli, Atlantic salmon parr (*Salmo salar*) could detect levels of copper in soft water (20 mg/dm^3 as $CaCO_3$) as low as 2,4 µg/dm^3 which was 0,05 of the threshold lethal concentration; in these laboratory experiments the fish were given a choice of clean or polluted water in a short tube with a sharp interface between the two solutions. Rainbow trout (*Salmo gairdneri*) apparently avoided even lower concentrations (0,1 mg Cu^{2+}/dm^3) in a Y-shaped maze in water having a hardness of 89,5 mg/dm^3 as $CaCO_3$ (Folmar, 1976). The increase in activity of the sensor fish in response to a lethal level of copper, in this instance, was probably an overall stress reaction, whereas the response to a sub-lethal concentration resulted from olfactory detection. The resumption of a normal behaviour pattern after three days exposure to the sub-lethal concentration possibly resulted from the fact that the toxicant adversely affected the chemical receptor system involved. Experiments on the effects of heavy metals on the palatal chemo-receptors of common carp (*Cyprinus carpio*) showed that a concentration of 6,4 mg Cu^{2+}/dm^3 depressed the response of the sugar and salt receptors (Hidaka, 1970). Also olfactory responses of sockeye salmon (*Oncorhynchus nerka*), coho salmon (*Oncorhynchus kisutch*) and

TABLE 34. Hourly activity levels for a sensor fish before and after exposure to 1,0 Cu^{2+}/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								230	202	361	312	274	322	242	233	255	249	279	314	170	96	104	105	69
2	92	48	83	56	123	218	180	206	275	281	292	244	186	189	176	172	201	152	105	179	166	166	35	96
3	136	65	108	83	89	207	214	271	261	288	216	283	183	224	204	137	142	201	141	147	90	102	110	78
4	123	60	89	69	128	204	213	309	264	243	259	238	317	269	293	260	275	233	164	76	82	68	39	29
5	80	56	61	202	97	249	302	255	304	324	371	365	324	339	332	305	307	269	240	175	108	142	105	92
6	53	76	57	31	85	135	338	308	309	312	300	262	329	340	288	304	238	224	234	201	177	98	107	80
7	83	83	38	50	92	71	274	255	247	294	324	310	282	298	188	156	194	101	130	256	202	89	63	116
8	37	101	182	97	82	101	173	*513*	*627*	*781*	*613*	*514*	*412*	*391*	*288*	*346*	*384*	*481*	*516*	*623*	*614*	*412*	*302*	*220*
	130	*141*	*162*	*63*	*41*	*28*	*17*	*13*	*8*	*9*	*13*	*2*												
a	86	70	88	84	99	169	242	262	266	300	296	282	278	272	245	227	229	208	190	172	132	110	81	80
b	35	18	47	56	19	67	63	38	36	37	49	44	65	58	60	71	55	64	75	55	49	33	34	27
c	155	105	181	195	136	300	366	337	337	373	392	368	406	384	362	366	337	332	337	279	227	175	147	133

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 35. Hourly activity levels for a sensor fish before and after exposure to 0,1 mg Cu^{2+}/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								149	235	218	209	236	90	186	151	140	226	288	238	242	110	106	122	118
2	98	44	46	35	102	157	165	222	145	271	231	323	243	209	209	435	368	368	237	212	150	110	130	92
3	108	69	96	57	107	245	72	93	276	474	485	197	113	281	386	148	301	203	191	58	30	33	40	55
4	38	62	47	47	29	62	65	190	518	546	582	609	560	550	515	511	434	219	427	106	31	35	3	1
5	53	8	1	7	38	94	137	294	140	400	453	436	422	368	362	222	198	268	167	212	34	1	3	8
6	1	2	11	4	1	49	180	199	167	211	216	169	331	323	140	265	276	169	234	272	202	66	36	36
7	41	24	30	34	41	29	218	176	229	168	177	164	319	579	215	208	253	249	197	301	213	68	35	29
8	35	29	25	62	67	27	85	*312*	*461*	*584*	*612*	*658*	*702*	*859*	*916*	*803*	*831*	*791*	*694*	*704*	*729*	*819*	*820*	*616*
9	*534*	*323*	*104*	*79*	*42*	*21*	*80*	*104*	*201*	*218*	*226*	*334*	*313*	*361*	*271*	*293*	*314*	*248*	*196*	*171*	*94*	*62*	*71*	*53*
10	*83*	*42*	*62*	*84*	*61*	*80*	*94*	*206*	*332*	*412*	*438*	*318*	*276*	*337*	*263*	*271*	*217*	*194*	*204*	*122*	*71*	*23*	*34*	*40*
11	*41*	*24*	*36*	*51*	*58*	*94*	*132*	*274*	*416*	*378*	*401*	*321*	*264*	*281*	*341*	*233*	*252*	*216*	*246*	*193*	*154*	*101*	*73*	*62*
12	*56*	*25*	*4*	*7*	*43*	*83*	*102*	*162*	*214*	*294*	*306*	*274*	*288*	*353*	*207*	*257*	*231*	*231*	*273*	*201*	*112*	*82*	*53*	*41*
	39	*48*	*21*	*33*	*74*	*161*	*194*																	
a	53	34	37	35	55	95	132	189	244	327	336	305	297	357	283	276	294	252	242	200	110	60	53	48
b	38	26	31	28	39	80	59	62	131	146	165	166	166	155	141	143	82	65	86	88	81	40	52	43
c	127	84	98	80	132	252	248	311	501	614	659	630	623	661	558	556	456	380	411	373	268	138	155	133

a, b and c are, respectively, the mean, standard deviation and 95% confidence limit

Figures in italics represent post-exposure activity levels.

rainbow trout to food extracts, amino acids and hand rinses were extinguished after more than 12 h exposure of the fish to a concentration of 40 µg Cu^{2+}/dm^3 (Hara, 1972).

A lethal concentration of *cadmium* (0,5 mg/dm^3) induced a gradual increase in the activity of a sensor fish, the designated critical response level being exceeded within 11 h exposure (Figure 37, Table 36). Subsequently, activity decreased slightly before resuming a high level which was maintained for over 24 h before it decreased gradually as the sensor fish became more and more torpid. The sensor fish died after being exposed to the toxic effect for 49 h . The response to a sub-lethal quantity of cadmium (0,1 mg/dm^3) was similar to that for copper (Table 37). The critical response level was exceeded after 16 h exposure. Thereafter the sensor fish reverted to a diurnal rhythm within 24 h exposure and assumed its normal behaviour pattern after 48 h .

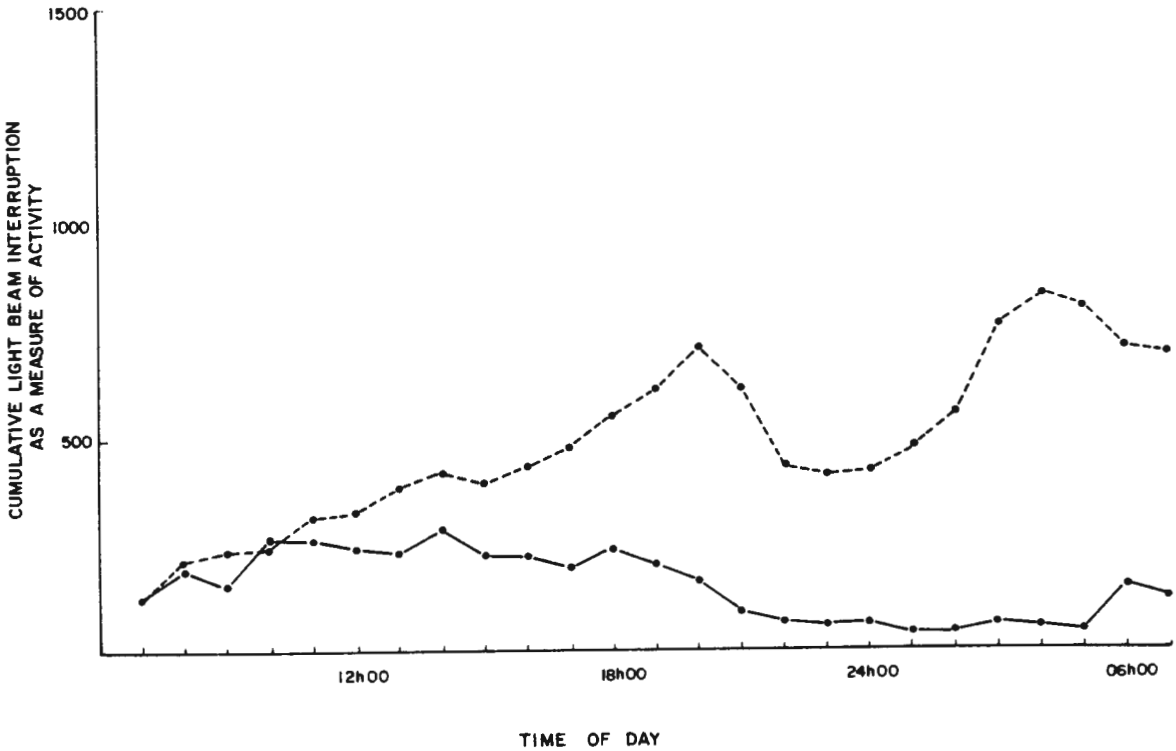

FIGURE 37. The effect of 0,5 mg Cd^{2+}/dm^3 (broken line) on the normal activity pattern of *Micropterus salmoides* (continuous line).

TABLE 36. Hourly activity levels for a sensor fish before and after exposure to 0,5 mg Cd^{2+}/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								188	119	174	167	189	72	149	120	112	190	180	230	194	88	84	98	94
2	37	34	78	81	70	126	132	116	178	217	184	258	194	167	167	348	190	294	294	169	119	88	104	73
3	77	55	86	86	46	196	58	220	74	379	338	158	90	225	308	118	153	241	162	46	24	66	80	111
4	47	50	74	58	47	125	52	414	152	437	465	487	448	440	412	409	342	347	175	84	31	71	3	1
5	1	8	53	38	7	188	110	112	235	320	362	348	337	294	290	178	134	158	214	170	34	1	0	8
6	11	0	1	1	4	98	144	134	159	169	173	135	264	258	112	212	187	220	135	217	161	66	36	73
7	30	48	41	41	68	158	174	168	140	134	142	131	255	463	172	166	157	202	199	240	170	68	71	59
8	51	58	71	54	48	155	168	*201*	*232*	*241*	*311*	*326*	*383*	*419*	*394*	*436*	*481*	*553*	*612*	*714*	*621*	*438*	*414*	*423*
9	*481*	*560*	*771*	*832*	*812*	*714*	*698*	*614*	*518*	*432*	*289*	*318*	*271*	*269*	*191*	*94*	*182*	*192*	*216*	*143*	*121*	*98*	*74*	*62*
	58	*41*	*14*	*83*	*103*	*81*	*79*	*3*																
a	36	36	58	51	41	149	120	193	151	261	262	244	237	285	226	220	193	235	201	160	90	63	56	60
b	26	23	29	29	27	36	49	105	50	117	125	132	133	124	113	115	69	66	52	70	62	29	43	41
c	86	82	115	108	93	219	216	399	249	491	507	503	498	529	506	445	329	364	303	298	212	120	141	141

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 37. Hourly activity levels for a sensor fish before and after exposure to 0,1 mg Cd^{2+}/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								690	406	369	450	597	319	270	338	317	316	246	101	115	56	62	70	68
2	55	26	1	16	94	393	354	498	503	383	484	400	352	367	378	259	215	145	125	51	47	59	62	36
3	41	57	55	28	59	306	492	344	232	280	342	273	279	235	212	165	168	95	43	49	16	51	2	2
4	75	30	74	65	105	302	261	442	342	324	317	278	251	291	285	321	405	367	335	20	142	53	123	126
5	196	135	1	93	209	269	439	353	362	293	288	319	262	301	411	358	337	280	230	191	72	147	83	47
6	42	19	112	136	43	162	347	341	320	333	296	275	272	264	247	296	259	252	265	57	105	105	114	118
7	39	129	129	188	238	194	237	216	213	310	175	141	144	255	142	122	148	254	141	37	65	17	5	37
8	13	9	37	150	221	193	324	*423*	*391*	*414*	*426*	*434*	*398*	*461*	*503*	*515*	*543*	*521*	*604*	*586*	*614*	*672*	*753*	*804*
9	*916*	*802*	*791*	*774*	*683*	*621*	*515*	*471*	*403*	*396*	*352*	*316*	*301*	*294*	*288*	*241*	*245*	*224*	*193*	*181*	*162*	*98*	*74*	*60*
10	*51*	*48*	*50*	*83*	*98*	*194*	*281*	*384*	*412*	*317*	*318*	*337*	*294*	*272*	*299*	*286*	*291*	*291*	*146*	*92*	*71*	*64*	*53*	*50*
11	*64*	*68*	*102*	*113*	*157*	*209*	*294*	*321*	*333*	*286*	*341*	*363*	*261*	*206*	*271*	*280*	*304*	*267*	*132*	*71*	*42*	*24*	*21*	*30*
12	*48*	*59*	*41*	*62*	*102*	*261*	*372*	*472*	*361*	*214*	*294*	*301*	*194*	*253*	*304*	*253*	*243*	*221*	*204*	*76*	*53*	*21*	*43*	*52*
	58	*74*	*83*	*92*	*96*	*231*	*406*																	
a	66	58	58	97	138	260	351	412	340	327	336	326	268	283	288	263	264	234	177	74	72	71	66	62
b	60	53	50	65	82	82	91	151	100	38	104	142	65	43	95	88	94	89	103	59	41	42	48	46
c	184	161	157	223	299	420	529	708	535	402	540	604	396	368	474	434	448	409	378	191	152	154	159	151

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

Hyperactivity in response to cadmium poisoning has been reported for bluegill, *Lepomis macrochirus*, (Cearley and Coleman, 1974; Eaton, 1974), for largemouth bass, *Micropterus salmoides*, (Cearley and Coleman, 1974), and for flagfish, *Jordanella floridae*, (Spehar, 1976). Ellgaard, Tusa and Malizia (1978) have also described an increase in the locomotor activity of bluegill at concentrations of cadmium of 0,1 and 0,25 mg/dm^3 which were not lethal to the fish within two weeks. There are almost no data available on the effects of sub-lethal concentrations of cadmium on the behaviour of fish. However, a consideration of the results would indicate that the effect of cadmium is similar to that of copper. The apparent acclimation of the sensor fish to sub-lethal levels of both toxicants probably being due to the inhibition of olfactory sensor mechanisms.

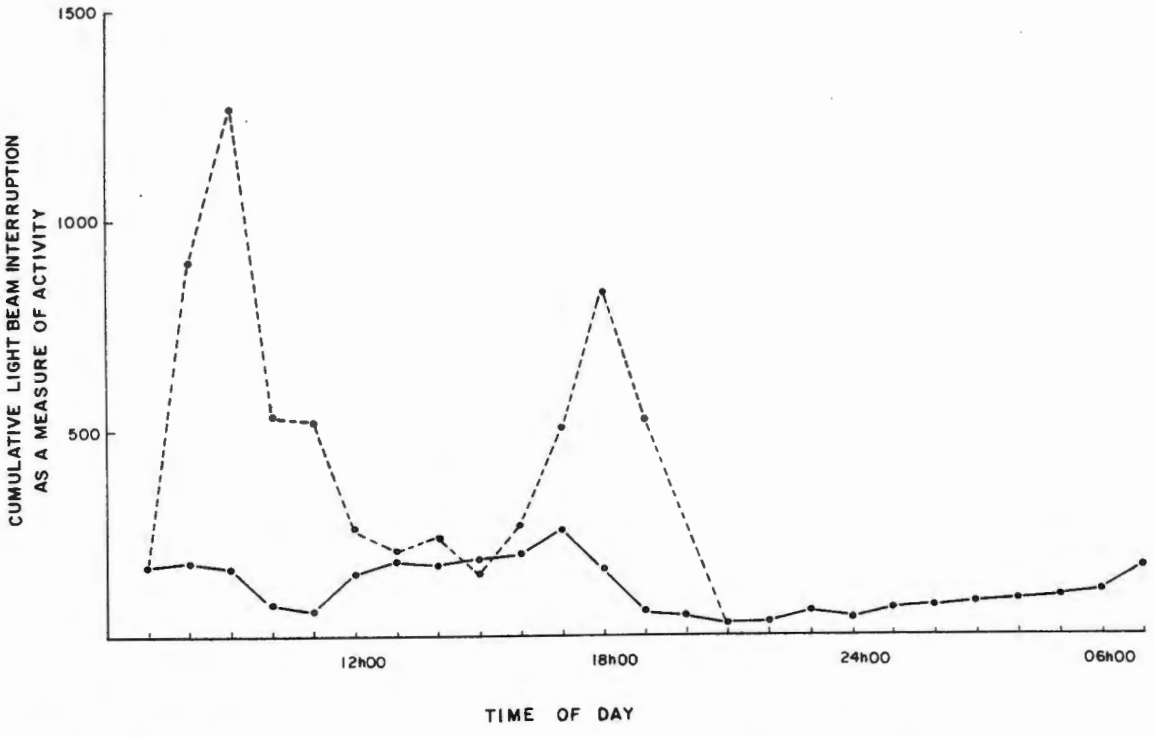

FIGURE 38. The effect of 1,0 mg Hg^{2+}/dm^3 (broken line) on the normal activity pattern of *Micropterus salmoides* (continuous line).

Exposure of a sensor fish to a lethal level of *mercury* (1,0 mg/dm^3) resulted in an activity rate increase to approximately eight times normal levels within two h . This instantaneous increase in locomotory activity was followed by an immediate sudden decrease (Figure 38, Table 38). A

second heightened level of activity was achieved before the expected, but not gradual, passivity leading to mortality. Sensor fish response to a sub-lethal level of mercury (0,01 mg/dm^3) was similar to that of the other heavy metals tested (Table 39). The critical response level was exceeded after 18 h exposure. Activity remained at a high level well into the second day of exposure, assuming a normal diurnal behaviour pattern after 72 h . However, in contrast to the effects of copper and cadmium, the activity level fell markedly after five days of exposure.

Research by Lindahl and Schwanbom (1971) upon the ability of fish to remain upright in a rotary flow of water has indicated that sub-lethal mercury poisoning induces disturbances in the central nervous system resulting in inhibition of neuro-muscular transmission. This apparent narcotic effect would explain the progressive langour induced by the sub-lethal concentration of mercury used in the test, described above. It is arguable, therefore, whether the sensor animal would function adequately if maintained in this environment for a long period, and its ability to survive would be seriously impaired.

Fish exposed to concentrations of *phenol* that are lethal within a few days soon become excited, swimming rapidly and becoming more sensitive to outside stimili (Veselov, 1957). This response was elicited in a sensor fish exposed to 5,0 mg phenol/dm^3, where the activity increased to more than five times the normal level within three h exposure (Figure 39) Table 40). Except for a short period of fatigue the sensor fish maintained a high activity rate for 48 h before the usual gradual decrease to fatality. On exposure to a sub-lethal level of phenol (1,0 mg/dm^3) the sensor fish exhibited an activity level exceeding the designated response limit within five h . A very high level of locomotory activity was, thereafter, maintained throughout the five day exposure period (Table 41).

Hasler and Wisby (1949) showed that the bluntnose minnow (*Pimephales notatus*) could be trained to detect phenol at concentrations below 0,01 mg/dm^3 (some individuals being capable of detecting as little as

TABLE 38. Hourly activity levels for a sensor fish before and after exposure to 1,0 mg Hg^{2+}/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								133	155	140	108	174	158	197	167	121	131	185	35	102	45	31	75	101
2	92	122	135	133	135	153	93	232	127	55	93	227	196	155	119	111	248	134	50	56	28	25	32	67
3	83	105	71	119	125	143	236	212	219	64	20	210	240	152	202	213	463	135	40	38	50	36	46	28
4	63	127	78	133	85	88	216	253	175	50	66	86	164	142	163	280	389	164	45	25	16	29	24	10
5	57	34	117	48	80	106	190	114	193	56	10	85	162	139	90	323	282	193	128	33	29	26	161	55
6	88	10	55	37	33	113	140	146	117	71	56	69	186	209	252	304	234	124	29	32	13	29	43	14
7	27	51	49	52	147	54	163	161	160	101	63	205	168	230	342	48	89	228	73	51	14	33	29	17
8	23	53	33	74	47	85	157	*908*	*1266*	*531*	*520*	*261*	*207*	*241*	*153*	*268*	*502*	*831*	*529*	*2*				
a	62	71	77	85	93	106	170	179	164	77	59	151	182	175	191	200	262	166	57	48	28	30	59	42
b	28	46	37	42	44	34	48	53	36	33	36	68	29	37	85	108	132	38	34	26	15	4	48	34
c	117	162	149	168	180	173	265	283	234	141	129	285	238	246	358	411	522	241	124	99	57	37	153	108

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 39. Hourly activity levels for a sensor fish before and after exposure to 0,01 mg Hg^{2+}/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								146	259	214	251	147	43	409	96	70	246	197	133	250	88	129	77	87
2	40	99	324	173	239	220	153	437	201	404	267	156	142	201	106	158	184	129	231	79	21	7	13	12
3	20	18	19	20	49	154	101	154	91	182	138	103	71	50	78	13	146	77	65	44	19	78	92	38
4	105	12	17	25	23	79	52	124	145	198	218	206	204	167	136	53	141	190	202	174	104	54	6	1
5	22	15	6	18	37	121	128	127	238	127	133	162	113	133	174	169	118	108	157	84	4	9	6	3
6	6	10	2	3	24	65	104	53	64	52	31	28	39	51	58	86	24	42	6	10	87	9	8	14
7	13	18	6	16	32	53	37	18	11	14	14	15	39	18	16	20	13	39	21	65	208	11	11	5
8	1	3	13	4	15	67	41	*121*	*134*	*181*	*253*	*193*	*134*	*157*	*161*	*184*	*196*	*231*	*241*	*302*	*218*	*246*	*332*	*409*
9	*432*	*479*	*516*	*494*	*481*	*471*	*496*	*584*	*602*	*512*	*316*	*212*	*94*	*162*	*114*	*94*	*81*	*86*	*94*	*96*	*71*	*46*	*41*	*39*
10	*31*	*23*	*13*	*43*	*76*	*134*	*112*	*131*	*156*	*184*	*163*	*124*	*86*	*138*	*94*	*36*	*114*	*138*	*153*	*117*	*92*	*53*	*56*	*51*
11	*43*	*21*	*24*	*35*	*64*	*142*	*134*	*204*	*231*	*212*	*182*	*131*	*98*	*129*	*73*	*42*	*73*	*114*	*121*	*92*	*73*	*28*	*32*	*32*
12	*35*	*7*	*9*	*29*	*52*	*103*	*96*	*151*	*163*	*146*	*101*	*77*	*74*	*117*	*64*	*29*	*82*	*106*	*128*	*83*	*53*	*36*	*19*	*24*
	14	*28*	*58*	*64*	*61*	*92*	*42*																	
a	30	25	55	37	60	108	88	151	144	170	150	117	93	147	95	81	125	112	116	101	76	42	30	23
b	36	33	119	61	80	61	45	136	93	128	101	72	63	134	52	62	83	65	88	83	71	47	37	31
c	99	90	288	156	216	228	177	417	327	420	349	257	217	409	196	203	288	238	289	263	214	135	104	83

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

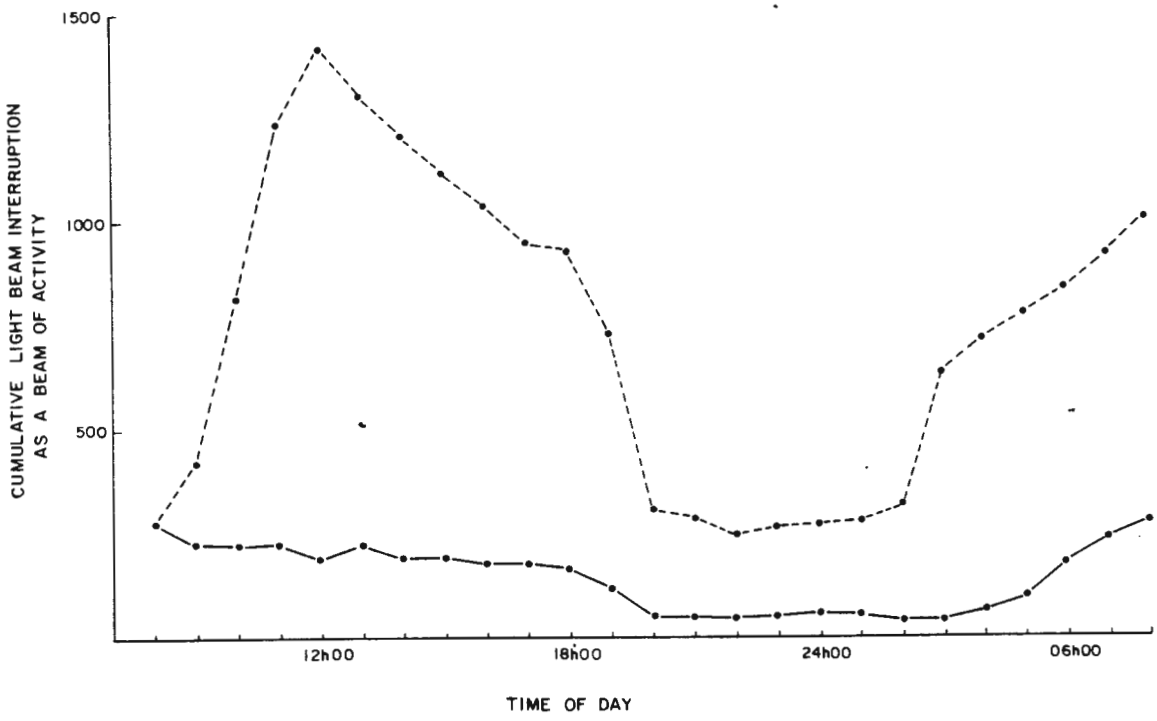

FIGURE 39. The effect of 5,0 mg Phenol/dm^3 (broken line) on the normal activity pattern of *Micropterus salmoides* (continuous line).

0,5 µg/dm^3) and to distinguish between phenol and *o*-chlorophenol. Yet, when given the choice of clean water or water containing phenol in a small horizontal tube, minnow (*Phoxinus phoxinus*) did not choose clean water in preference to either 400 or 4 mg/dm^3 of phenol (Jones, 1951), and rainbow trout (*Salmo gairdneiri*) did not avoid the lower concentrations (0,001 to 10 mg/dm^3) that were used by Sprague and Drury (1969). On the other hand, Ishio (1965), using a different kind of apparatus, presented a very brief summary of pooled data for several species, including carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*), showing that the median position occupied by the fish in his gradient channel coincided with concentrations of phenol (15 mg/dm^3) that were only slightly lower than the lowest lethal levels. Evidence of avoidance of phenolic effluents under field conditions is also conflicting. Kalabina (1935) reported that fish would leave parts of a river containing 0,2 to 10,0 mg/dm^3 of phenol, whereas Shelford (1917) said that they would tend to enter and remain in portions of a stream polluted with gas liquor. Definite conclusions cannot, therefore, be drawn about avoidance reactions of fish from this conflicting and sparse evidence. Nevertheless, it would seem that the sensor fish, exposed to a

TABLE 40. Hourly activity levels for a sensor fish before and after exposure to 5,0 mg C_6H_5OH/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									270	246	300	212	398	180	225	211	211	230	67	37	45	47	41	76
2	36	17	1	16	62	262	236	460	335	255	322	234	266	244	252	172	143	96	83	31	24	41	39	34
3	27	38	36	18	39	204	328	332	154	186	228	186	182	156	141	110	112	63	28	16	2	2	34	32
4	50	20	49	42	70	201	174	229	228	216	211	234	185	194	190	214	270	244	223	94	84	82	35	20
5	130	90	1	62	139	179	292	294	241	195	192	174	212	200	274	238	224	186	153	48	31	55	98	127
6	42	19	74	92	43	108	231	235	213	222	197	181	183	176	164	197	172	168	177	70	78	76	70	57
7	39	86	86	125	158	129	158	227	142	206	116	96	94	170	94	81	98	169	94	43	37	5	17	37
8	13	9	37	100	147	128	216	144	*413*	*814*	*1236*	*1418*	*1306*	*1214*	*1117*	*1032*	*946*	*931*	*728*	*303*	*286*	*242*	*263*	*271*
9	*278*	*314*	*632*	*714*	*766*	*832*	*916*	*1004*	*1126*	*1286*	*1431*	*1310*	*1330*	*1107*	*942*	*838*	*714*	*743*	*707*	*761*	*782*	*743*	*763*	*778*
10	*780*	*694*	*666*	*632*	*518*	*431*	*483*	*518*	*493*	*374*	*321*	*211*	*194*	*96*	*74*	*101*	*94*	*72*	*38*	*12*	*8*	*14*	*6*	*13*
	4	*2*																						
a	48	40	41	65	94	173	234	274	226	218	224	188	217	189	191	175	176	165	118	48	43	44	48	55
b	38	34	33	42	52	55	60	101	66	25	70	48	95	29	64	58	63	66	69	26	29	31	27	37
c	123	107	105	148	196	280	352	472	356	268	360	281	403	244	316	289	299	294	252	99	100	105	101	127

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 41. Hourly activity levels for a sensor fish before and after exposure to 1,0 mg C_6H_5OH/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								122	162	50	102	237	23	89	78	135	136	34	78	227	717	363	90	235
2	56	58	156	44	76	34	193	102	400	206	191	191	418	332	273	139	363	374	444	244	57	225	169	52
3	12	36	39	61	52	138	237	534	356	499	502	309	235	249	162	151	261	97	162	280	97	51	3	37
4	113	103	37	110	137	265	398	472	364	458	534	508	534	433	534	429	344	379	425	225	233	293	156	180
5	270	50	253	329	37	352	423	447	468	504	370	463	449	409	521	434	384	405	273	153	116	270	185	140
6	286	319	258	266	259	352	348	429	467	442	417	398	429	395	408	418	377	330	271	296	171	281	394	320
7	279	331	62	70	74	140	384	352	300	337	366	342	339	385	299	363	394	222	113	277	283	130	307	140
8	63	82	56	25	96	38	51	*416*	*503*	*573*	*664*	*771*	*816*	*864*	*909*	*971*	*1003*	*1412*	*918*	*897*	*971*	*958*	*966*	*943*
9	*977*	*918*	*894*	*903*	*991*	*976*	*1013*	*912*	*1142*	*963*	*889*	*900*	*831*	*871*	*998*	*934*	*914*	*957*	*906*	*916*	*977*	*1041*	*933*	*984*
10	*963*	*842*	*1032*	*971*	*872*	*931*	*923*	*888*	*913*	*874*	*846*	*965*	*883*	*962*	*911*	*861*	*933*	*912*	*879*	*880*	*910*	*791*	*843*	*882*
11	*894*	*831*	*919*	*963*	*904*	*996*	*963*	*846*	*946*	*913*	*904*	*931*	*901*	*917*	*846*	*853*	*963*	*984*	*991*	*979*	*931*	*841*	*793*	*786*
12	*881*	*946*	*873*	*814*	*918*	*842*	*901*	*973*	*832*	*903*	*912*	*843*	*794*	*773*	*694*	*712*	*847*	*902*	*921*	*846*	*853*	*803*	*817*	*801*
	794	*883*	*714*	*796*	*704*	*810*	*803*																	
a	154	140	123	130	104	188	291	351	360	357	355	350	347	327	325	296	323	263	252	243	239	230	186	158
b	120	128	99	119	75	136	136	172	106	172	157	115	171	122	173	146	94	148	145	48	225	106	130	99
c	389	392	317	363	252	455	557	689	568	693	663	576	681	566	664	582	506	554	536	338	680	439	442	352

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

sub-lethal concentration of phenol in the present study, was able to appreciate the presence of phenol for a long period of time, suggesting that the toxicant had no adverse effect upon the olfactory apparatus. The high levels of activity noted throughout the exposure period would indicate a continuing escape reaction which may be due, in part, to the cumulative pathological disturbance experienced by the sensor fish as well as olfactory perception.

Exposure of a sensor fish to an acute concentration of *ammonia* (5,0 mg/dm^3) produced a response within six h (Figure 40, Table 42). This toxic level did not prove lethal during the five day exposure period but activity rates did not assume either the pre-exposure rhythm or level. Sensor fish response to a sub-lethal level (0,5 mg/dm^3) was similar to that for phenol in that the sensor fish exhibited a heightened activity rate for the whole exposure period (Table 43).

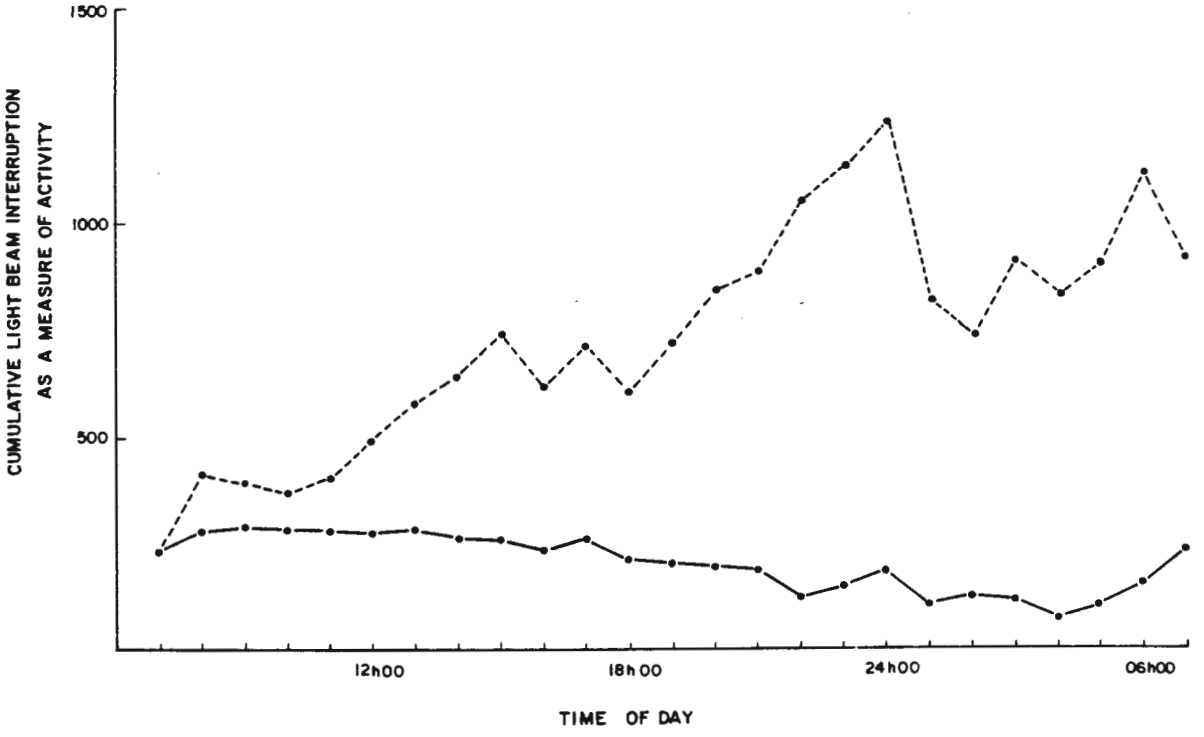

FIGURE 40. The effect of 5,0 mg Ammonia/dm^3 (broken line) on the normal activity pattern of *Micropterus salmoides* (continuous line).

Both Jones (1948), using stickleback (*Gasterosteus aculeatus*), and Summerfelt and Lewis (1967), using green sunfish (*Lepomis cyanellus*), found that these fish were repelled by lethal solutions of ammonia in a gradient tank. Green sunfish were not repelled by concentrations in which the fish showed obvious signs of distress and stickleback were

TABLE 42. Hourly activity levels for a sensor fish before and after exposure to 5,0 mg Ammonia/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								97	129	81	190	18	40	71	62	108	108	28	62	182	574	188	72	290
2	124	45	47	30	36	34	155	82	320	153	152	334	165	265	218	111	290	299	355	195	46	42	135	180
3	39	12	36	21	49	110	190	427	285	401	247	188	399	199	129	121	208	78	130	224	78	30	3	41
4	75	90	82	110	88	212	318	378	291	427	406	427	366	346	427	343	275	303	340	180	186	144	125	234
5	202	216	40	30	263	281	338	358	374	296	371	359	403	327	416	347	307	324	218	122	92	112	148	216
6	206	229	255	207	213	281	307	343	373	333	318	271	354	316	326	334	301	264	217	236	137	256	315	224
7	49	223	264	59	56	112	278	282	240	293	274	343	269	308	239	290	315	178	90	221	226	112	245	104
8	45	51	66	38	25	38	41	*416*	*394*	*371*	*402*	*496*	*581*	*642*	*738*	*618*	*714*	*602*	*718*	*843*	*886*	*1047*	*1130*	*1234*
9	*818*	*742*	*912*	*834*	*906*	*1114*	*917*	*903*	*884*	*798*	*712*	*696*	*643*	*631*	*574*	*638*	*784*	*690*	*683*	*574*	*496*	*532*	*584*	*681*
10	*761*	*842*	*861*	*716*	*734*	*796*	*720*	*717*	*723*	*756*	*803*	*777*	*735*	*771*	*834*	*863*	*791*	*774*	*778*	*598*	*633*	*684*	*690*	*672*
11	*684*	*717*	*803*	*733*	*774*	*814*	*786*	*736*	*677*	*684*	*774*	*783*	*773*	*787*	*821*	*828*	*818*	*791*	*784*	*735*	*616*	*554*	*603*	*670*
12	*778*	*802*	*741*	*782*	*614*	*712*	*743*	*752*	*746*	*751*	*732*	*751*	*764*	*702*	*735*	*741*	*741*	*883*	*840*	*818*	*772*	*746*	*731*	*770*
	731	*634*	*718*	*696*	*612*	*734*	*692*																	
a	106	124	113	71	104	153	232	281	287	283	280	277	285	262	260	236	258	211	202	194	191	126	149	184
b	73	95	102	67	95	106	108	138	85	126	92	137	137	97	139	117	75	118	116	39	180	79	104	85
c	249	311	312	203	290	360	445	551	454	530	461	545	554	452	531	465	405	443	428	270	544	282	353	351

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 43. Hourly activity levels for a sensor fish before and after exposure to 0,5 mg Ammonia/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								346	304	542	469	412	483	363	349	383	374	419	472	255	145	157	158	103
2	139	72	125	84	185	327	271	309	414	421	444	366	280	284	264	209	302	228	108	269	250	251	53	145
3	204	98	167	125	133	312	321	406	392	432	324	371	275	336	306	205	213	301	212	221	136	158	165	117
4	185	91	133	104	193	306	320	463	397	365	389	307	476	404	440	390	413	350	296	115	123	103	59	44
5	40	28	92	303	147	374	453	383	457	486	557	548	486	509	499	408	410	404	360	262	162	213	108	139
6	79	114	86	46	128	203	507	463	413	469	450	393	493	510	432	456	357	337	351	302	265	147	160	120
7	125	124	57	76	138	106	411	382	371	442	486	465	423	447	283	234	292	152	196	385	303	133	94	125
8	56	151	273	145	123	151	260	*313*	*404*	*516*	*523*	*551*	*585*	*564*	*534*	*418*	*477*	*479*	*514*	*535*	*574*	*598*	*612*	*704*
9	*712*	*697*	*617*	*633*	*674*	*712*	*746*	*778*	*801*	*831*	*828*	*774*	*783*	*716*	*699*	*704*	*732*	*755*	*733*	*718*	*736*	*802*	*694*	*686*
10	*666*	*774*	*812*	*849*	*900*	*812*	*779*	*766*	*779*	*848*	*793*	*718*	*742*	*802*	*774*	*717*	*709*	*738*	*694*	*705*	*775*	*683*	*657*	*594*
11	*637*	*786*	*794*	*801*	*817*	*823*	*753*	*704*	*759*	*783*	*717*	*725*	*695*	*712*	*663*	*700*	*676*	*707*	*694*	*801*	*765*	*731*	*704*	*633*
12	*694*	*634*	*687*	*585*	*479*	*381*	*368*	*564*	*635*	*664*	*660*	*589*	*591*	*603*	*651*	*683*	*691*	*581*	*632*	*681*	*655*	*584*	*551*	*530*
	518	*491*	*408*	*377*	*284*	*256*	*283*																	
a	118	97	133	126	150	254	363	393	393	451	446	409	417	408	368	326	337	313	285	258	198	166	114	113
b	63	40	71	85	28	101	95	57	47	56	74	78	98	86	90	106	73	96	122	82	73	50	48	34
c	242	174	273	292	205	452	549	505	485	560	590	562	608	577	544	535	479	500	525	419	340	264	208	179

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

attracted to sub-lethal levels. Hepher (1959) observed that carp (*Cyprinus carpio*) avoided local high concentrations of ammonia after it had been applied as a fertilizer in pond culture. There is no evidence to suggest that fish avoid sub-lethal levels of this poison. However it would seem that the similarity in sensor fish response to intoxication by both phenol and ammonia indicates a combination of physiological stress and olfactory perception to be the initiators of the alarm response.

A lethal level of *cyanide* (0,1 mg/dm^3) produced an instantaneous increase in sensor fish locomotory activity, the critical response level being exceeded within one h of exposure (Figure 41, Table 44). Subsequent to a sharp decrease, due possibly to fatigue, the activity rate remained at a high level until death ensued after 43 h exposure. A fairly quick response was also obtained from the sensor fish exposed to a sub-lethal cyanide concentration (0,05 mg/dm^3), the critical limit being exceeded within five h . Thereafter the sensor fish resumed its normal behaviour pattern after 48 h exposure, but within five days its locomotory rate had decreased to well below normal levels (Table 45).

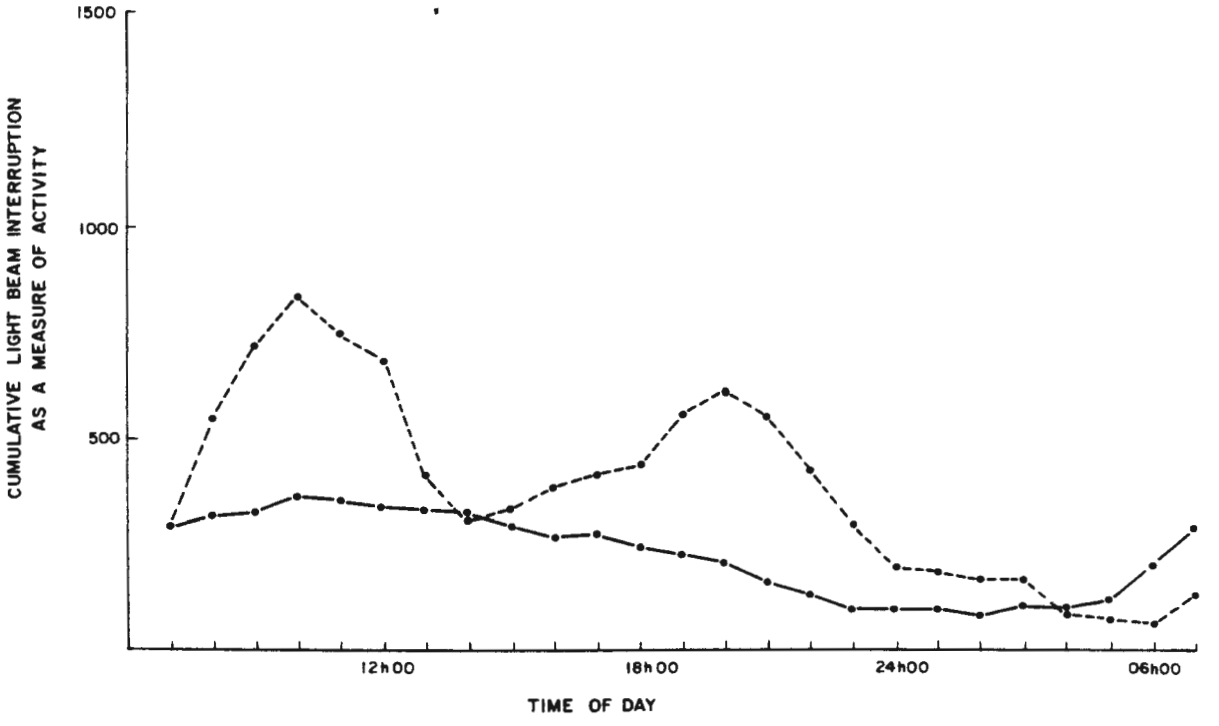

FIGURE 41. The effect of 0,1 mg CN^-/dm^3 (broken line) on the normal activity pattern of *Micropterus salmoides* (continuous line).

TABLE 44. Hourly activity levels for a sensor fish before and after exposure to 0,1 mg CN^-/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1								276	243	434	375	329	386	290	279	306	299	335	378	204	116	125	126	83
2	111	57	100	68	148	262	216	247	330	337	351	293	224	227	212	207	241	182	127	215	200	200	42	116
3	163	78	130	100	107	249	256	325	314	345	259	340	220	269	244	164	170	241	169	177	109	122	132	94
4	148	72	107	84	154	245	256	370	317	292	311	285	381	323	352	312	330	280	197	92	98	82	48	35
5	32	22	74	242	117	299	362	306	365	389	446	439	389	407	399	366	368	323	288	210	130	171	127	111
6	63	92	69	37	103	163	406	370	371	375	360	314	394	408	345	364	285	269	280	242	212	118	128	96
7	100	99	46	61	110	85	328	306	297	353	389	372	338	358	226	187	234	122	157	308	242	106	76	140
8	45	121	218	116	99	121	208	*542*	*717*	*833*	*749*	*683*	*413*	*318*	*336*	*384*	*414*	*432*	*561*	*612*	*553*	*423*	*304*	*196*
9	*184*	*171*	*163*	*84*	*71*	*64*	*132*	*214*	*111*	*96*	*74*	*53*	*24*	*38*	*21*	*16*	*18*	*18*	*14*	*12*	*8*	*6*	*10*	*3*
	2	*2*																						
a	95	77	106	101	120	203	290	314	320	361	356	339	333	326	294	272	276	250	228	207	158	132	97	96
b	50	31	57	67	22	80	76	46	43	45	59	53	78	69	72	85	66	76	90	65	58	40	40	33
c	193	139	217	233	163	361	439	404	404	448	472	443	486	462	435	439	405	400	404	335	272	211	176	161

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 45. Hourly activity levels for a sensor fish before and after exposure to 0,05 mg CN^-/dm^3

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1							192	324	547	314	268	184	53	119	88	512	303	246	166	313	111	161	96	108
2	50	124	406	216	248	275	127	251	183	335	505	196	177	133	197	252	231	161	289	98	53	18	17	31
3	25	23	24	25	64	193	65	114	193	173	228	130	88	97	66	64	182	97	81	56	48	97	114	96
4	132	15	8	25	58	99	160	182	152	273	159	258	255	170	211	210	176	238	252	212	130	67	16	11
5	55	15	6	18	47	152	132	298	158	167	246	204	142	212	107	166	148	136	196	106	4	19	6	13
6	6	10	2	3	60	81	47	82	67	39	66	71	98	73	50	127	61	105	16	25	109	29	8	35
7	13	18	6	16	32	53	52	20	22	34	36	39	99	41	31	44	33	98	53	81	261	28	27	35
8	1	3	13	4	15	67	*201*	*242*	*463*	*494*	*601*	*742*	*818*	*779*	*631*	*647*	*589*	*603*	*677*	*647*	*618*	*597*	*609*	*511*
9	*583*	*566*	*517*	*495*	*502*	*434*	*391*	*231*	*304*	*316*	*192*	*98*	*74*	*61*	*55*	*177*	*152*	*97*	*103*	*91*	*46*	*23*	*38*	*121*
10	*143*	*112*	*103*	*97*	*111*	*134*	*133*	*204*	*216*	*183*	*154*	*136*	*128*	*130*	*94*	*231*	*216*	*158*	*121*	*54*	*38*	*28*	*14*	*49*
11	*56*	*41*	*37*	*25*	*67*	*54*	*114*	*177*	*194*	*301*	*171*	*162*	*94*	*88*	*31*	*184*	*171*	*136*	*94*	*72*	*76*	*71*	*51*	*39*
12	*61*	*32*	*25*	*38*	*74*	*83*	*128*	*186*	*177*	*284*	*136*	*112*	*114*	*97*	*72*	*201*	*74*	*54*	*71*	*34*	*15*	*3*	*8*	*28*
	24	*15*	*4*	*43*	*71*	*91*																		
a	40	30	66	44	75	131	111	182	189	191	215	155	130	121	107	196	162	154	150	127	102	60	41	47
b	46	42	150	76	78	80	57	115	170	123	156	78	68	58	71	158	93	64	104	101	83	53	45	39
c	130	112	360	194	228	289	222	407	522	432	521	308	263	234	246	506	345	280	353	324	264	164	129	123

a, b and c are, respectively, the mean, standard deviation and upper 95% confidence limit.

Figures in italics represent post-exposure activity levels.

Whether fish can detect the presence of cyanide in solution does not appear to have been tested. However, an effective way to evaluate the effects of environmental pollutants upon fish activity has been to measure their swimming ability, a response which has important ecological implications considering migration, maintaining position in a current or movements required in predator - prey interactions. Various studies illustrate the profound effect cyanide has on the swimming ability of fish. Cichlids (Leduc, 1966), rainbow trout, *Salmo gairdneri*, (Speyer, 1975), coho salmon, *Oncorhynchus kisutch*, (Broderius, 1970) and brook trout, *Salvelinus fontinalis*, (Neil, 1957) elicited 60, 75, 85 and 95 per cent reductions in swimming ability, respectively, when exposed to concentrations of cyanide ranging from 0,01 to 0,1 mg/dm^3. Resumption of normal swimming ability, after return to clean water, was a very slow process, taking 15 to 20 days (Neil, 1957; Broderius, 1970).

These results are indicative of very serious metabolic impairment by cyanide, and of inhibition of the oxidative pathways responsible for the maintenance of swimming. However, since the inhibitory action of cyanide on cytochrome oxidase is reversible (Stannard and Horecker, 1948) and since cyanide is a non-cumulative cytoplasmic poison (Hewitt and Nicholas, 1963), one would expect a rapid recovery after removal from a toxic environment unless there had been structural damage caused to the fish by the toxicant.

A disadvantage of assessing an effluent utilizing biological monitoring techniques; based upon either respiratory or locomotory responses to intoxication; compared with physical-chemical techniques, is that the precise identity of the toxicant cannot be determined. However, the fact that sensor fish exhibit multifarious locomotory responses to different toxicants (Table 46) may enable a decision to be made regarding the type of toxic effect being applied to the sensor animal. Thus one may be able to differentiate between heavy metals, which inhibit olfactory function; phenolics, ammonia and possibly other organic toxicants, which exert their influence through pathologic stress; and metabolic depressants, such as mercury and cyanide.

TABLE 46. The effect of sub-lethal intoxication upon the daily average activity levels, measured as cumulative light beam interruption, of *Micropterus salmoides*

Toxicant concentration (mg/dm^3)	Daily average activity levels					
		Days post-exposure				
	X	1	2	3	4	5
0,01 Hg^{2+}	91	302	153	98	92	76
0,1 Cu^{2+}	179	546	168	175	189	173
0,1 Cd^{2+}	196	590	213	208	187	192
1,0 C_6H_5OH	256	885	941	900	896	822
0,5 Ammonia (as N)	277	571	765	746	667	548
0,05 CN^-	118	565	136	107	106	85

The X column represents the daily average activity levels for the seven days standardization period before toxicant application.

The response of sensor fish to increasing concentrations of a number of toxicants was used to establish the threshold limits of detection. In each case three individuals were exposed to successively increased concentrations and a further three individuals were exposed to that concentration designated as the threshold, in order to confirm that limit (Table 47).

Responses were obtained from the more sensitive individuals in concentrations as low as 25 µg Cu^{2+}/dm^3; 100 µg Cd^{2+}/dm^3; 50 µg Hg^{2+}/dm^3; 250 µg C_6H_5OH/dm^3; 500 µg Ammonia (as N)/dm^3; and 10 µg CN^-/dm^3. The levels of intoxication producing alarm conditions, as defined for industrial situations, were, generally, higher by a factor of two. Sensor fish exposed directly to the established threshold limits confirmed in all cases that the procedure of exposing the experimental animals to successively higher concentrations did not influence the final result.

CONCLUSIONS

The results of this investigation indicated that lethal and sub-lethal concentrations of a number of toxicants do alter the movement patterns of fish, supporting the validity of the use of this parameter as a basis

TABLE 47. Elapsed times to behavioural response by *Micropterus salmoides* exposed to various degrees of intoxication

Toxicant	Concentration (mg/dm^3)	Times to response (h) for sensor fish 1	2	3
Copper	0,025	-	11	-
	0,05	13	7	14
	0,05	*9*	*14*	*16*
Cadmium	0,1	18	-	-
	0,25	4	13	15
	0,25	*17*	*9*	*17*
Mercury	0,05	22	16	-
	0,1	8	4	13
	0,05	*19*	-	-
	0,1	*10*	*13*	*12*
Phenol	0,25	19	-	-
	0,5	7	9	4
	0,5	*10*	*6*	*7*
Ammonia	0,5	-	23	-
	1,0	12	7	17
	1,0	*6*	*5*	*20*
Cyanide	0,01	22	-	-
	0,05	11	17	19
	0,05	*13*	*19*	*18*

Only those toxicant concentrations in which any sensor fish response was observed, within 24 h exposure, are represented.

Figures in italics represent responses by separate groups of sensor fish, in each case, utilized for confirmation of threshold levels.

for a biological monitoring technique. It is problematical, however, whether the apparatus described would function adequately in an industrial environment. Compared with the system designed to continuously monitor sensor fish opercular rhythms, the behavioural unit is not as cost effective. The size of the sensor chambers themselves would entail a larger capital outlay for the construction of laboratory facilities for their accommodation. Selection of sensor fish was restricted to those with a length of less than 160 mm (the distance between each vertical group of infra-red emitters) so that no two beams could be triggered at the same time, thus limiting ones choice of test animal. Further, the infra-red emitters and sensors had to be realigned after any disturbance to, or movement of, the chambers.

Similar monitoring equipment used by Waller and Cairns (1972) was limited by turbidity. Turbidimetric determinations made, by the authors, under normal operating conditions showed that erroneous counts were registered when the turbidity of the water reached 15 (NTU). The levels of turbidity over which such a system could operate successfully could be increased significantly by providing better light beam - photocell alignment. But, 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.

Aberrations in fish movement patterns do, nevertheless, give a reliable index of premortal toxicity. Table 48 compares the performance of activity and respiratory (opercular rhythm) responses in monitoring similar nominal concentrations of a number of toxicants. Where the response depends partially or completely upon olfactory sensitivity rather than direct physiological impairment, such as with phenol and ammonia toxicity, the activity response proved more successful. It was also determined that lower concentrations of these toxicants were able to be detected using the system based upon movement pattern response to intoxication (Table 49). It is evident, therefore, that a chamber designed to monitor locomotory behaviour patterns, without the disadvantages described above, could be compatible with that used to monitor opercular rhythms. The combination of the two monitoring systems would allow for simultaneous monitoring of two parameters, increasing the effectiveness of both systems.

TABLE 48. Relationship between times for opercular rhythm and activity responses for *Micropterus salmoides* subjected to differing toxicant regimes

Nominal toxicant concentration (mg/dm^3)		Time for response (h)	
		Opercular rhythm	Activity
1,0	Cu^{2+}	2	1
0,1	Cu^{2+}	4	6
0,5	Cd^{2+}	10	11
0,1	Cd^{2+}	13	16
5,0	Phenol	6	2
1,0	Phenol	18	5
5,0	Ammonia	19	6
0,5	Ammonia	-	16
0,1	Cyanide	1	1
0,05	Cyanide	5	5

TABLE 49. Threshold opercular rhythm and activity responses of *Micropterus salmoides* to intoxication

Toxicant	Lowest concentration (mg/dm^3) causing response in 60% of sensor fish within 24-h exposure	
	Opercular rhythm	Activity
Copper	0,05	0,05
Cadmium	0,1 - 0,25	0,25
Mercury	0,01	0,05 - 0,1
Phenol	1,0	0,5
Ammonia	5,0	1,0
Cyanide	0,005 - 0,01	0,05

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SECTION IV

THE RELATIONSHIP BETWEEN CONTINUOUS BIOLOGICAL MONITORING AND WATER QUALITY CRITERIA FOR CHRONIC EXPOSURE

INTRODUCTION

A great deal of progress has been made in determining biologically safe concentrations of toxicants for fish. Along with the standards for the chronic exposure of fish to toxicants, there must be safeguards against the development of acutely toxic conditions resulting from either industrial or municipal "accidental spills" or from changes in the total environment of the organism due to a combination of normal environmental variation and industrial processes.

Continuous physical and chemical monitoring systems can be used to detect, almost instantaneously, changes in individual environmental factors. However, aquatic organisms respond to the collective impact of these environmental factors, and this cannot be predicted from physical and chemical data alone.

The techniques described in this study permit the continuous monitoring of either fish movement patterns or breathing. Both techniques detected effects when test species were exposed to lethal and sub-lethal concentrations of a number of toxicants. Further, it has been shown that the use of the techniques, to monitor industrial and municipal discharges, would provide protection for fish, in receiving systems, from acute effects. Whether this level of protection suffices to safeguard species against the long-term chronic effects of intoxication has, however, not been established. In order to investigate this facet of continuous biomonitoring, the relationship between the criteria for detecting response to intoxication and that toxic concentration which chronically affects fish life needed to be examined.

The detection limits of a biological monitoring system could be related to criteria for chronic exposure by conducting additional growth and reproduction experiments. Two conditions, however, had to be satisfied in order to correlate long-term effects of toxicants with their proportionate relationship to automatic biomonitoring sensitivity levels. Firstly, it was necessary that the sensor fish selected be easily maintained and bred under laboratory conditions; and, secondly, that a monitoring unit be designed to accommodate that sensor.

The common guppy (*Poecilia reticulata*) was selected as the experimental animal, for several reasons. The species is hardy and can easily be reared and maintained in the laboratory in large numbers. The female is viviparous and produces broods about once a month. A constant supply of fish was thus obtainable from stock laboratory aquaria, and the effects of toxicants on the development of the young, without a direct contact to the eggs, could be examined. The biomonitoring system, used to compare immediate responses of sensor fish to intoxication with long-term effects, was based on locomotor behaviour patterns rather than respiration, as the previous unit employed to monitor that parameter had proved unsuitable for exploitation in industrial situations. The monitoring system sensed activity by means of ultrasonic echoes rather than light beams, and was found to be an improvement in all respects.

EFFECTS OF ACUTE AND CHRONIC EXPOSURE TO A NUMBER OF TOXICANTS ON SURVIVAL, GROWTH AND REPRODUCTION IN THE GUPPY (*POECILIA RETICULATA*)

The investigation was undertaken essentially to examine the effects of chronic exposure to sub-lethal levels of a number of toxicants on reproduction and growth in the guppy, *Poecilia reticulata*, in order to determine those toxic concentrations which may be regarded as safe, or maximum acceptable levels, in the environment. To evaluate these effects the 96-h LC50, i.e. the concentration of a toxicant lethal to one-half of a test population in four days, was initially determined for all toxicants. The sub-lethal levels, to which experimental fish were exposed for long periods, were established as fractions of the respective median lethal levels.

THE ACUTE TOXICITY OF CADMIUM, COPPER, MERCURY, PHENOL, AMMONIA AND CYANIDE TO *POECILIA RETICULATA*

TEST PROCEDURE

A two-test sequence was employed to estimate the acute effects of the toxicants: a preliminary, short-term (24 h), range finding test to define the range of toxicant dilutions to be used in the definitive test, and a more rigorous long-term definitive test conducted over a 96-h period (using the range of toxicant dilutions determined by the range finding test) to arrive at the acute toxicity expressed as a LC50.

For the range finding test, three fish were placed in 2 dm^3 capacity containers and exposed, under static conditions, to five widely spaced dilutions of the toxicant for a period of 24 h .

For the definitive test, 20 fish were exposed to each toxicant concentration flowing into and out of 40 dm^3 chambers on a once through basis for the duration of the test. Toxicant dilutions were precisely controlled using the proportional constant flow diluter described previously. Test solutions were checked for toxicant concentration, pH (adjusted to approximately 7,0), oxygen concentration, and temperature (controlled at 22 ±1°C) once every six h for the duration of each test. All these parameters remained within their defined limits throughout the test periods (Table 50). Dechlorinated municipal tap water was used as the

diluent, and although no chemical analyses were conducted during the tests, it may be assumed that its constitution was similar to that of the diluent water used, from the same source, for the long-term studies.

The test organisms were two-week old guppies which had been reared from 20 breeding sets of adults, one breeding set consisted of two male and five female fish. An adequate supply of test fish were constantly available. The adult fish were obtained from a local supplier and maintained in the laboratory for two months before any of their broods were used. Fish were fed once to twice daily *ad libitum* with a mixture of dried commercial food, high in protein content. Experimental fish, however, were not fed for 48 h before, nor during, a test. The animals were transferred from stock holding tanks to the test concentrations by dip netting. Not more than 20% of organisms transferred to one toxicant concentration being taken from any given net capture.

The definitive determination of each LC50 employed a control (diluent water only) and at least five toxicant concentrations in a logarithmic series. Tests were considered valid when at least one concentration killed more than 65% of the fish exposed to it, and one concentration, other than the control, killed less than 35% of the fish. A test was not acceptable when more than 10% of the fish died in the control. The number of dead fish in each container were counted 24, 48, 72 and 96-h after the beginning of the test, and removed at least once every 24 h . The criterion for death was cessation of movement, especially gill movement, and no reaction to gentle prodding.

CALCULATION OF THE LC50

For each set of data the 96-h LC50 and its 95% confidence limits were calculated on the basis of the nominal toxicant concentrations in the test solutions. The method used was based upon that of Litchfield and Wilcoxon (1949) and employed the following steps.

1. The data was tabulated to show the toxicant concentrations used, the total number of fish exposed to those concentrations, the number of affected fish and the observed percent-affected fish. Not more than two consecutive 100% affects at high toxicant concentrations or more than two consecutive 0% affects at low concentrations were listed.

TABLE 50. Experimental toxicant concentrations for median lethal tests

Toxicant	Nominal concentration (mg/dm^3)	Measured concentration (mg/dm^3) Mean	Range	Dissolved oxygen** (mg/dm^3)	Temperature** °C	pH**
Cadmium	0,16	0,13	0,10 - 0,17			
	0,25	0,26	0,22 - 0,29			
	0,39	0,37	0,35 - 0,39			
	0,63	0,60	0,58 - 0,63			
	1,0	1,12	1,03 - 1,48	6,3	22	7,2
	1,6	1,65	1,58 - 1,70			
	2,5	2,53	2,49 - 2,57			
	3,9	3,71	3,53 - 3,92			
	6,3	6,23	5,98 - 6,51			
	10,0	9,83	9,64 -10,11			
Copper	0,16	0,17	0,14 - 0,2			
	0,25	0,23	0,2 - 0,27			
	0,39	0,36	0,33 - 0,39	6,4	22	7,3
	0,63	0,61	0,58 - 0,65			
	1,0	1,12	0,94 - 1,23			
	1,6	1,78	1,54 - 1,96			
Mercury	0,01	0,008	0,006- 0,12			
	0,025	0,03	0,025- 0,035			
	0,04	0,043	0,038- 0,049			
	0,06	0,061	0,058- 0,063			
	0,1	0,09	0,08 - 0,12			
	0,16	0,14	0,12 - 0,16	6,3	21	7,2
	0,25	0,27	0,25 - 0,30			
	0,39	0,41	0,36 - 0,44			
	0,63	0,66	0,63 - 0,69			
	1,0	1,13	0,94 - 1,23			
	6,3	6,9	6,0 - 7,2			
Phenol	6,3	6,7	6,4 - 7,1			
	10,0	10,8	10,4 -11,1			
	16,0	15,8	15,6 -16,0	6,8	22	7,4
	25,0	23,8	23,4 -24,8			
	39,0	41,2	38,1 -44,3			
	63,0	67,4	60,2 -71,4			
Ammonia	2,5	2,1	1,9 - 2,3			
	3,9	4,1	3,7 - 4,6			
	6,3	6,0	5,7 - 6,3			
	10,0	11,1	10,4 -11,9			
	16,0	15,8	15,0 -16,3	6,1	22	7,8
	25,0	27,3	25,1 -28,1			
	39,0	39,2	38,2 -41,2			
	63,0	67,1	65,3 -71,7			
Cyanide	0,016	*				
	0,025	*				
	0,039	*				
	0,063	0,051	0,05 - 0,053			
	0,1	0,09	0,08 - 0,11	6,2	22	7,1
	0,16	0,16	0,14 - 0,18			
	0,25	0,29	0,27 - 0,33			
	0,39	0,44	0,40 - 0,48			
	0,63	0,65	0,63 - 0,67			
	1,0	0,98	0,92 - 1,12			

* Below limit of detection

** Average values for the test

2. The percent affected fish were plotted against the toxicant concentration on two-cycle logarithmic probability paper (except for the 0% and 100% affect values) and a temporary straight line fitted through the points, particularly those in the region of 40% and 60% affects.

3. The straight line was used to read and list "expected" percent affects for each toxicant concentration, disregarding the "expected" percent value for any of the concentrations less than 0,01 or more than 99,99. The expected-percent-affect was used to calculate a "corrected" value for each 0% and 100% affect obtained in the test (Table 51). These values were plotted on the logarithmic probability paper and the fit of the line to the completely plotted data inspected. Whenever the fit was unsatisfactory the line was redrawn to obtain a new set of expected values.

4. The difference between each observed (or corrected) value and its corresponding expected value was listed. Each difference and the corresponding expected value were used to read and list the contributions to Chi-square (Chi^2) from Figure 42. The contributions to Chi^2 were summed and the total multiplied by the average number of fish per toxicant concentration, *viz.* the number of fish used in K concentrations divided by K, where K is the number of percent-affected fish values plotted. The product was the "calculated" Chi^2 of the line. The degrees of freedom (N) were two less than the number of points plotted, i.e. $N = K - 2$. When the calculated Chi^2 was less than the Chi^2 given in Table 52 for N degrees of freedom, the data were non-heterogeneous and the line was a good fit. However, whenever the calculated Chi^2 was greater than the Chi^2 given in Table 52 for N degrees of freedom, the data were heterogeneous and the line was not a good fit, and the toxicity test was repeated.

5. The confidence limits of the LC50 were determined by reading, from the fitted line, the toxicant concentrations for the corresponding 16, 50 and 84% affects (LC16, LC50, LC84). The slope function was calculated as:

$$S = \frac{LC84/LC50 + LC50/LC16}{2}$$

TABLE 51. Corrected values of 0 % or 100 % effect

Expected value	Corrected value									
	0	1	2	3	4	5	6	7	8	9
0	-	0,3	0,7	1,0	1,3	1,6	2,0	2,3	2,6	2,9
10	3,2	3,5	3,8	4,1	4,4	4,7	4,9	5,2	5,5	5,7
20	6,0	6,2	6,5	6,7	7,0	7,2	7,4	7,6	7,8	8,1
30	8,3	8,4	8,6	8,8	9,0	9,2	9,3	9,4	9,6	9,8
40	9,9	10,0	10,1	10,2	10,3	10,4	10,4	10,4	10,4	10,5
50	-	89,5	89,6	89,6	89,6	89,7	89,7	89,8	89,9	90,0
60	90,1	90,2	90,4	90,5	90,7	90,8	91,0	91,2	91,4	91,6
70	91,7	91,9	92,2	92,4	92,6	92,8	93,0	93,3	93,5	93,8
80	94,0	94,3	94,5	94,8	95,1	95,3	95,6	95,9	96,2	96,5
90	96,8	97,1	97,4	97,7	98,0	98,4	98,7	99,0	99,3	99,7

TABLE 52. Values of Chi^2 (p = 0,05)

Degrees of freedom (N)	Chi^2
1	3,84
2	5,99
3	7,82
4	9,49
5	11,1
6	12,6
7	14,1
8	15,5
9	16,9
10	18,8

From the tabulation of data N^1 was determined, being defined as the total number of test fish used within the percent-affected-fish interval of 16% and 84%. The exponent, $2,77/N^1$, for the slope function was calculated together with the factor f_{LC50}, used to establish the confidence limits for the LC50

$$f_{LC50} = S^{(2,77/\sqrt{N^1})}$$

The confidence limits were calculated as follows:

(a) Upper limit for 95% probability = LC50 x f_{LC50}

(b) Lower limit for 95% probability = LC50/f_{LC50}

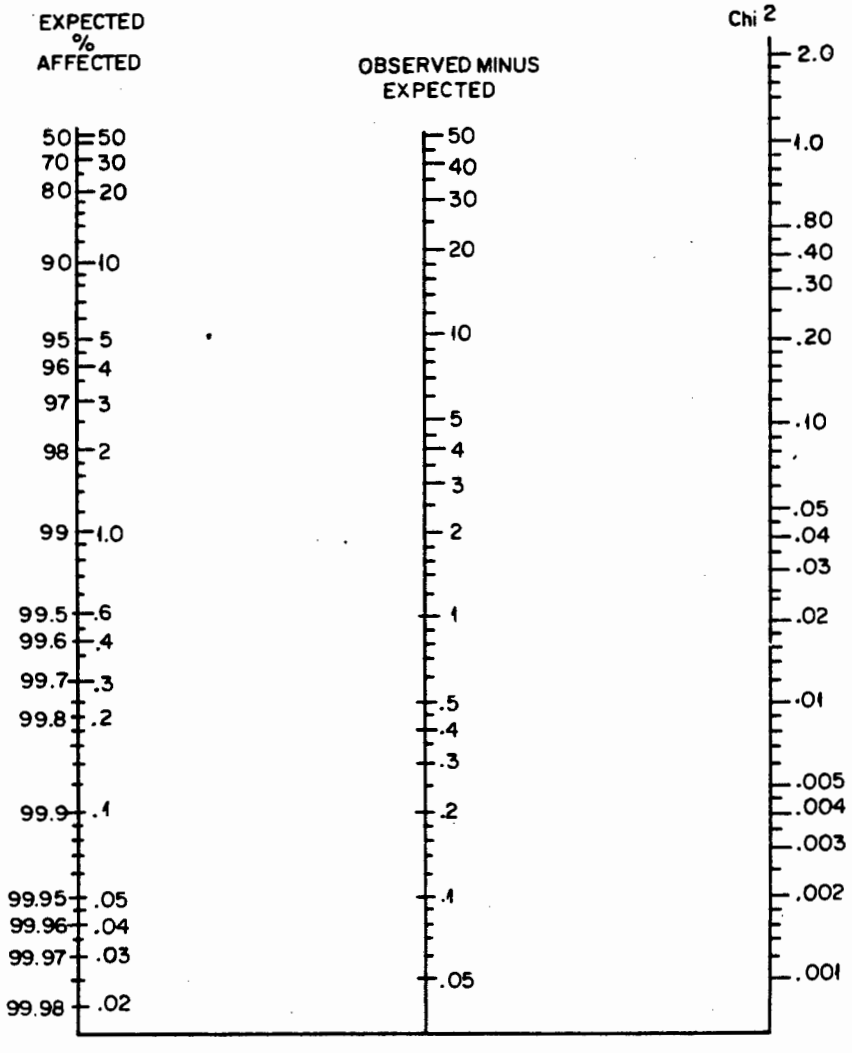

FIGURE 42. Nomograph for obtaining Chi^2 from expected % affected and observed-minus-expected (Step 4).

DISCUSSION OF RESULTS

Range finding tests indicated the LC50s for cadmium and copper to be between 0,1 and 10,0 mg/dm^3, for mercury and cyanide between 0,01 and 1,0 mg/dm^3 and for phenol and ammonia between 1,0 and 100,0 mg/dm^3. Accordingly, test fish were exposed to logarithmic series of toxic concentrations between and including those designated to elicit a 0% and 100% effect. The data were plotted (Figures 43 to 48), tabulated and the expected figures read from the graphs (Tables 53 to 58). In one case, cyanide, a second test had to be performed as the first produced heterogeneous results for the Chi^2 calculation in determining goodness of fit of the plotted line. The effect of 6,3 mg/dm^3 of mercury was tested separately from other concentrations of the toxicant in order to satisfy the requirement for 100% mortality.

The relationships between calculated and tabular Chi^2 were determined to establish the goodness of fit of the plotted lines (Table 59) and the LC50s for cadmium, copper, mercury, phenol, ammonia and cyanide were calculated to be 1,85; 0,55; 0,2; 15,0; 15,2 and 0,13 mg/dm^3 respectively. The 95% probability limits were determined according to the procedure outlined above (Table 60).

Information regarding the effects of short-term exposures of fish to toxicants is important in that such exposures can arise from a spill of a chemical into a body of water. Such information is, generally, supplied by the acute mortality test. Most acute mortality tests determine the level of a toxic agent which kills 50% of the subjects in two to seven. This test has achieved wide-spread acceptance among fish toxicologists because it is easier, quicker, less costly and more dramatic than any other kind of test. In addition, the acute mortality test has shown that great differences can exist between different species of fish and that water quality and temperature can have an important effect on the results of toxicity tests.

However, at least as important as the effects of short-term exposures are the effects of long-term exposures of fish to toxicants. As the human population increases, the quantity and sources of pollutants are growing. As water pollution control becomes a reality, discharges of

TABLE 53. Data analysis for the determination of the median lethal effect of cadmium on *Poecilia reticulata*

Cadmium concentration (mg/dm^3)	Number of fish	Number of affected fish	Observed % affected fish	Expected %	Observed minus expected	Chi^2
0,16	20	0	0	discarded		
0,25	20	0	0(1,0)[a]	3,0	2,0	0,014
0,39	20	1	5	7,0	2,0	0,006
0,63	20	3	15	15,0	-	-
1,0	20	6	30	28,0	2,0	0,002
1,6	20	8	40	44,0	4,0	0,007
2,5	20	12	60	60,5	0,5	0,01
3,9	20	14	70	75,0	5,0	0,014
6,3	20	18	90	87,0	3,0	0,008
10,0	20	20	100(98,0)[a]	94,0	4,0	0,02
				TOTAL		0,081

a = "corrected" affected values from Table 51.

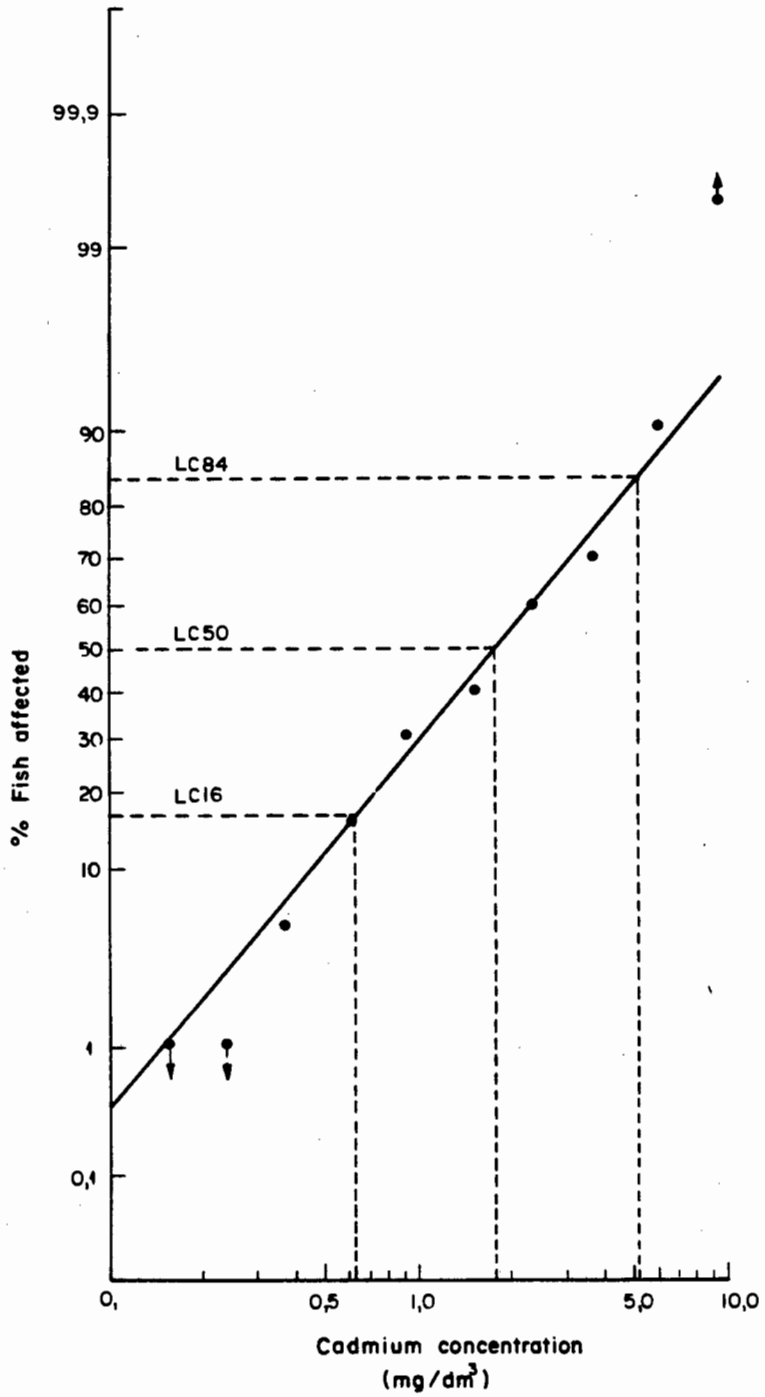

FIGURE 43. Plotted data and fitted line for log-toxicant concentration versus percent survival of *Poecilia reticulata* exposed to cadmium.

TABLE 54. Data analysis for the determination of the median lethal effect of copper on *Poecilia reticulata*

Copper concentration (mg/dm^3)	Number of fish	Number of affected fish	Observed % affected fish	Expected %	Observed minus expected	Chi^2
0,16	20	0	0(0,1)[a]	0,4	0,3	0,002
0,25	20	1	5	4,5	0,5	0,005
0,39	20	6	30	22,0	8,0	0,04
0,63	20	11	55	63,0	8,0	0,025
1,0	20	18	90	90,0	-	-
1,6	20	20	100(99,7)[a]	98,9	0,8	0,007
				TOTAL		0,079

a = "corrected" affected values from Table 51.

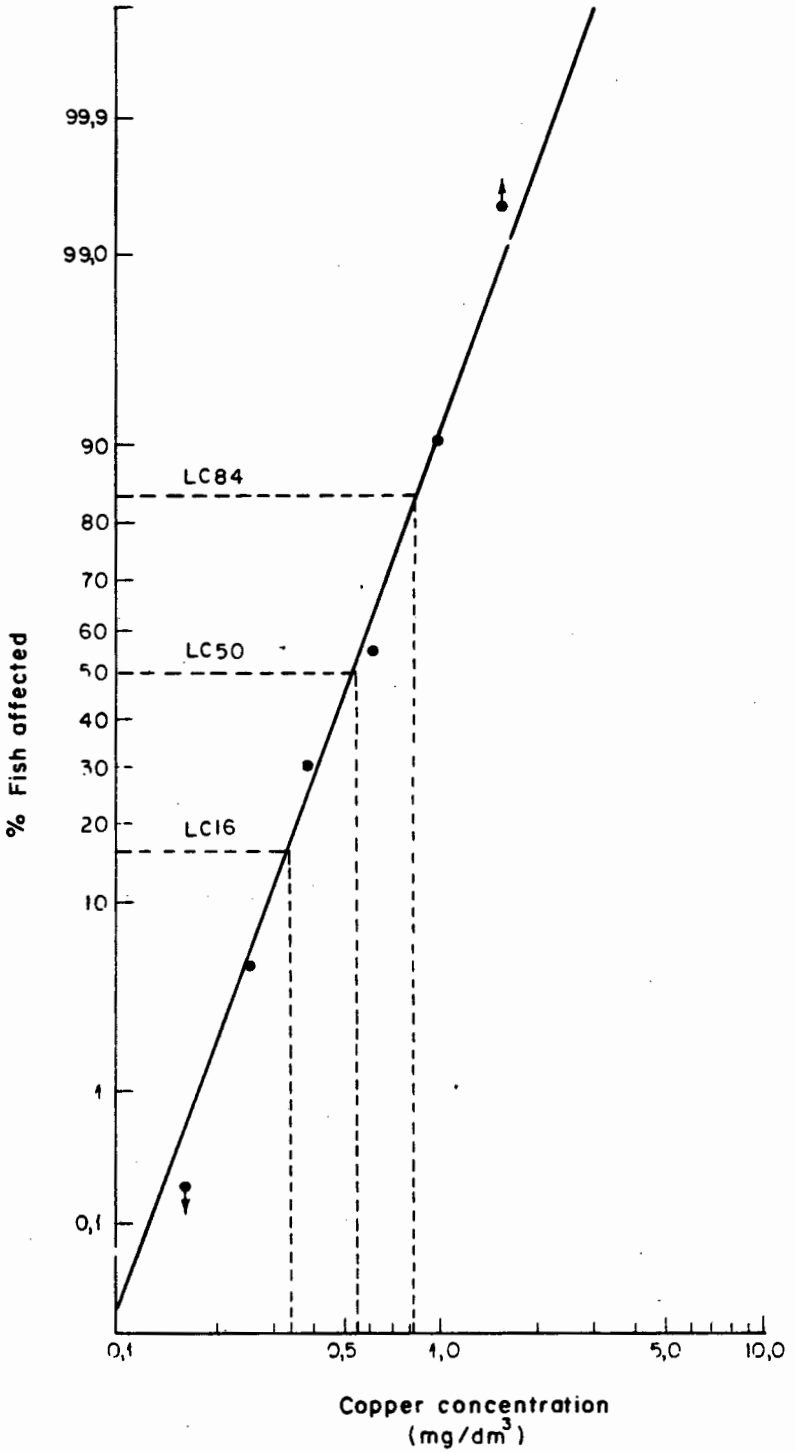

FIGURE 44. Plotted data and fitted line for log-toxicant concentration versus percent survival of *Poecilia reticulata* exposed to copper.

TABLE 55. Data analysis for the determination of the median lethal effect of mercury on *Poecilia reticulata*

Mercury concentration (mg/dm^3)	Number of fish	Number of affected fish	Observed % affected fish	Expected %	Observed minus expected	Chi^2
0,01	20	0	0(1,3)[a]	4,0	2,7	0,02
0,025	20	2	10	10,5	0,5	0,001
0,04	20	1	5	aberrant value		
0,06	20	6	30	23,0	7,0	0,03
0,1	20	8	40	33,5	10,5	0,05
0,16	20	8	40	44,0	4,0	0,006
0,25	20	10	50	55,0	5,0	0,01
0,39	20	14	70	65,5	4,5	0,009
0,63	20	14	70	75,5	5,5	0,019
1,0	20	18	90	87,0	3,0	0,008
6,3	20	20	100(99,3)[a]	98,0	1,3	0,009
				TOTAL		0,162

a = "corrected" affected values from Table 51.

Percent affected organisms at the 0.,04 concentration was obviously an abberant value and was omitted when fitting the line.

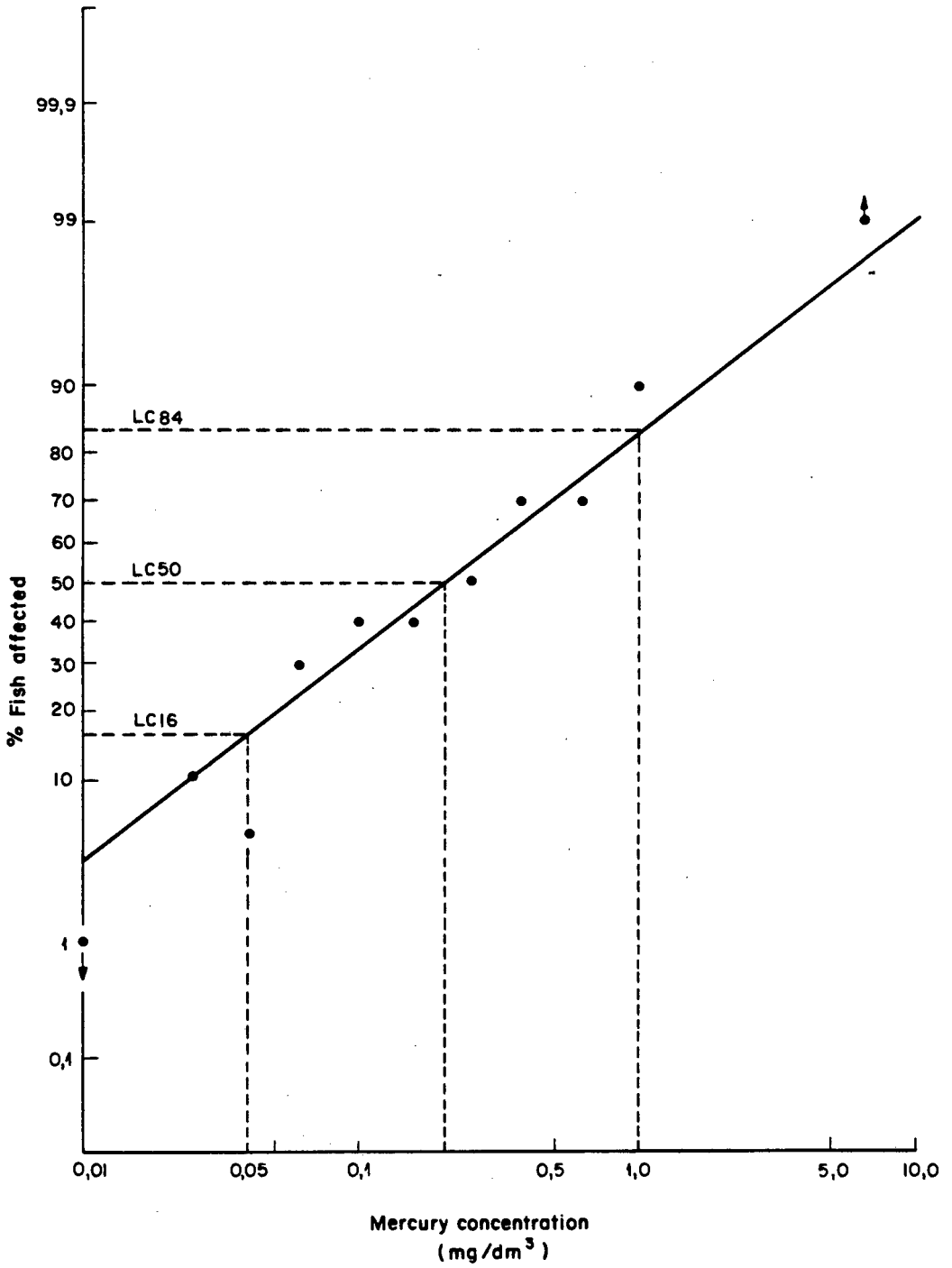

FIGURE 45. Plotted data and fitted line for log-toxicant concentration versus percent survival of *Poecilia reticulata* exposed to mercury.

TABLE 56. Data analysis for the determination of the median lethal effect of phenol on *Poecilia reticulata*

Phenol concentration (mg/dm^3)	Number of fish	Number of affected fish	Observed % affected fish	Expected %	Observed minus expected	Chi^2
6,3	20	0	0(1,2)[a]	3,5	2,3	0,015
10,0	20	4	20	18,5	1,5	0,002
16,0	20	12	60	55,0	5,0	0,012
25,0	20	16	80	86,5	6,5	0,04
39,0	20	20	100(99,3)[a]	98,0	1,3	0,009
63,0	20	20		discarded		
					TOTAL	0,078

a = "corrected" affected values from Table 51.

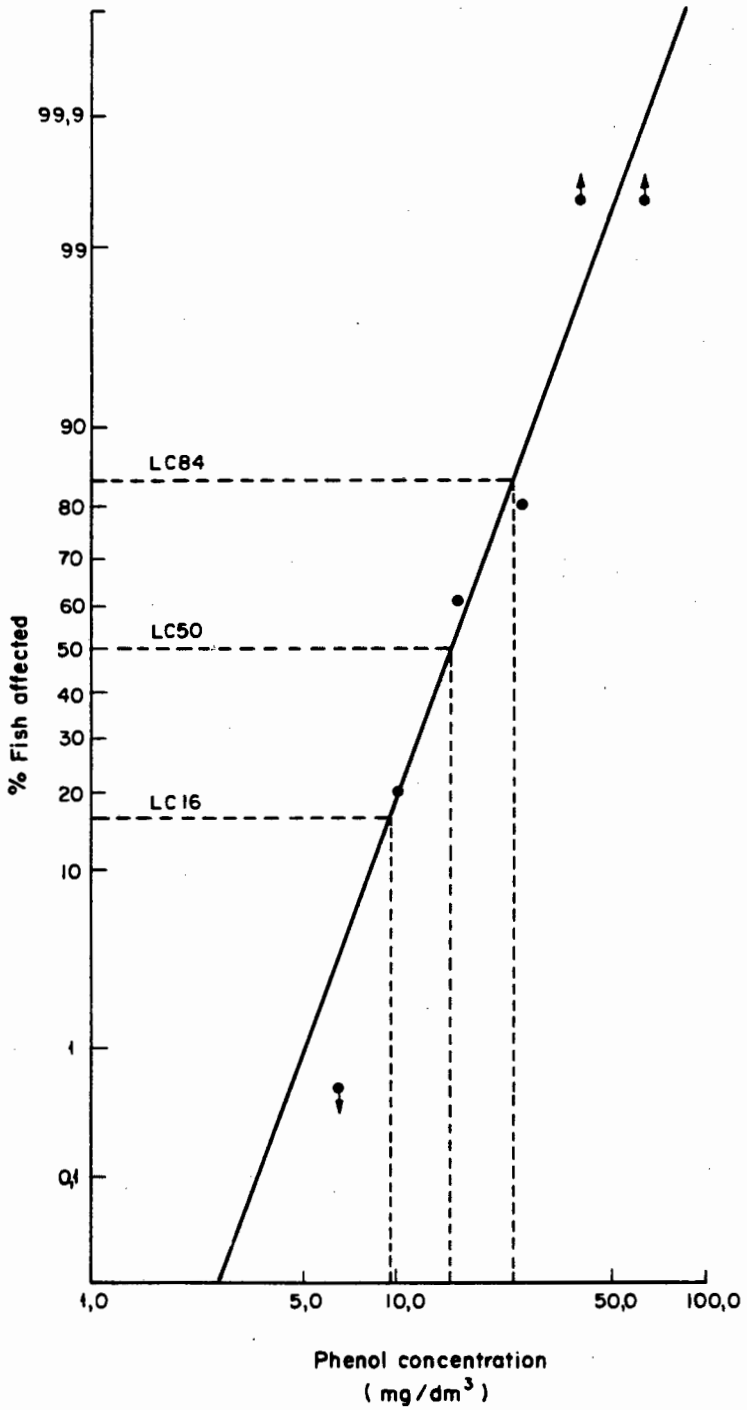

FIGURE 46. Plotted data and fitted line for log-toxicant concentration versus percent survival of *Poecilia reticulata* exposed to phenol.

TABLE 57. Data analysis for the determination of the median lethal effect of ammonia (as N) on *Poecilia reticulata*

Ammonia concentration (mg/dm^3)	Number of fish	Number of affected fish	Observed % affected fish	Expected %	Observed minus expected	Chi^2
2,5	20	0	0	discarded		
3,9	20	0	0(0,6)[a]	1,7	1,1	0,008
6,3	20	2	10	8,5	1,5	0,003
10,0	20	4	20	26,0	6,0	0,02
16,0	20	8	40	54,0	14,0	0,08
25,0	20	16	80	78,0	2,0	0,002
39,0	20	18	90	96,5	6,5	0,1
63,0	20	20	100(99,6)[a]	98,8	0,8	0,003
				TOTAL		0,216

a = "corrected" affected values from Table 51.

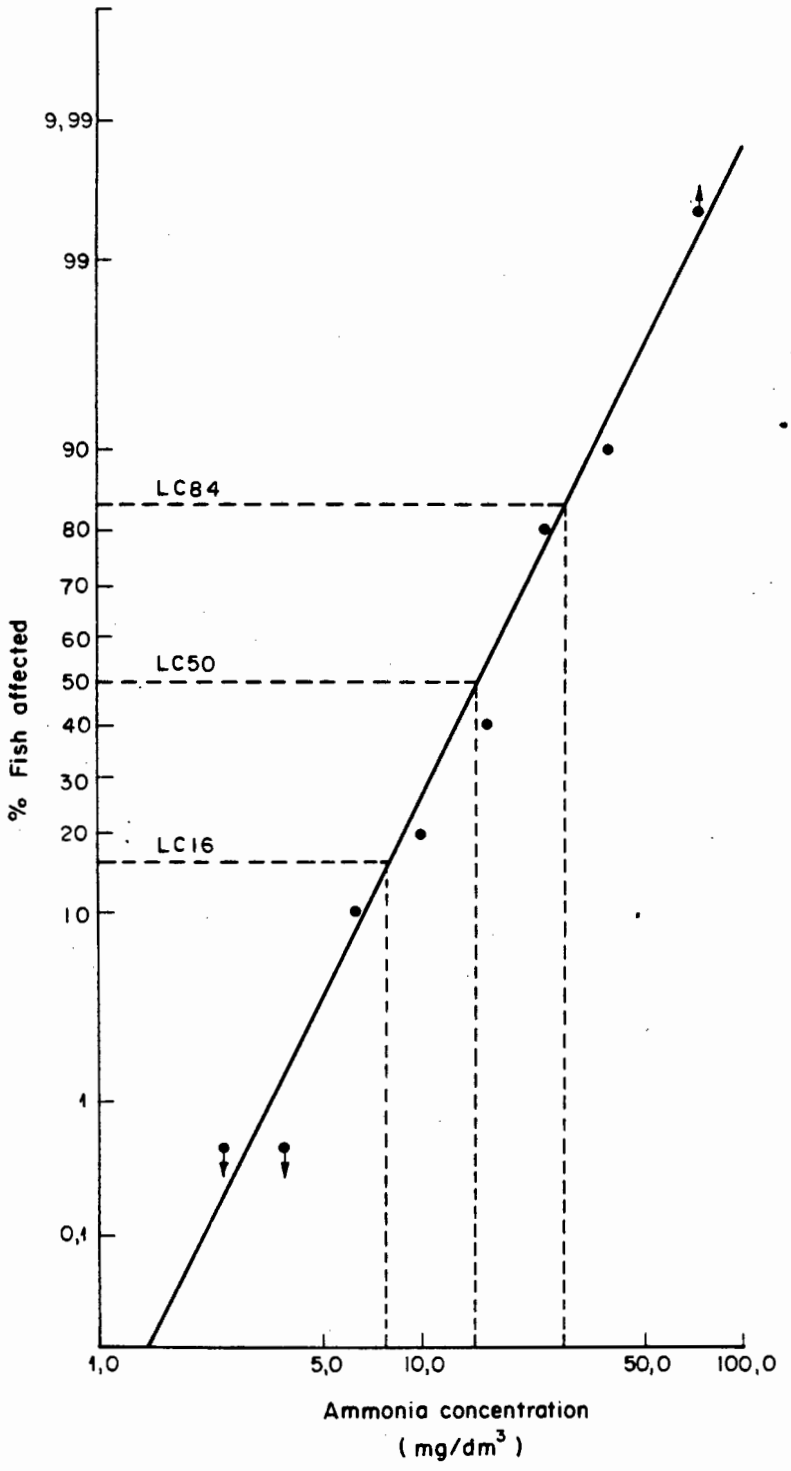

FIGURE 47. Plotted data and fitted line for log-toxicant concentration versus percent survival of *Poecilia reticulata* exposed to ammonia.

TABLE 58. Data analysis for the determination of the median lethal effect of cyanide on *Poecilia reticulata*

Cyanide concentration (mg/dm^3)	Number of fish	Number of affected fish	Observed % affected fish	Expected %	Observed minus expected	Chi^2
0,016	20	0	0	discarded		
0,025	20	0	0	discarded		
0,039	20	0	0(4,7)[a]	15,0	10,3	0,08
0,063	20	5	25	28,0	3,0	0,004
0,1	20	10	50	41,0	9,0	0,035
0,16	20	12	60	57,0	3,0	0,004
0,25	20	13	65	71,0	6,0	0,018
0,39	20	16	80	83,5	3,5	0,008
0,63	20	17	85	88,5	3,5	0,012
1,0	20	20	100(98,7)[a]	96,0	2,7	0,02
					TOTAL	0,181

a = "corrected" affected values from Table 51.

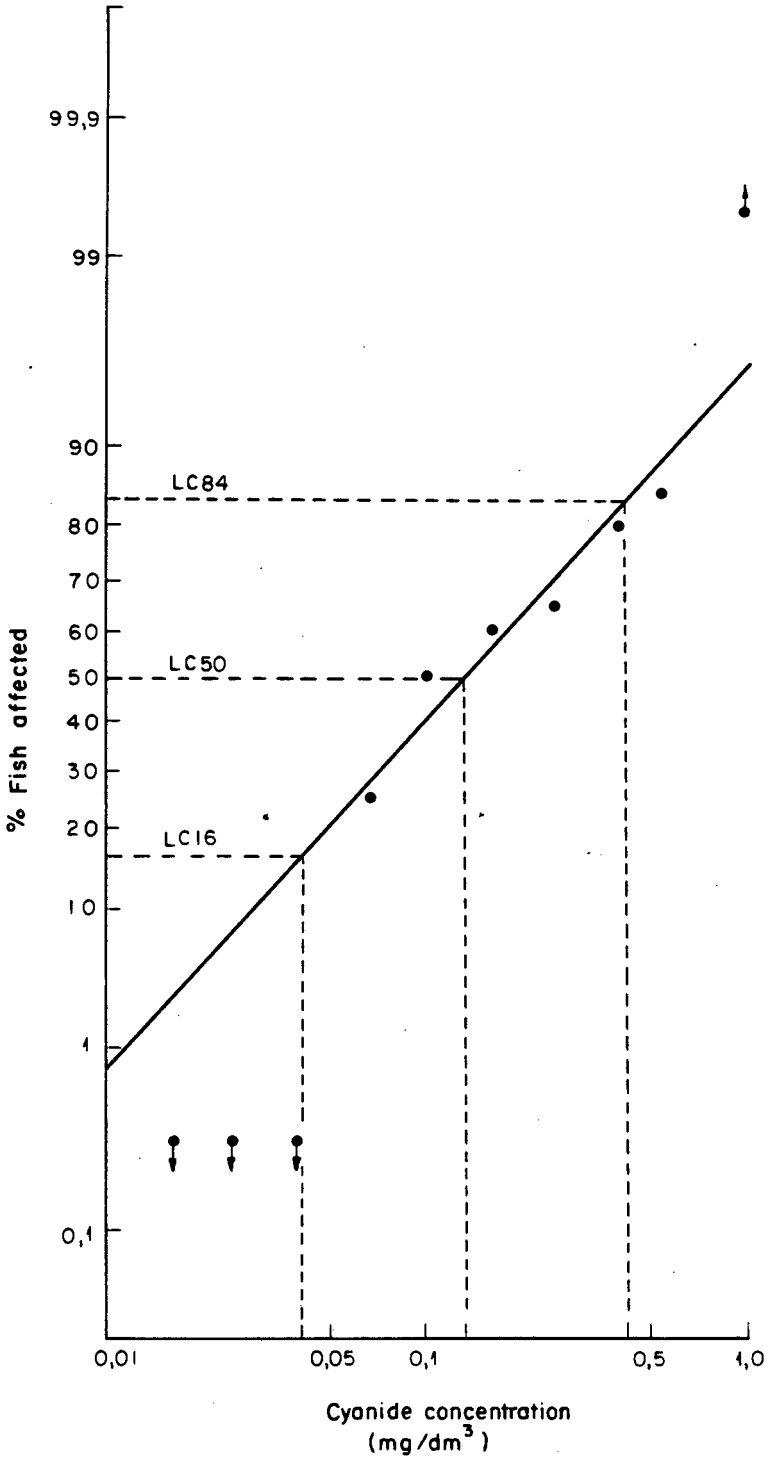

FIGURE 48. Plotted data and fitted line for log-toxicant concentration versus percent survival of *Poecilia reticulata* exposed to cyanide.

TABLE 59. The relationships between calculated and tabular Chi^2 and the determination of heterogeneity of data

Toxicant	Total No of fish in 'K' concentrations	Calculated Chi^2*	Degrees of freedom	Tabular Chi^2**
Cadmium	20(180/9)	1,62	7	14,10
Copper	20(120/6)	1,58	4	9,49
Mercury	20(220/11)	3,24	9	16,90
Phenol	20(100/5)	1,56	3	7,82
Ammonia	20(140/7)	4,32	5	11,10
Cyanide	20(160/8)	3,62	6	12,60

* Where the calculated Chi^2 is less than the tabulated Chi^2 the lines were assumed to be a good fit.

** From Table 52.

TABLE 60. Calculation of the 95% probability limits for the respective median lethal concentrations

Toxicant	LC84	LC50	LC16	S	N^1	f_{LC50}	95% probability limits	
							Upper	Lower
Cadmium	5,5	1,85	0,66	2,88	80	1,39	2,57	1,33
Copper	0,82	0,55	0,35	2,28	40	1,44	0,79	0,38
Mercury	1,0	0,2	0,039	5,0	120	1,5	0,3	0,13
Phenol	23,0	15,0	9,5	1,56	60	1,17	17,55	12,82
Ammonia	27,5	15,2	7,9	1,85	60	1,25	19,0	12,2
Cyanide	0,41	0,13	0,04	3,2	100	1,38	0,18	0,09

high levels of toxic agents are more and more being replaced by long-term discharges of low levels. Thus, fish are being increasingly exposed to low levels of pollution for long periods of time, and applied fish toxicologists are becoming more and more concerned about the effects of long-term low level exposures. Although fish are probably rarely exposed to a constant low level of a toxicant continuously over a long period of time in a field situation, this is a practical and useful way to approach the problem from both legal and toxicological viewpoints.

Because of the advantages and acceptance of the acute mortality test, the first attempts to determine long-term safe concentrations were based on it. The test is severely limited for this use because first, a level that kills 50% of the subjects cannot be considered safe, and second, the test ignores all of the possible adverse effects other than death. However, two ways have been suggested to overcome these limitations. One involves the use of application factors. Hart *et al.* (1945) proposed a formula for the calculation of a safe concentration from results of acute mortality tests. Later, Henderson and Tarzwell (1957) suggested that an application factor of one tenth might estimate the safe concentration in some cases. Such application factors are essentially arbitrary attempts to extrapolate from a toxicity test to a real-life situation.

Some fish toxicologists have suggested that the problems associated with using the acute mortality test for estimating long-term safe concentrations can be avoided by using other acute toxicity tests and making the assumption that any toxicant-induced change from normal in a species is deleterious to the species. This philosophy can be extended to apply to whole aquatic ecosystems, which would imply that any man-caused change in the biological structure of an aquatic ecosystem should be considered detrimental. Investigators have little trouble showing that a change from normal will occur in a species or an ecosystem under specified conditions but they have much trouble showing the significance of the change. Fish and aquatic ecosystems can adapt to some changes from normal, and certain changes may even be beneficial. Changes from normal should not be considered detrimental to aquatic life until such changes can be shown to cause adverse effects on important organisms. It is necessary, therefore, to conduct toxicological tests which bear a direct relationship to a species ability to function successfully in the aquatic ecosystem. Such tests must consider species survival, growth and reproductive potential. Although acute toxicity tests do not cover all these parameters they, at least, indicate toxicant concentration levels which should be applied to the organism in conducting long-term tests.

THE EFFECTS OF SUB-LETHAL CONCENTRATIONS OF SEVERAL TOXICANTS ON GROWTH OF THE GUPPY (*POECILIA RETICULATA*)

EXPERIMENTAL PROCEDURE

This present investigation was undertaken to determine the effects of cadmium, copper, mercury, phenol, ammonia and cyanide, in sub-lethal concentrations, on fish growth. Information obtained from preliminary acute toxicity tests, in the laboratory, was used as bases for the amounts of toxicants to employ.

The toxicant concentrations employed were approximately 20, 10, 5 and 1% of the respective 96-h LC50s. Static tests were conducted in 40 dm^3 glass aquaria, as there were insufficient continuous flow modules available to conduct the 25 experimental determinations simultaneously. The fish were maintained in 30 dm^3 of solution made up by adding predetermined quantities of 1 g/dm^3 toxicant to diluent water to establish the required concentrations. One control solution consisting of diluent water only (Table 61) was employed. Once every two days one-half of the solution in the experimental and control aquaria was removed and replaced with fresh solution. A filtering system was used on each aquarium with glass wool employed to remove debris. All aquaria were aerated with an air stone and covered to prevent evaporation. The temperature of the water was maintained between 21 and 23 °C and the pH regulated to 7,0. These parameters, together with oxygen and toxicant concentrations were checked once a day (Table 62).

Newborn fish were obtained from gravid females which had previously been placed in breeding traps. Within one or two days of being born, the young were added to the experimental or control aquaria, thus the exact age of the fish was always known. In certain cases a brood from a single female was used for a single group; however, if several females delivered young on the same day the broods were mixed and individuals randomly assigned to groups. Between 15 to 32 fish of the same age were added to a single aquarium depending upon the number of fish available. Control and experimental tests were started within a 10-day time span. The fish were fed once to twice daily *ad libitum* with a mixture of dried commercial food, high in protein content (Tetramin). The fish were observed daily for the appearence of sexual maturity and the presence of any dead fish.

TABLE 61. Routinely determined characteristics of dilution water (Each figure being the mean of 90 analyses)

Characteristics	Mean	S.D.	Range
Hardness (mg/dm^3 as $CaCO_3$)	87,74	9,12	83,4 - 94,7
Chemical Oxygen Demand	10,61	0,31	10 - 11
Sodium (mg Na^+/dm^3)	13,14	1,41	12 - 14
Potassium (mg K^+/dm^3)	2,51	0,25	2,4 - 2,7
Calcium (mg Ca^{2+}/dm^3)	24,12	0,61	23 - 25
Magnesium (mg Mg^{2+}/dm^3)	6,51	0,26	6 - 7
Ammonia (mg N/dm^3)	always less than 0,2		
Orthophosphate (mg P/dm^3)	always less than 0,2		
Dissolved Oxygen (mg/dm^3)	always more than 7,0		
Cadmium (mg Cd^{2+}/dm^3)	always less than 0,005		
Copper (mg Cu^{2+}/dm^3)	always less than 0,025		
Mercury (mg Hg^{2+}/dm^3)	always less than 0,001		
Cyanide (mg CN^-/dm^3)	always less than 0,05		
Phenol (mg C_6H_5OH/dm^3)	always less than 0,01		
Electrical Conductivity (mS/m)	23,82	0,21	23,6 - 24,2

TABLE 62. Toxicant concentrations during experiment

Toxicant	Nominal concentration (mg/dm^3)	Measured concentration ($\bar{x} \pm 1$ SD)	pH
Cadmium (Cd^{2+})	0,4	0,38(0,02)	7,0
	0,2	0,21(0,01)	7,1
	0,1	0,1 (0,01)	6,9
	0,02	0,02(0,001)	7,0
Copper (Cu^{2+})	0,1	0,13(0,02)	7,0
	0,05	0,05(0,002)	7,0
	0,025	-	7,0
	0,005	-	7,0
Mercury (Hg^{2+})	0,04	0,036(0,004)	6,9
	0,02	0,022(0,002)	6,9
	0,01	0,014(0,002)	7,1
	0,002	0,002(-)	7,0
Phenol (C_6H_5OH)	3,0	3,42(0,6)	7,0
	1,5	1,61(0,9)	7,0
	0,75	0,82(0,1)	6,8
	0,15	0,18(0,03)	7,0
Ammonia (as N)	3,0	3,13(0,2)	7,0
	1,5	1,53(0,4)	7,2
	0,75	0,71(0,1)	7,2
	0,15	-	6,9
Cyanide (CN^-)	0,03	-	7,1
	0,015	-	7,0
	0,007	-	7,0
	0,0015	-	7,0

- Below limit of detection

S.D. - Standard deviation

After 30 days in the toxic and control solutions, the fish groups were weighed at 15-day intervals until the 90th day. Each fish was weighed individually on an analytical balance by the following procedure. First the whole group was removed from the aquarium and transferred to a five dm^3 beaker containing the solution in which they were living. Exactly one cm^3 of water was added to a small dry glass cup and weighed. This was repeated three times to obtain an average weight for the cup. The fish were captured, blotted on absorbent paper and transferred to the cup containing one cm^3 of the solution in which the fish had been living. Each fish was weighed to the nearest 0,1 mg .

DISCUSSION OF RESULTS

General observations on size, activity, behaviour and sexual maturity were made in both control and experimental solutions. Size differences in affected groups were invariably obvious before 60 days, particularly regarding fish exposed to the higher toxicant concentrations. Deaths occurred in the fish groups exposed to 0,4 mg Cd^{2+}/dm^3 (2 fish), 0,04 mg Hg^{2+}/dm^3 (4 fish) and 0,02 mg Hg^{2+}/dm^3 (2 fish). Mortality in these groups occurred predominently in small fish; consequently, the weights at any one time interval did not reveal the absolute extent of stunting in these fish groups (Table 63). The markedly undersized fish in the affected groups were comparatively inactive and did not feed normally on the surface and appeared to have difficulty in swimming and maintaining equilibrium.

At maturity male guppies develop prominent colouration and a gonopodium which easily distinguishes them from the females. The females have no marked external sex characteristics; however, the continued growth of the female makes them larger than the male which attains a constant size at maturity. In this study differences between the sexes were not usually obvious in the higher toxicant concentrations and only the male characteristics could be observed with any certainty. Using the male external secondary sex characteristics as a basis for the presence of sexual maturity, it was evident that groups in the toxic solutions were not only stunted in growth but sexual maturity was also retarded or prevented.

TABLE 63. Median and range of fish weights (mg) at 15 day intervals when exposed to different degrees of intoxication

Toxicant	Concentration (mg/dm^3)	Number of fish	Weight of fish at day 30	45	60	75	90
Control	-	23	21,6 (10,1-39,4)	31,8 (15,8-52,1)	42,7 (20,4-78,8)	51,5 (22,1-96,1)	55,9 (23,4-105,8)
Cadmium	0,02	18	20,4 (11,8-35,6)	32,4 (16,2-50,5)	44,8 (18,1-69,4)	49,5 (20,2-88,8)	54,4 (21,8-94,6)
	0,1	21	18,1 (7,1-24,9)	24,7 (9,3-32,6)	30,7 (9,5-42,3)	34,5 (9,8-47,7)	37,1* (10,1-53,8)
	0,2	28	15,8 (6,4-18,4)	17,5 (6,8-23,4)	21,7 (7,3-34,0)	25,3 (8,1-42,6)	30,6* (8,8-49,6)
	0,4	25	13,2 (6,1-17,3)	16,4 (6,4-21,2)	18,7 (6,8-25,8)	20,2 (7,1-31,0)	21,2* (7,2-32,6)
Copper	0,005	18	22,8 (11,6-37,3)	33,2 (16,1-50,2)	44,6 (21,3-81,8)	53,2 (23,0-101,2)	58,4 (25,6-110,2)
	0,025	19	19,2 (10,8-34,1)	29,6 (14,8-42,3)	38,9 (19,1-77,7)	48,1 (21,3-84,6)	53,6 (24,6-97,8)
	0,05	26	19,4 (10,8-33,8)	28,6 (14,1-40,6)	41,5 (18,6-81,4)	49,8 (22,8-86,4)	54,4 (26,7-101,6)
	0,1	24	15,1 (7,7-22,4)	18,2 (8,1-27,3)	23,7 (10,8-46,4)	31,6 (11,6-61,2)	37,3* (14,7-72,3)
Mercury	0,002	21	19,2 (10,1-32,2)	28,6 (11,2-38,4)	34,9 (12,6-48,2)	38,2 (13,6-56,4)	41,4* (15,8-64,7)
	0,01	19	15,3 (6,9-23,5)	19,1 (7,1-32,4)	24,9 (7,8-41,2)	27,5 (8,2-42,5)	28,3* (8,3-47,6)
	0,02	26	13,3 (5,8-19,9)	18,2 (6,1-23,4)	21,5 (6,8-29,6)	23,1 (7,1-37,8)	24,7* (8,0-44,8)
	0,04	31	9,5 (4,6-17,3)	10,3 (4,8-21,4)	11,6 (5,1-25,6)	12,6 (5,4-31,2)	13,1* (5,4-33,4)
Phenol	0,15	23	22,6 (11,6-41,2)	32,6 (16,1-53,8)	43,1 (20,3-76,6)	48,9 (22,4-88,4)	54,2 (25,3-98,6)
	0,75	15	19,7 (8,3-25,2)	29,1 (14,3-37,7)	38,3 (16,2-51,6)	45,1 (19,2-69,4)	52,8 (21,8-88,8)
	1,5	28	17,5 (7,4-23,1)	25,2 (8,6-32,8)	32,4 (9,5-47,3)	36,3 (10,1-56,6)	40,4* (12,6-61,7)
	3,0	26	16,8 (6,8-23,2)	23,1 (7,1-26,8)	28,1 (9,2-38,4)	30,3 (10,2-44,3)	31,8* (11,1-53,6)
Ammonia	0,15	21	24,9 (11,9-42,3)	35,2 (12,2-51,6)	51,5 (14,6-78,8)	62,1 (19,7-94,3)	69,3* (27,5-123,2)
	0,75	21	24,5 (11,6-38,1)	32,5 (12,6-51,2)	41,8 (12,8-64,4)	48,6 (16,2-78,8)	52,6 (21,2-99,3)
	1,5	18	19,2 (10,2-29,3)	29,3 (12,6-38,4)	37,9 (15,3-49,4)	46,0 (18,8-58,6)	50,0 (21,3-76,4)
	3,0	24	14,9 (8,4-21,3)	19,7 (8,8-25,6)	25,1 (8,9-33,3)	28,2 (9,1-41,6)	32,6* (9,7-51,8)
Cyanide	0,0015	18	23,8 (11,2-39,3)	33,3 (12,6-53,2)	45,2 (15,6-68,9)	53,1 (19,8-81,4)	57,7 (21,3-104,3)
	0,007	23	22,7 (10,9-41,6)	30,8 (12,6-49,8)	41,5 (14,8-65,3)	46,2 (18,1-79,9)	50,3 (20,4-96,3)
	0,015	26	14,9 (7,3-19,8)	16,8 (7,5-21,2)	18,1 (7,8-22,1)	21,2 (8,0-26,3)	23,1* (8,6-31,2)
	0,03	29	16,8 (7,1-21,3)	18,0 (7,8-25,8)	19,1 (8,6-29,7)	19,9 (8,8-31,2)	20,8* (8,9-31,2)

* Significant difference to control $P<0,05$

Cadmium is an extremely dangerous cumulative poison. All concentrations of this metal, above 0,1 mg/dm^3, induced an insidious progressive adverse chronic effect upon growth in the test fish (Figure 49). The

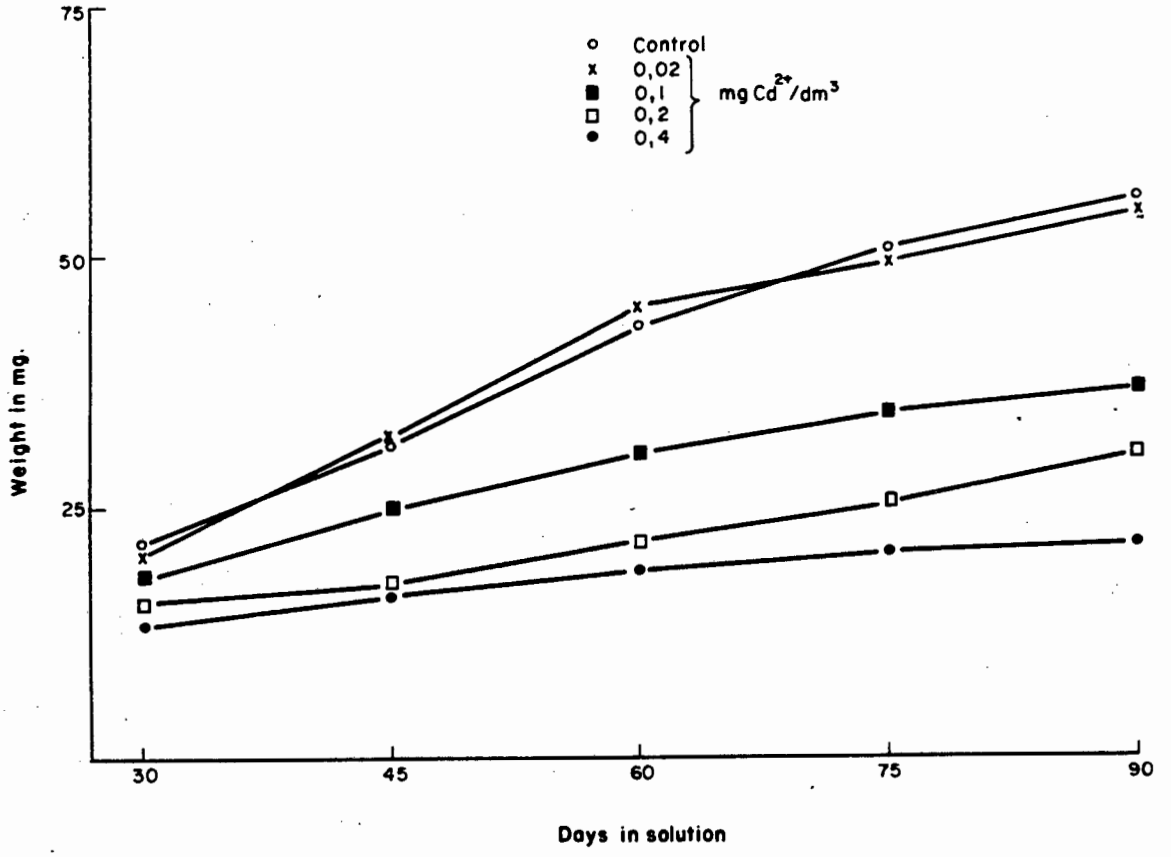

FIGURE 49. Effect of different concentrations of cadium upon growth of *Poecilia reticulata*.

maximum no effect level upon fish growth was found to lie between 1 and 5% of the 96-h LC50. Investigations into the chronic effects of cadmium intoxication have mainly been concerned with bioconcentration of the metal in fish tissue. However, the few results obtained regarding the effect of cadmium on growth indicate safe levels to lie between 5 and 10% of the 96-h LC50. Kumada *et al.* (1972) found no significant mortality, and no effect on growth of rainbow trout exposed to a concentration of 5 µg Cd^{2+}/dm^3 over a period of 30 weeks. Long-term constant-flow tests have been carried out on under-yearling rainbow

trout over a period of 65 weeks in hard water (250 mg/dm^3 as $CaCO_3$) at nominal concentrations of cadmium of 0,2, 5 and 8 µg Cd^{2+}/dm^3 (V.M. Brown, Water Research Centre, U.K., personal communication). All the fish survived and their growth was apparently not affected by the cadmium. The highest concentrations noted in these investigations approximate to 5 to 10% of the 96-h LC50. The chronically safe levels for growth in fathead minnows (*Pimephales promelas*) in hard water lie between 0,06 and 0,03 mg Cd^{2+}/dm^3 (Pickering and Gast, 1972), being 7 and 3% of the 96-h LC50 respectively (Jones, 1964).

Several long-term studies have been made on the effects of chronic exposure to *copper* solutions on the survival and growth of fish species. McKim and Benoit (1971) found that, in the laboratory, the growth and survival of yearling brook trout was affected at a copper concentration of 32,5 µg/dm^3 over an eight-month exposure period. A transient effect of copper on growth rate was noted at concentrations of 3,4 to 32,5 µg/dm^3, but after 23 weeks the growth rate of brook trout was equal to that of the controls at concentrations up to 9,5 µg/dm^3. It was concluded that the maximum concentration of copper which did not significantly affect brook trout under these conditions was 0,1 to 0,17 of the 96-h LC50.

Tests with bluegill, over a 24-month exposure period, showed that survival was reduced at copper levels greater than 40 µg/cm^3 and that growth was retarded at 77 to 162 µg/dm^3 (Benoit, 1975). The level of 40 µg/dm^3 was 0,04 of the 96-h LC50 value for this species.

Similar tests with fathead minnow exposed for 11 months to copper solutions in soft water (hardness 31,4 mg/dm^3 as $CaCO_3$) showed that at levels up to 10,6 µg/dm^3 growth was normal (Mount and Stephan, 1969); this level was 0,13 of the 96-h LC50. However, tests in hard water (198 µg/dm^3 as $CaCO_3$) showed that growth was unaffected below the range 14,5 to 33,0 µg Cu^{2+}/dm^3 which was 0,03 to 0,07 of the 96-h LC50 value of 450 µg/dm^3 (Mount, 1968).

The effect of copper upon growth of guppies (Figure 50) would indicate this species to be less susceptible to the metal than others. However, several authors have indicated that sub-lethal effects measured in

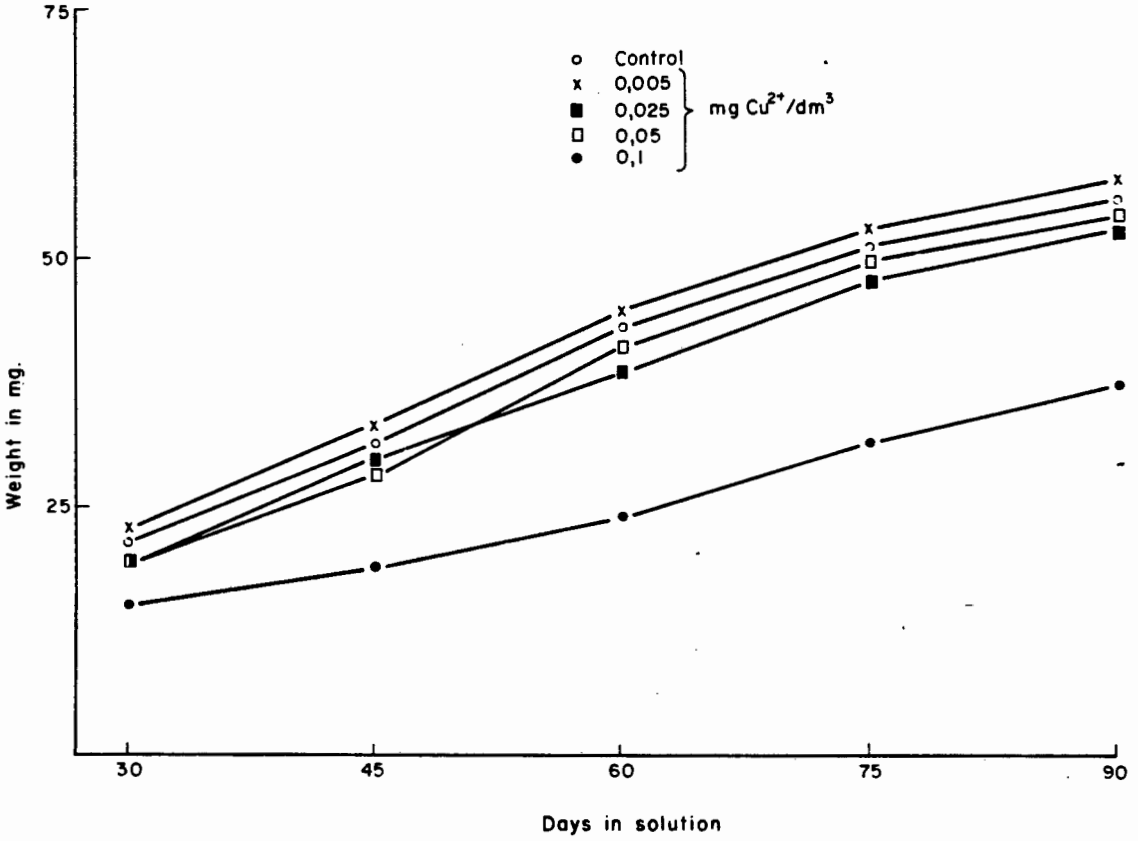

FIGURE 50. Effect of different concentrations of copper upon growth of *Poecilia reticulata*.

copper solutions are transitory, persisting for a few days only, which would imply that some acclimation takes place. Except for work on the long-term effects of copper solutions on the fathead minnow (Mount, 1968) and bluegill (Benoit, 1975) in hard waters, concentrations below 0,1 of the 96-h LC50 appear to exert no measurable effect.

Very little is known of the effect of *mercury* upon growth of fish, investigators having concentrated their research into the bioaccumulatory effects of the metal. A consideration of the results obtained in the present study (Figure 51) would, however, seem to indicate that mercury is infinitely toxic. All concentrations used affected growth of guppies adversely, the progressive harmful effects being shown especially in the lowest concentrations where fish growth rate decreased markedly over a period of time.

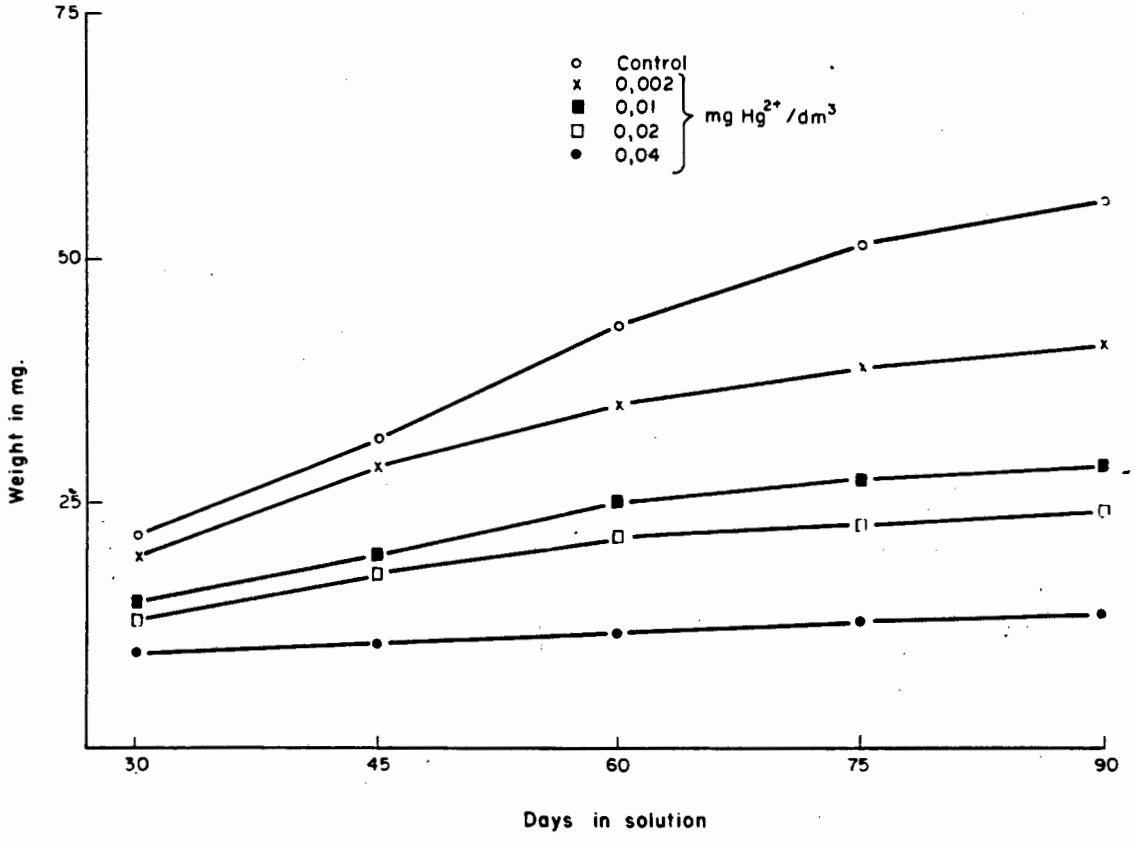

FIGURE 51. Effect of different concentrations of mercury upon growth of *Poecilia reticulata.*

Mikriakov (1969) observed a loss of weight of common carp exposed for two months to 12,5 mg/dm^3 *phenol*. Stepanov and Flerov (1969) reported that guppies kept for a year in 12,5 mg/dm^3 phenol first spawned at the age of five months, compared with 10 months for the controls, but there was no reduction in growth. This latter observation contrasts markedly with the results obtained in the present study, where concentrations of phenol above 1,5 mg/dm^3 inhibited growth (Figure 52). V.M. Brown (personal communication) studied the effect on rainbow trout of concentrations of 1, 2, 3, 4 and 5 mg/dm^3 of phenol over a period of 18 weeks and found that there was a 20% reduction in growth at 1 mg/dm^3 and greater reductions at higher concentrations.

Recent developments in high density fish culture, involving the recirculation of water, have given rise to renewed interest in the toxicity to fish of metabolic by-products, including *ammonia*. Burrows (1964) stated that chinook salmon were adversely affected when the un-ionized ammonia

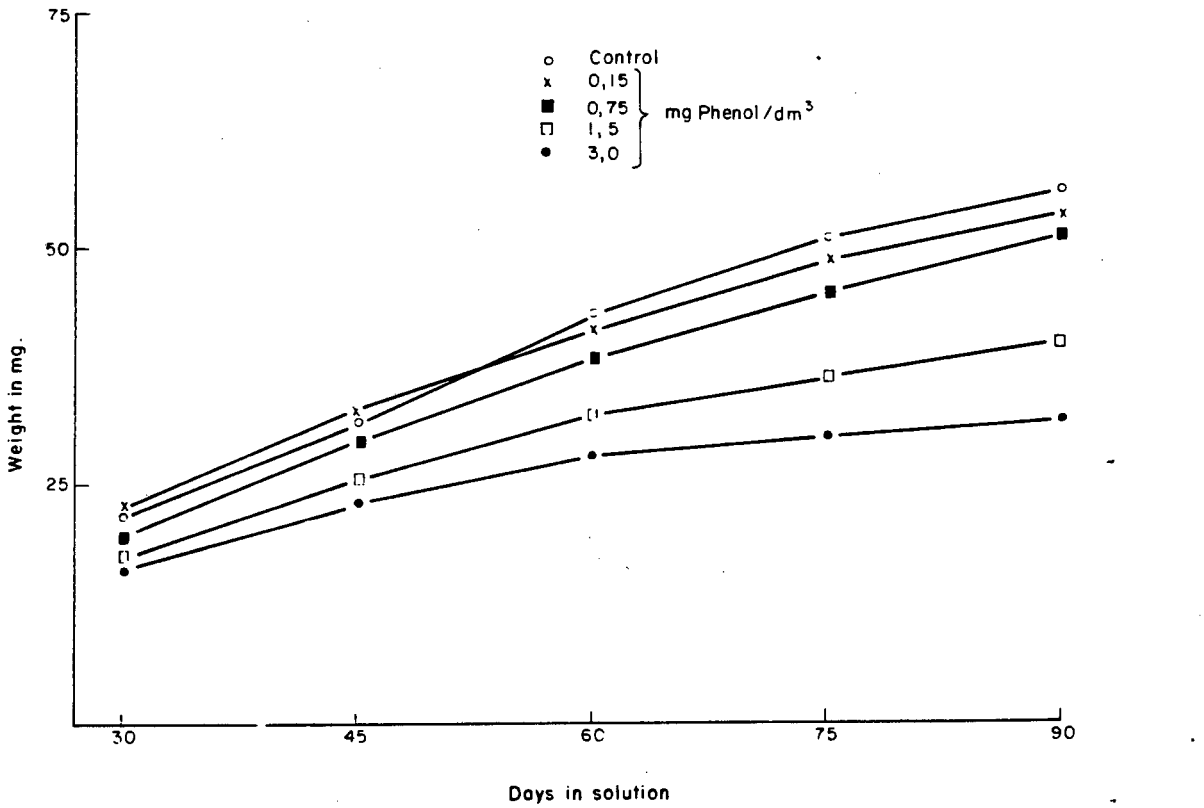

FIGURE 52. Effect of different concentrations of phenol upon growth of *Poecilia reticulata*.

concentration became greater than 0,005 mg/dm^3. A similar upper limit has been quoted by Liao and Mayo (1972) for salmonids reared in intensive culture systems with recirculating water, but with no supporting experimental evidence. More recently, Robinson-Wilson and Seun (1975) studied the growth rate of chinook salmon in ammonia solutions at 15 °C and found that growth was affected only at concentrations of un-ionized ammonia greater than 0,026 mg NH_3/dm^3; below this value the salmon grew faster than those in clean water. Rainbow trout were also found to grow satisfactorilly in a recirculation unit where the total ammonia ranged from 4 to 23 mg NH_3/dm^3 at a pH range of 6,5 to 7,5 (Scott and Gillespie, 1972); assuming an average pH value of 7,0 the un-ionized ammonia concentrations ranged from 0,008 to 0,044 mg NH_3/dm^3. Schulze-Wiehenbrauck (1976) found that exposure to un-ionized ammonia concentrations of 0,05 to 0,13 mg NH_3/dm^3 initially limited the growth of rainbow trout, but after two weeks the subsequent growth rate was good; concentrations greater than 0,13 mg NH_3/dm^3 inhibited growth rate. In these

experiments there was some evidence of acclimation to ammonia toxicity. The growth of rudd was reduced at un-ionized ammonia concentrations above 0,19 mg NH_3/dm^3 but was increased at or below 0,1 mg NH_3/dm^3 (Department of the Environment, U.K., 1971). Similar data have been obtained for channel catfish *(Ictaluras punctatus)* by Robinette (1976) as well as in the present study where the lowest ammonia concentration employed induced increased growth in guppies (Figure 53), whereas the highest had a decidedly adverse effect. The reason for this is not known. It is possible that fish become acclimatized to very low levels of ammonia and accommodate to the absorbtion of water due to increased permeability, retaining sufficient so as not to provide undue stress to the excretory system.

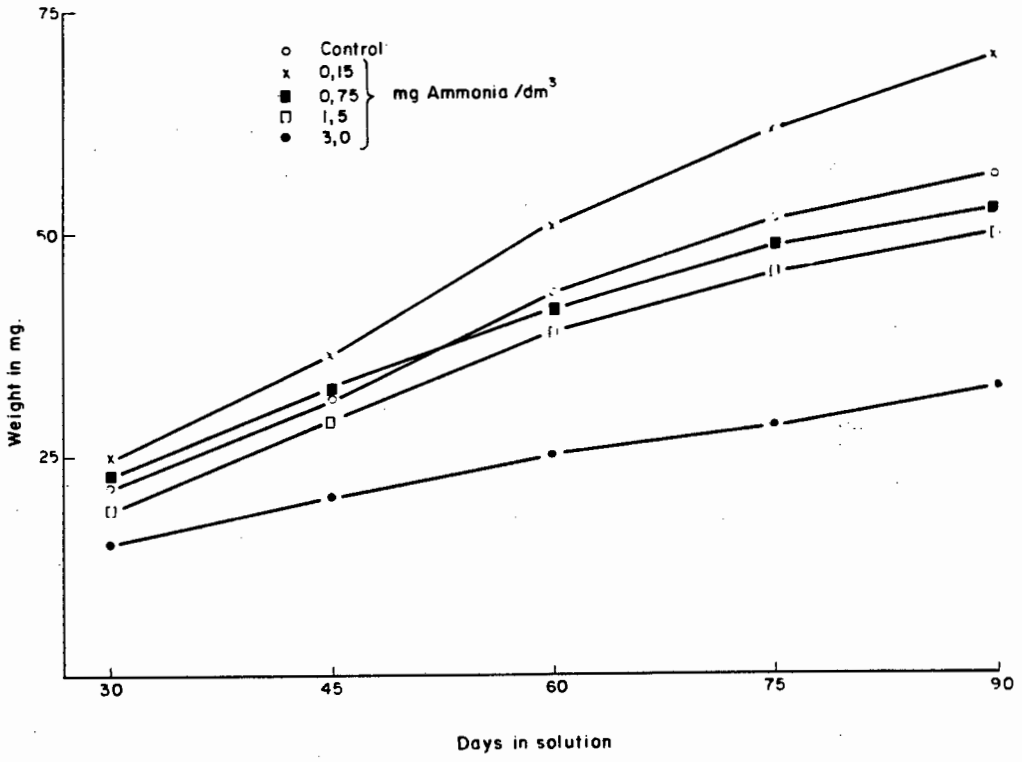

FIGURE 53. Effect of different concentrations of ammonia upon growth of *Poecilia reticulata.*

Cyanide concentrations of 0,03 and 0,015 mg/dm^3 (0,2 and 0,1 of the 96-h LC50) affected the growth rate of guppies adversely (Figure 54). The large significant difference between these concentrations and the highest no-effect concentration may indicate that the cut-off point between effect and no effect is marginal. Guppies were less sensitive to lethal and sub-lethal intoxication by cyanide than many other fish species (Jones, 1964).

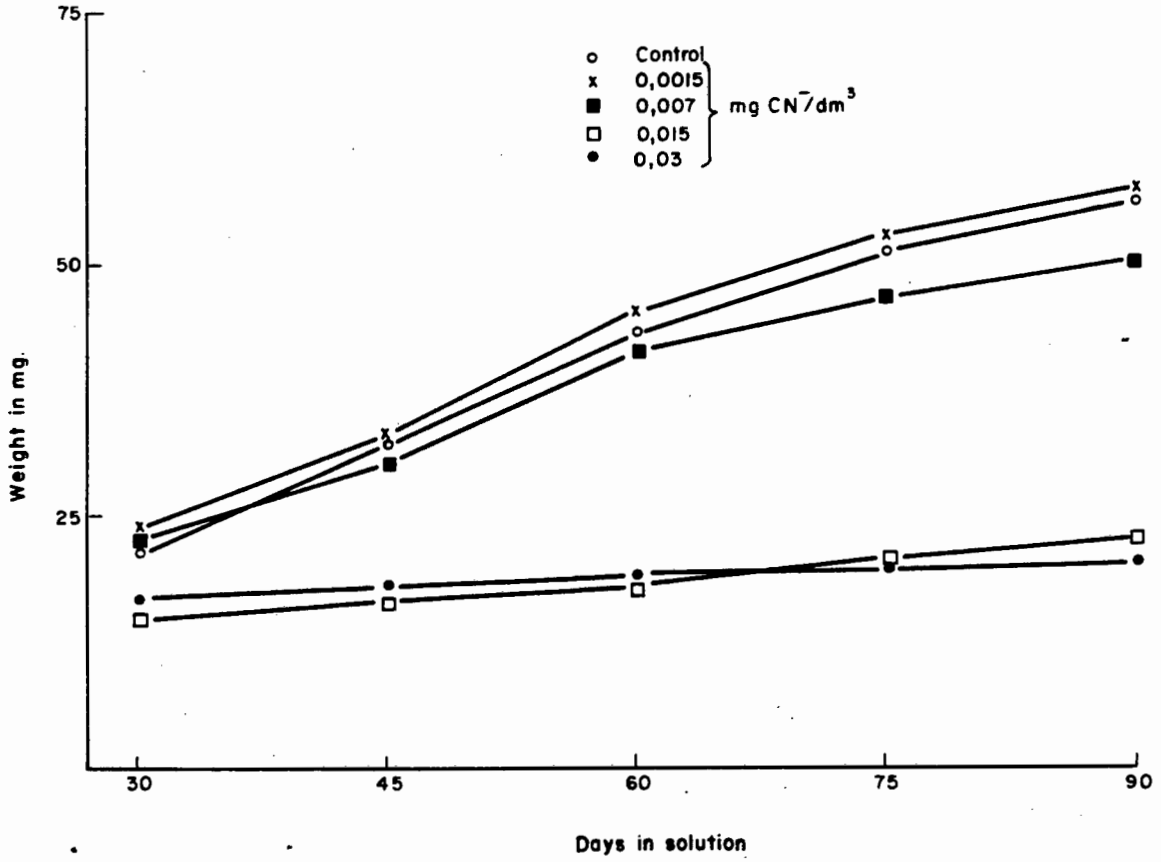

FIGURE 54. Effect of different concentrations of cyanide upon growth of *Poecilia reticulata*.

The allometric response of fish to respiratory depressants has long been established for reduced dissolved oxygen by Wells (1913) and for cyanide by Herbert and Merkens (1952). These authors showed a greater tolerance of smaller specimens to acutely lethal conditions. Hughes (1970) has shown a log-log linear relation between the gill area and the body weight with a slope of 0,8, demonstrating a larger weight-specific gill area in small fish compared with larger ones. Consequently, an increase in the size of the fish brings about an unproportional increase in the cost of ventilation. This lower respiration/body size capacity in larger fish would then make them more sensitive to a respiratory depressant such as cyanide. Cyanide could act as a regulator of the basal metabolic rate of fish, reducing maintenance requirements in small fish, such as the guppy, but increasing them in larger ones.

EFFECTS ON REPRODUCTION IN THE GUPPY (*POEÇILIA RETICULATA*) OF CHRONIC EXPOSURE TO SUB-LETHAL CONCENTRATIONS OF SEVERAL TOXICANTS

EXPERIMENTAL PROCEDURE

Females used in this study were 5-month old virgins that were 33,6 ±3,1 mm ($\bar{x}$ ±1 standard deviation) in standard length (from the tip of the snout to the end of the vertebral column). They were raised in the laboratory. It was necessary to use virgin females because brood size, i.e. number of young in the brood, in the guppy shows a cyclic variation, the value increasing to a maximum and then decreasing to a minimum (Rosenthal, 1952).

The test system was static and tanks held 5 dm^3 of test solution each. Dilution water was dechlorinated municipal tap water (Table 61) to which was added predetermined values of 1 g/dm^3 solutions to establish toxicant levels to span concentrations pinpointed as those producing minimum effect and maximum no-effect levels in the study on the effects of intoxication upon growth, described previously. Twelve toxicant concentrations were studied, being 0,1; 0,02; and 0,01 mg/dm^3 of cadmium (5, 1 and 0,5% of the 96-h LC50 respectively), 0,1 and 0,05 mg/dm^3 of copper (20 and 10% of the 96-h LC50), 0,001 mg/dm^3 of mercury (0,5% of the 96-h LC50), 1,5 and 0,75 mg/dm^3 of phenol (10 and 5% of the 96-h LC50), 3,0 and 1,5 mg/dm^3 of ammonia (as N) (20 and 10% of the 96-h LC50), and 0,015 and 0,007 mg/dm^3 of cyanide (10 and 5% of the 96-h LC50).

To begin the tests, 65 tanks were thoroughly cleaned and 5 dm^3 of dilution water was added to each. The tanks were gently aerated to maintain adequate oxygen levels, the temperature of the water was maintained between 21 and 23 °C, and the pH regulated to 7,0. All tests were carried out under natural conditions of photoperiod. One female was placed in each of the tanks and each tank was randomly assigned to one of thirteen groups. The females were then paired with selected males and they remained paired throughout the experiment. The selection of males was on the basis of courtship display. Males showing less than ten displays per minute were not used. Because parent guppies would eat their young, each pair was put into a breeding cage that was suspended in the tank. The cages were made of perspex (200 x 100 x 90 mm) with parallel rows of apertures (30 x 3 mm) in the base.

One day after the fish were paired the test concentrations were established in five tanks for each concentration. The remaining five tanks served as controls. To minimize handling the fish, one-half of the test solution was renewed twice a week. Fish were fed once or twice daily on a dry food preparation, Tetramin.

The experiment was run for four months, during which time four broods (representing four parturition periods) were produced. The record kept for each female consisted of the brood size, the date on which the brood was produced (from which the intervals between broods were calculated) and the standard length and condition of young at birth.

Significance was accepted at $P < 0,05$.

DISCUSSION OF RESULTS

Not one test concentration had any effect on the intervals between broods; between group differences at any parturition were not significant and although the intervals were shortest between pairing and the production of the first brood (Table 64), within group differences were also not significant.

The total number of young per female was affected by the highest concentration of all toxicants, except ammonia (Figure 55). Exposure to 0,1 mg Cd^{2+}/dm^3, 0,1 mg Cu^{2+}/dm^3, 0,001 mg Hg^{2+}/dm^3, 1,5 mg Phenol/dm^3 and 0,015 mg CN^-/dm^3 caused 64, 43, 28, 47 and 62% reductions respectively. The reduction was significant when fish exposed to these concentrations were compared with either control fish or those exposed to lower concentrations. The effect was not due to a decreased frequency of brood production in fish exposed to the highest concentrations since all fish produced four broods. Rather, it was the result of a marked reduction in the brood sizes of the females exposed to the highest concentrations.

Between group comparisons during each of the four parturition periods showed that the differences in brood size in females exposed to 0,1 mg Cd^{2+}/dm^3 were significant after the first parturition period

TABLE 64. Effect of chronic exposure to several toxicants on the interval (days) between broods over four parturition periods

Toxicant	Concentration (mg/dm^3)	1*	2**	3**	4**
Control	-	33,2	35,6	35,2	33,4
Cadmium	0,1	32,0	33,2	34,2	32,8
	0,02	29,2	30,4	31,2	31,8
	0,01	29,4	31,4	33,6	33,8
Copper	0,1	30,2	31,2	33,6	32,8
	0,05	29,8	32,4	32,8	31,6
Mercury	0,001	31,4	31,8	32,6	31,8
Phenol	1,5	32,2	33,6	35,4	34,6
	0,75	30,0	31,2	31,0	32,4
Ammonia	3,0	29,8	30,8	33,8	34,6
	1,5	29,4	31,6	32,4	32,4
Cyanide	0,015	32,4	33,2	35,8	34,2
	0,007	31,8	32,4	32,4	31,8

All figures are mean values.

* The interval represents the time between pairing and the appearence of the first brood.

** The interval represents the time between the appearence of young from a female in successive parturition periods.

(Figure 56). In the second parturition period, females in 0,1 mg Cu^{2+}/dm^3, 1,5 mg Phenol/dm^3 and 0,015 mg CN^-/dm^3 produced significantly smaller brood sizes (Figures 57, 59 and 61 respectively). Females exposed to 0,001 mg Hg^{2+}/dm^3 and 3,0 mg NH_3/dm^3 produced significantly smaller brood sizes in the third and fourth parturition periods respectively (Figures 58 and 60). Only live young were taken into consideration when recording brood size. In all effective concentrations, dead young were produced having the following abnormalities: they were less than four mm long, had not absorbed the yolk completely and had not uncurled from the embryonic posture. These individuals represented 12, 8, 10, 4, 10, 2 and 8% of the total number of young produced in 0,1 mg Cd^{2+}/dm^3, 0,02 mg Cd^{2+}/dm^3, 0,1 mg Cu^{2+}/dm^3, 0,001 mg Hg^{2+}/dm^3, 1,5 mg Phenol/dm^3, 3,0 mg NH_3/dm^3 and 0,015 mg CN^-/dm^3 respectively. In 0,1 mg Cd^{2+}/dm^3 and 0,1 Cu^{2+}/dm^3 there were three and two cases, respectively, in which the fertilized egg had not developed into a distinguishable embryo. Whenever abnormal young were produced, the brood also contained live and apparently normal individuals. There were no dead or abnormal young produced by control females or those exposed to the lower toxicant concentrations.

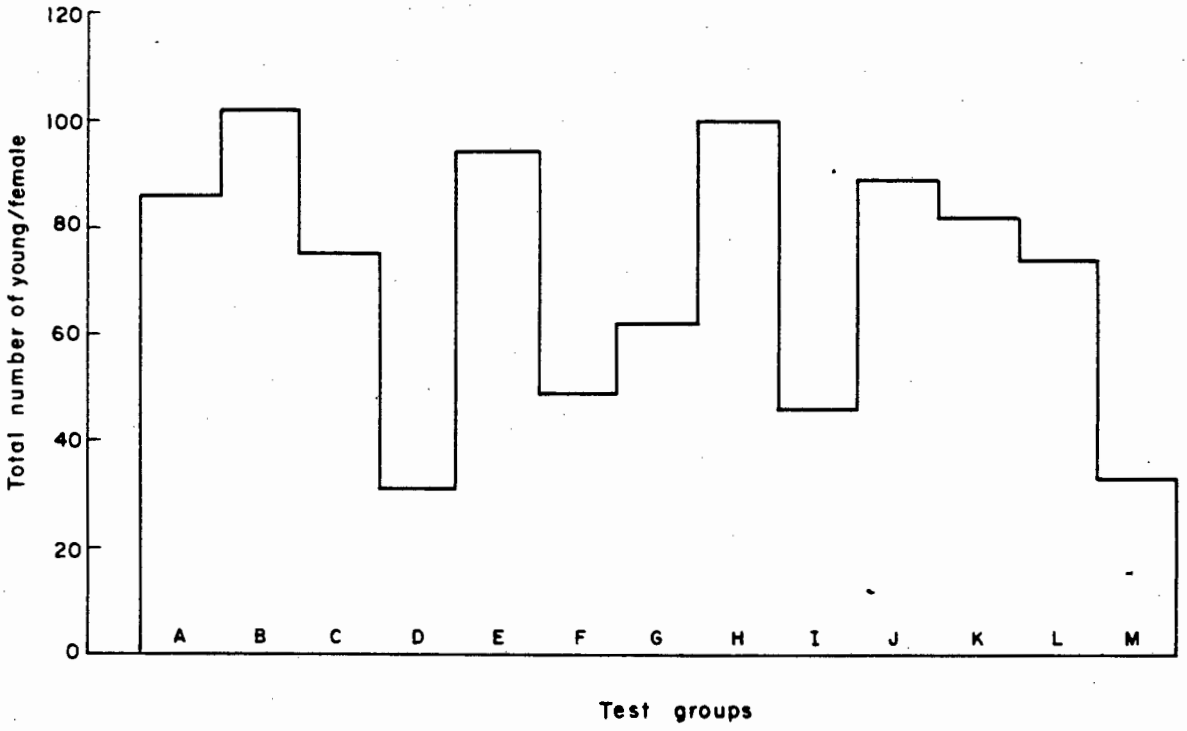

FIGURE 55. Effect of chronic exposure on the total number of young produced per female in four parturition periods. A = control, B = 0,01 mg Cd^{2+}/dm^3, C = 0,02 mg Cd^{2+}/dm^3, D = 0,1 mg Cd^{2+}/dm^3, E = 0,05 mg Cu^{2+}/dm^3, F = 0,1 mg Cu^{2+}/dm^3, G = 0,001 mg Hg^{2+}/dm^3, H = 0,75 mg C_6H_5OH/dm^3, I = 1,5 mg C_6H_5OH/dm^3, J = 1,5 NH_3-N/dm^3, K = 3,0 mg NH_3-N/dm^3, L = 0,007 mg CN^-/dm^3, M = 0,015 mg CN^-/dm^3.

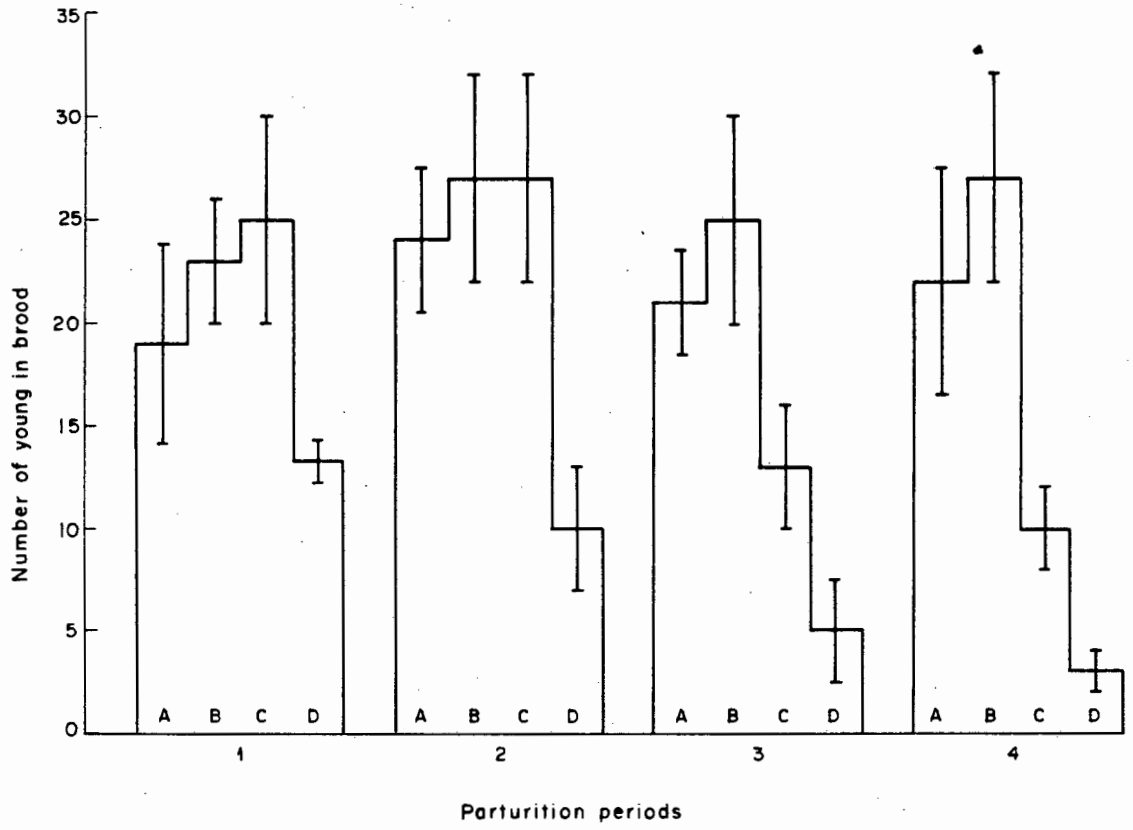

FIGURE 56. Changes in the brood sizes of female guppies during chronic exposure to cadmium during four parturition periods. Values are $\bar{x}$ ±1SD. A = control, B = 0,01 mg Cd^{2+}/dm^3, C = 0,02 mg Cd^{2+}/dm^3, D = 0,1 mg Cd^{2+}/dm^3.

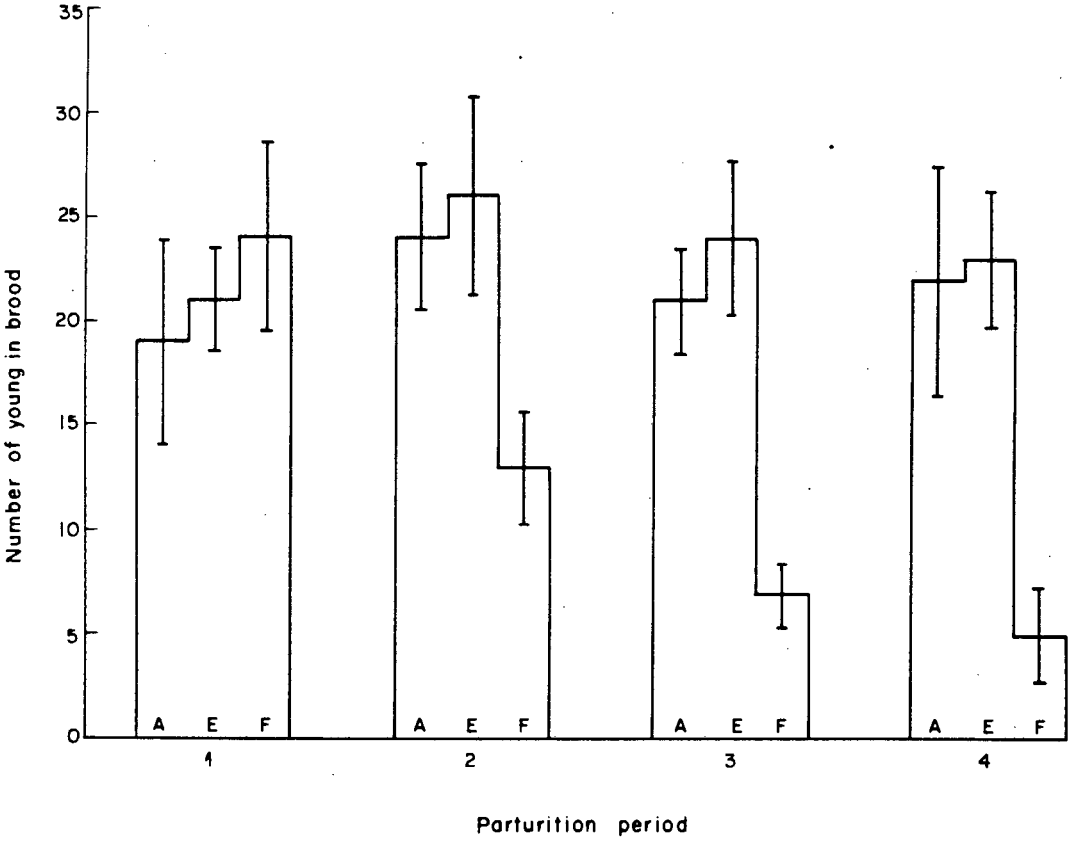

FIGURE 57. Changes in the brood sizes of female guppies during chronic exposure to copper during four parturition periods. Values are $\bar{x}$ ±1SD. A = control, E = 0,05 mg Cu^{2+}/dm^3, F = 0,1 mg Cu^{2+}/dm^3

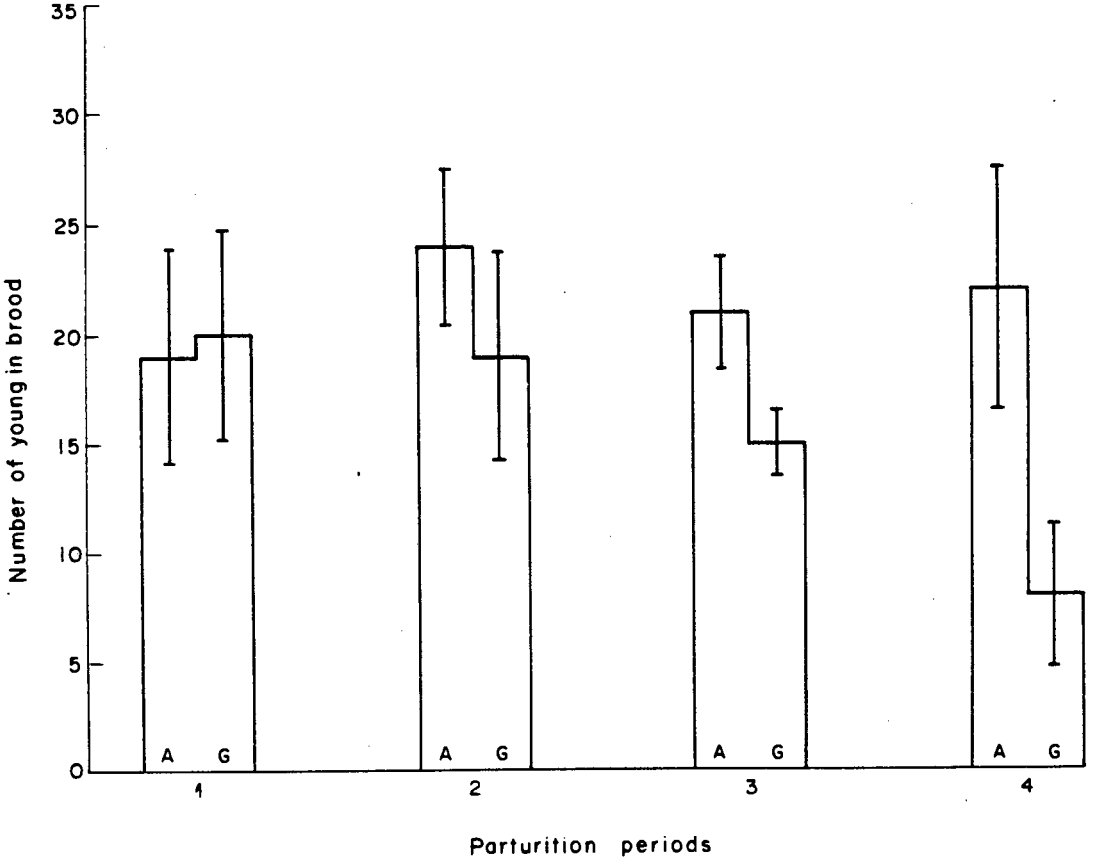

FIGURE 58. Changes in the brood sizes of female guppies during chronic exposure to mercury during four parturition periods. Values are $\bar{x}$ ±1SD. A = control, G = 0,001 mg Hg^{2+}/dm^3.

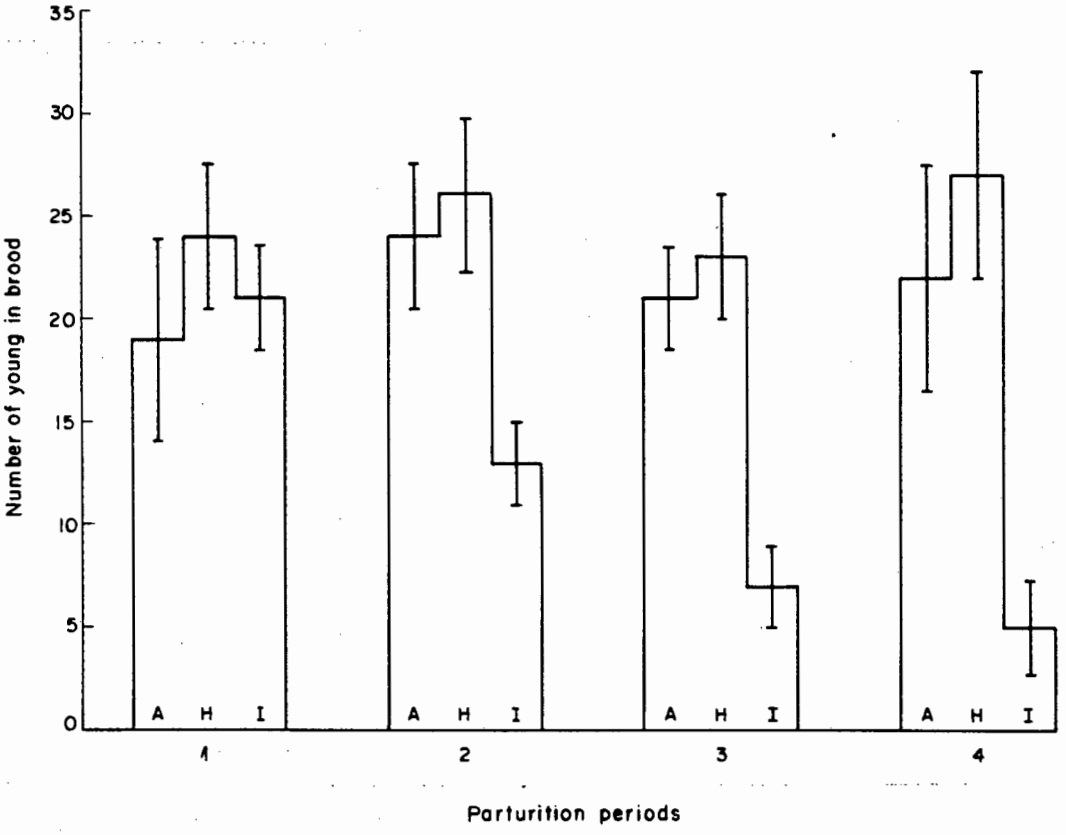

FIGURE 59. Changes in the brood sizes of female guppies during chronic exposure to phenol during four parturition periods. Values are x ±1SD. A = control, H = 0,75 mg C_6H_5OH/dm^3, I = 1,5 mg C_6H_5OH/dm^3.

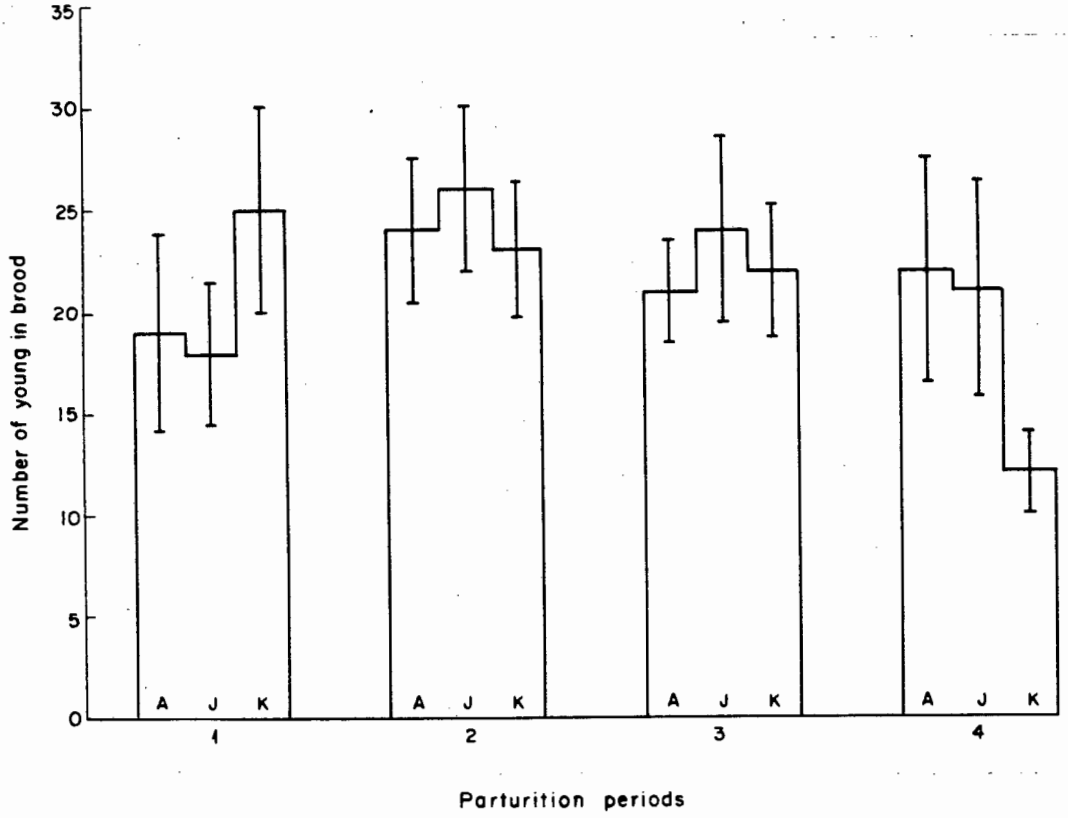

FIGURE 60. Changes in the brood sizes of female guppies during chronic exposure to ammonia during four parturition periods. Values are x ±1SD. A = control, J = 1,5 mg NH_3-N/dm^3, K = 3,0 mg NH_3-N/dm^3.

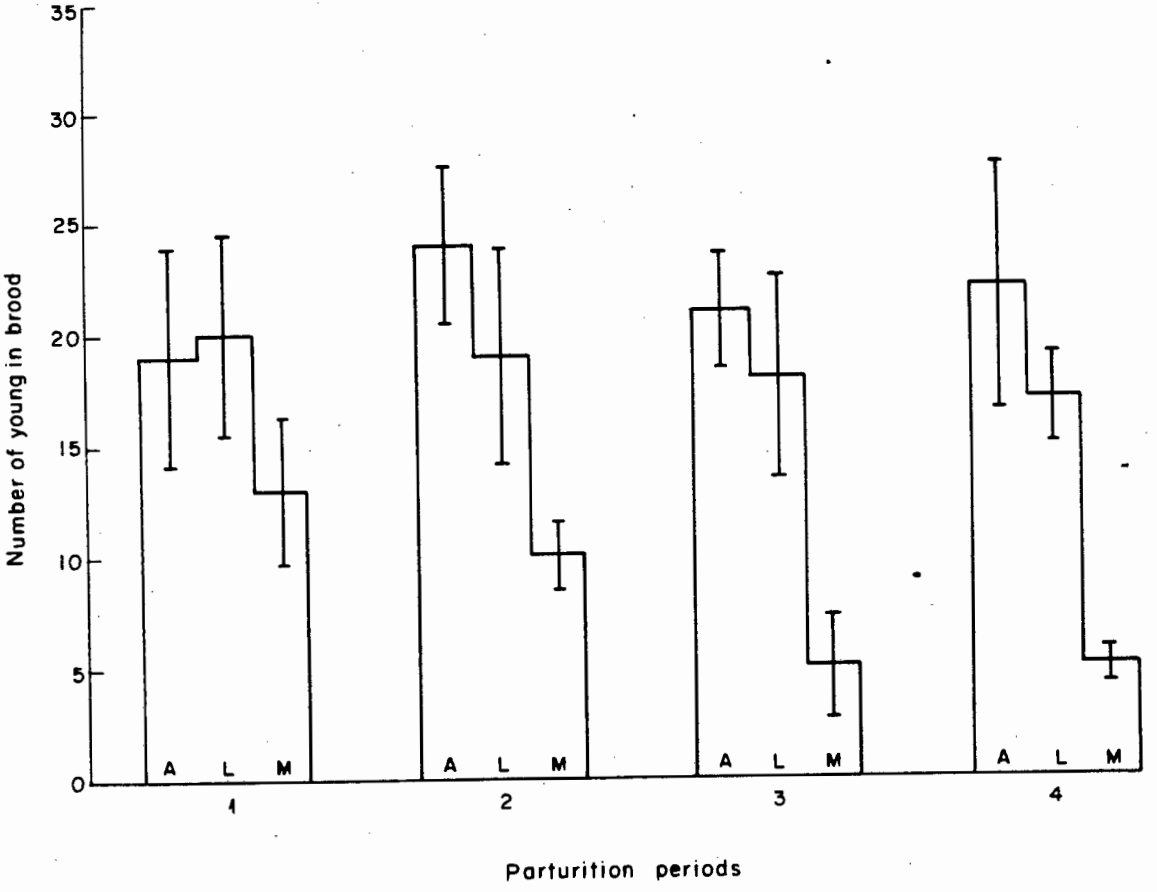

FIGURE 61. Changes in the brood sizes of female guppies during chronic exposure to cyanide during four parturition periods. Values are $\bar{x}$ ±1SD. A = control, L = 0,007 mg CN^-/dm^3, M = 0,015 mg CN^-/dm^3.

Dead young were also excluded from the determination of standard length of young at birth. Over the four parturition periods, females in the higher toxic concentrations produced significantly smaller young than control females and those in the lower concentrations. Those differences occurred as early as the first brood in females exposed to 0,01 mg Cu^{2+}/dm^3 and 0,015 mg CN^-/dm^3 (Figures 63 and 67). Females exposed to 0,1 and 0,02 mg Cd^{2+}/dm^3 (Figure 62), 1,5 mg Phenol/dm^3 (Figure 65) and 3,0 mg NH_3/dm^3 (Figure 66) produced significantly smaller young in the second brood whereas females exposed to 0,001 mg Hg^{2+}/dm^3 did not produce significantly smaller young until the fourth brood (Figure 64).

Chronic exposure, therefore, to toxicant levels of 0,02 mg Cd^{2+}/dm^3, 0,1 mg Cu^{2+}/dm^3, 0,001 mg Hg^{2+}/dm^3, 1,5 mg Phenol/dm^3, 3,0 mg NH_3/dm^3 and 0,015 mg CN^-/dm^3 significantly reduces brood size in the guppy.

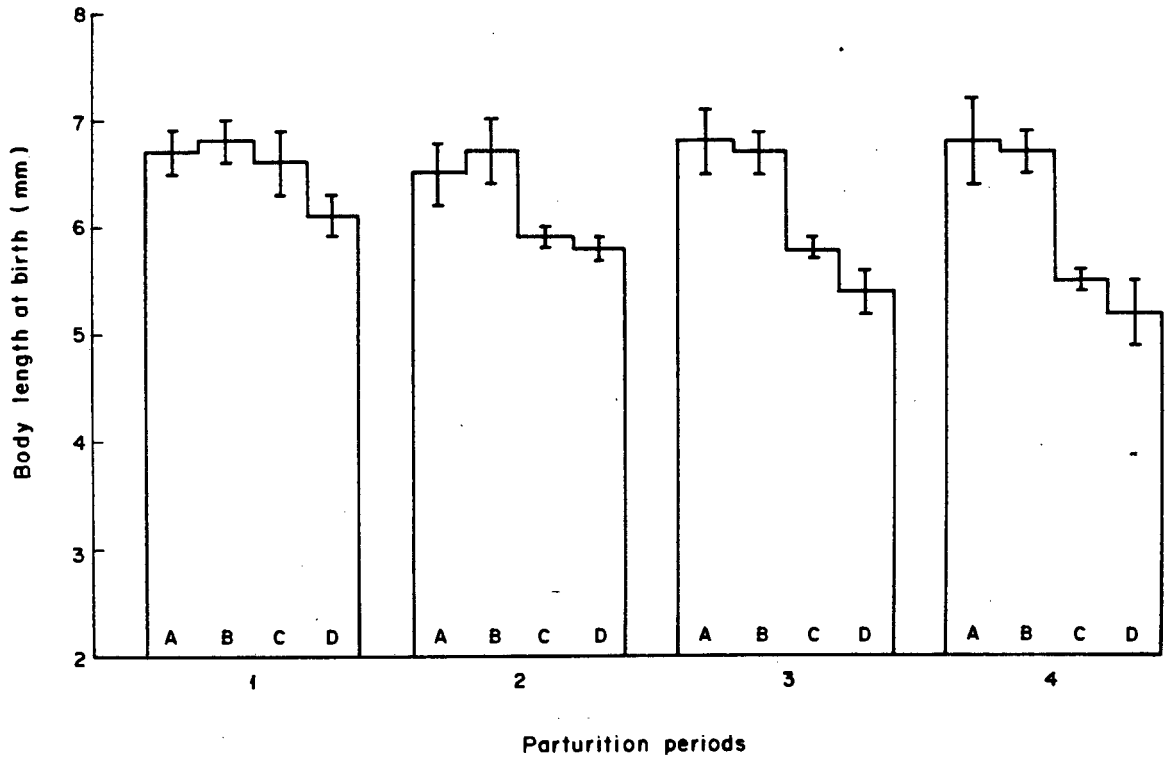

FIGURE 62. Effect of chronic exposure to cadmium on the standard length of young at birth during four parturition periods. Values are $\bar{x} \pm 1$SD. A = control, B = 0,01 mg Cd^{2+}/dm^3, C = 0,02 mg Cd^{2+}/dm^3, D = 0,1 mg Cd^{2+}/dm^3.

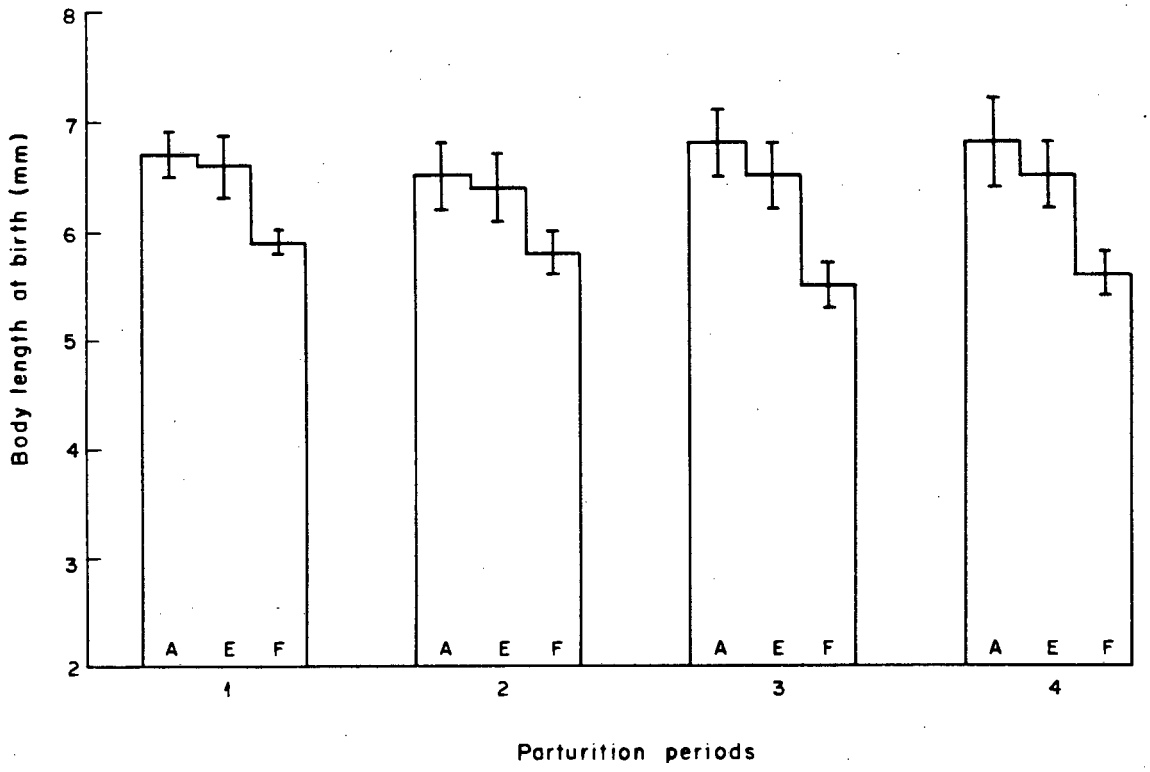

FIGURE 63. Effect of chronic exposure to copper on the standard length of young at birth during four parturition periods. Values are $\bar{x} \pm 1$SD. A = control, E = 0,05 mg Cu^{2+}/dm^3, F = 0,1 mg Cu^{2+}/dm^3.

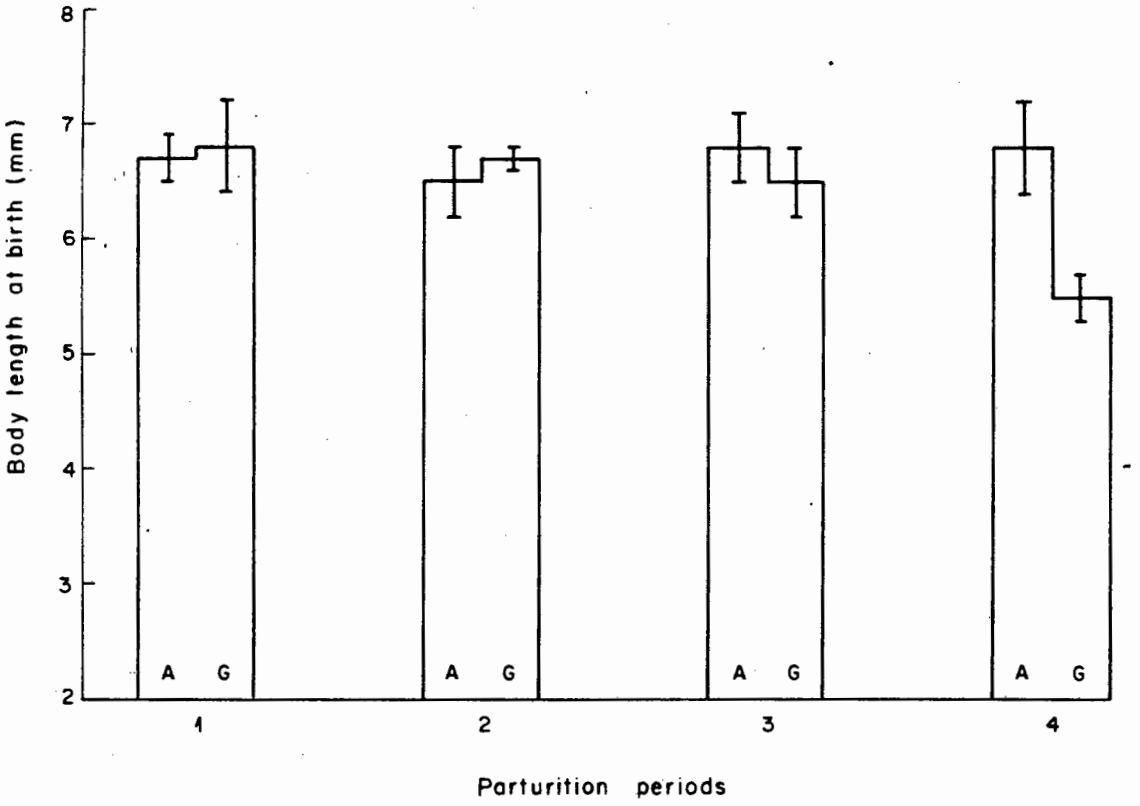

FIGURE 64. Effect of chronic exposure to mercury on the standard length of young at birth during four parturition periods. Values are $\bar{x}$ ±1SD. A = control, G = 0,001 mg Hg^{2+}/dm^3.

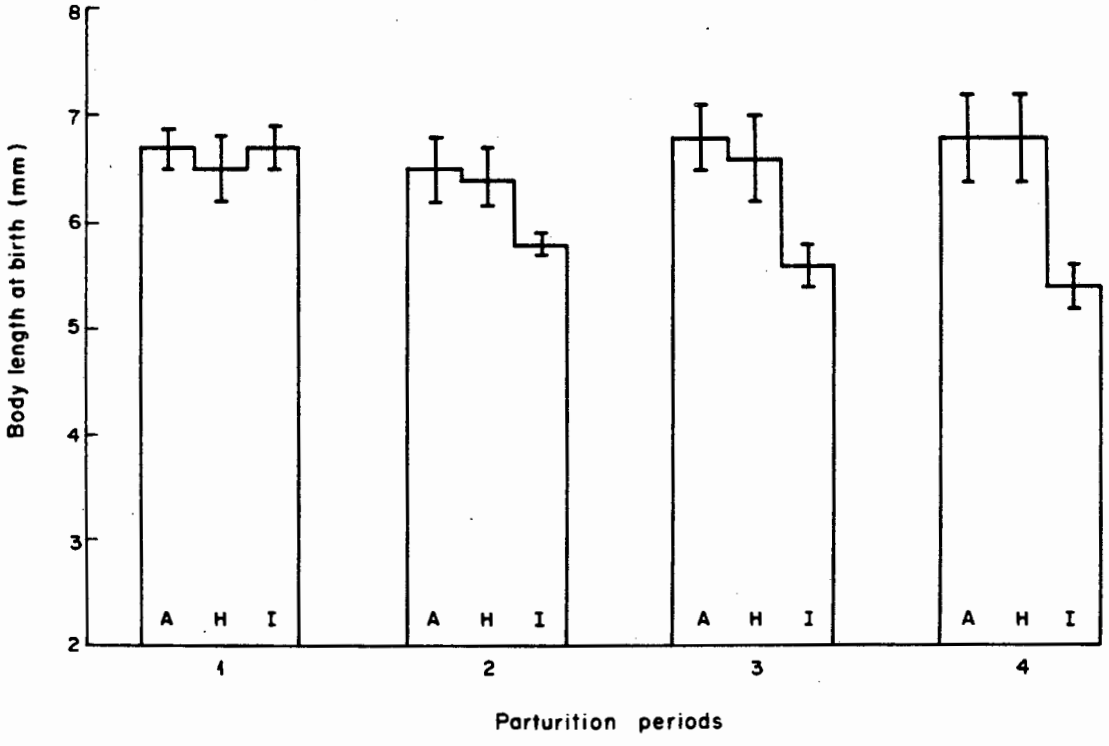

FIGURE 65. Effect of chronic exposure to phenol on the standard length of young at birth during four parturition periods. Values are $\bar{x}$ ±1SD. A = control, H = 0,75 mg C_6H_5OH/dm^3, I = 1,5 mg C_6H_5OH/dm^3.

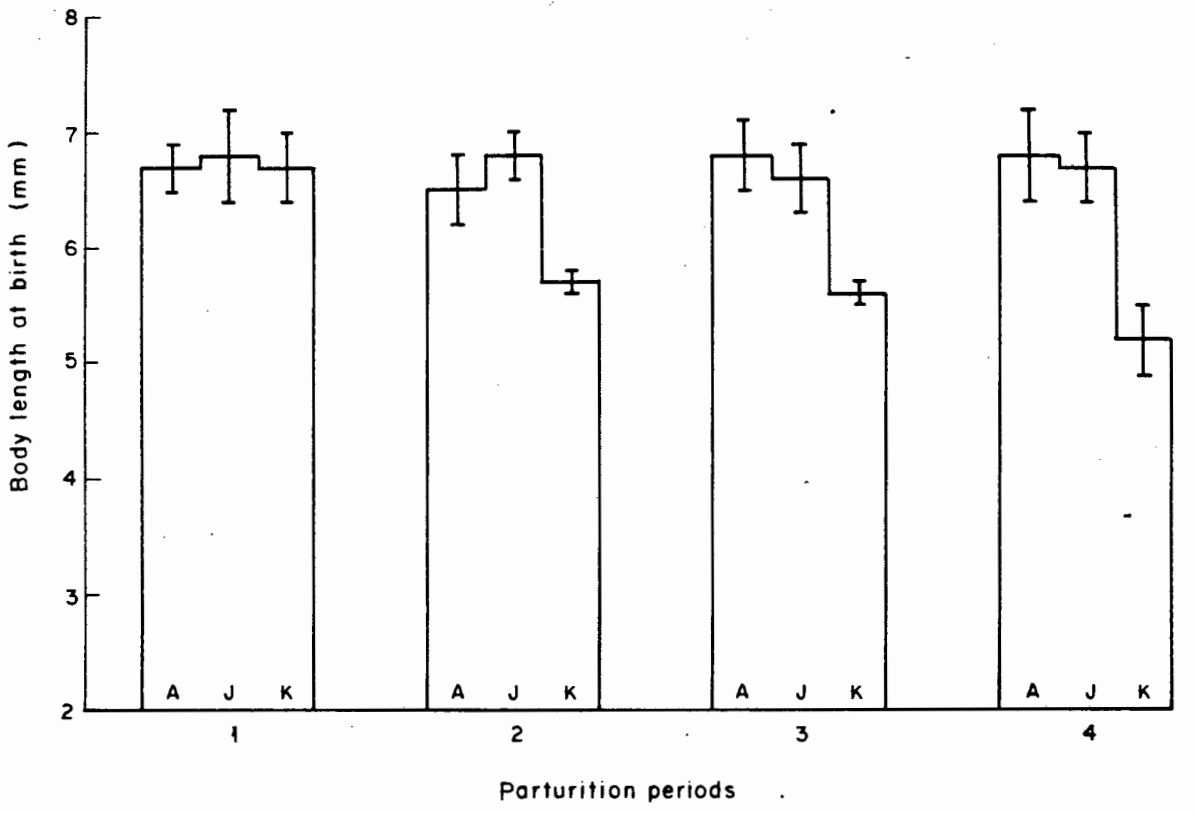

FIGURE 66. Effect of chronic exposure to ammonia on the standard length of young at birth during four parturition periods. Values are $\bar{x} \pm 1SD$. A = control, J = 1,5 mg NH_3-N/dm^3, K = 3,0 mg NH_3/dm^3.

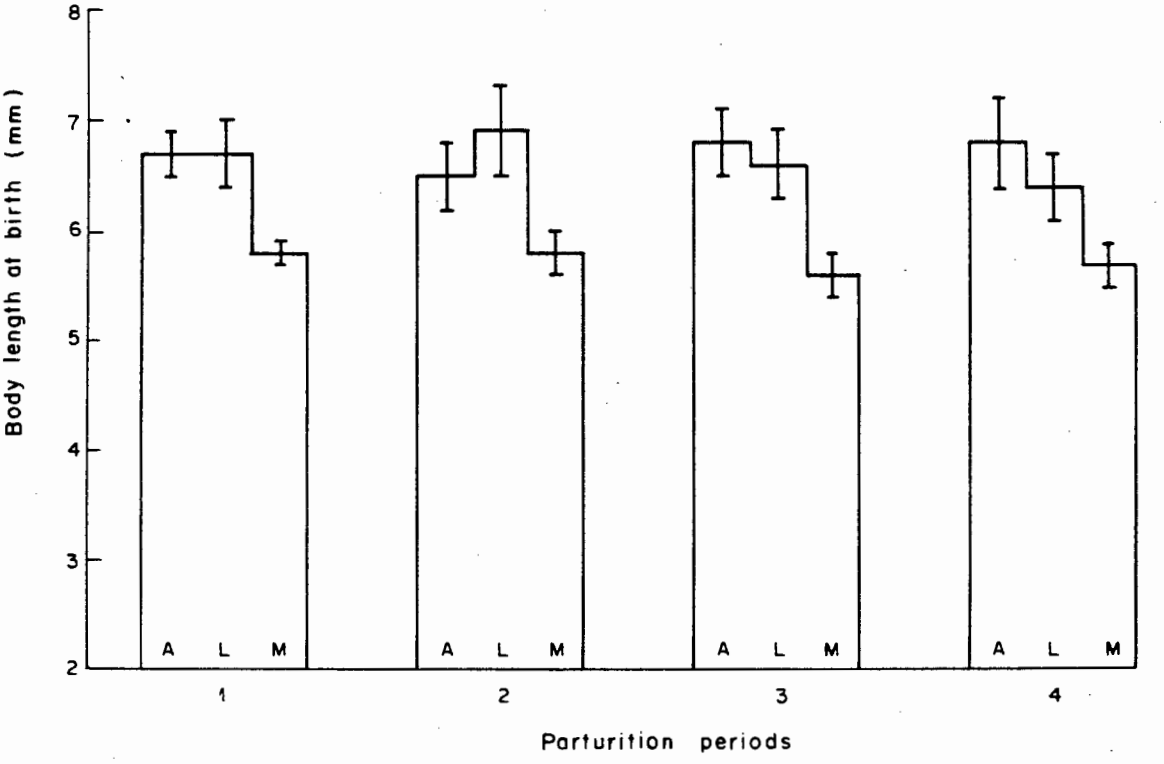

FIGURE 67. Effect of chronic exposure to cyanide on the standard length of young at birth during four parturition periods. Values are $\bar{x} \pm 1SD$. A = control, L = 0,007 mg CN^-/dm^3, M = 0,015 mg CN^-/dm^3.

The brood sizes recorded for test fish were not unusual and can occur under normal conditions (Rosenthal, 1952). The unusual feature was that most of the fish exposed to these concentrations consistently produced smaller brood sizes.

A direct relationship between fecundity and the amount of food consumed has been demonstrated in the guppy (Hester, 1963). Although the present investigation did not quantify food consumption, information in the literature (Bengtsson, 1974a) indicates that exposure to sub-lethal levels of toxicants could reduce the amount of food consumed. Also Hiltebran (1971) demonstrated a phosphate 'uncoupling' effect for low levels of a number of toxicants in the mitochondria of the bluegill (*Lepomis machrochirus*) liver.

It can be seen, therefore, that chronic toxic levels could decrease energy availability in exposed fish. Chronic exposure to cadmium, copper, phenol and mercury has been shown to cause degenerative changes. The question of additional tissue repairs therefore arises in exposed fish. Further, adjustment to the presence of ammonia or cyanide could call for the establishment of some kind of detoxifying mechanism. This might be energy consuming. If energy transformation is impaired while the energy cost of metabolism and tissue repair rises, it becomes obvious that exposed fish can only budget comparatively smaller amounts of energy for non-maintenance purposes including reproduction.

Nutrients required by the developing embroys for maintenance metabolism come from the mother guppy (Scrimshaw, 1945). If the energy transformation of the mother is impaired, the amount of nutrients made available to the young and the number of young she can successfully support could be reduced. In the present investigation, some abnormal young were produced by exposed females. Apart from being dead, these were smaller, had not uncurled from the embryonic posture and the yolk had not been completely absorbed. Since superfetation does not occur in the guppy, it is probable that the toxicants had the effect of slowing down, arresting or even reverting (dedifferentiation) development. As Rosenthal and Alderdice (1976) have pointed out, arrested development could result "if the available energy budget is sufficient only for

maintenance of cells in their current state of organization". One would, therefore, expect dedifferentiation to occur if the available energy is not sufficient to maintain the tissues at that state of development.

The developing young could accumulate toxicants from the mother during oogenesis or later in development, because of the intimate vascular connection between mother and embryo (Scrimshaw, 1945). If the levels are sufficiently high, the toxicants could have exerted the same 'uncoupling' effect that Hiltebran (1971) has shown in the bluegill.

Decreasing energy availability in the developing guppy resulting from one or more of the factors mentioned above could also account for the decreased size of young at birth in the exposed fish.

The present investigation indicates that the effect of chronic intoxication on the size of young at birth is a very sensitive parameter, occurring as early as the first brood. The interval between broods is the least sensitive probably because of the large variation that guppies show in nature (Yamagishi, 1976) with respect to this parameter.

CONCLUSION

The median lethal concentration (LC50) is a convenient reference point for expressing the acute lethal toxicity of a given toxicant to the average or typical test animal. Obviously it is in no way a safe concentration, although occasionally the two have been confused. Safe levels, which permit reproduction, growth and all other normal life processes, in the fish's natural habitat, have been shown, in the present study, to be much lower than the LC50.

Substantial data on long-term effects and safe levels are available for only a few toxicants. Information is now accumulating on the effect of toxicants on reproduction, an important aspect of all long-term toxicity tests. Other information is being gathered on sub-lethal effects on growth, performance, avoidance reactions and social behaviour of fish. Also important is the sensitivity of organisms at various life stages. Many organisms are most sensitive in the larval or fry stage: some are more sensitive in the egg and sperm stage.

It would be desirable if a single, universal, rapid, biological test could be used to measure directly sub-lethal effects of a pollutant. The present study suggests that continuous monitoring of respiratory and locomotory performance could be usefully employed in this respect. However, in a review of sub-lethal responses of fish, such as "coughing", swimming speed, avoidance behaviour, specific physiological and biochemical changes in various organisms, and histological changes, Sprague (1971) considered no single test to be meaningful for all kinds of pollutants and recommends that routine assessment and prediction of safe levels be made by carrying out bioassays for acute lethal toxicity and multiplying the lethal concentration by a suitable application factor.

Nevertheless, it is important, within the confines of the present investigation into the effective use of real-time monitoring systems, to appreciate the level of control for sub-lethal toxic effects which may be achieved, and to judge whether or not this control is sufficient for long-term ecosystem protection.

AN ULTRASONIC DETECTOR FOR MONITORING LOCOMOTORY ACTIVITY IN SMALL FISH

Although laboratory experiments and field observations have indicated that the effects of certain environmental contaminents on the behaviour of aquatic organisms can be ecologically significant, these effects have rarely been used as criteria for establishing specific effluent standards. This situation is due in part to the lack of sufficient methods to quantitatively measure behavioural effects of pollutants. This shortcoming has critically hampered the incorporation of laboratory data into water quality criteria and has emphasized the need for long term methods of monitoring. The purpose of this section is to describe the development of a system to monitor activity in fish which minimizes the disadvantages in the recording and analyzing of swimming behaviour experienced with previous models; to describe how this parameter was quantified and to provide examples of how it has been used to document changes in the swimming behaviour of small fish, caused by alterations in water quality due to lethal and sub-lethal toxicant applications.

DESIGN PHILOSOPHY

To the biologist wishing to observe continuously and objectively the activity of captive fish the range of methods available is rather limited. Visual observations cannot be made in darkness, are difficult for long periods and often very subjective. Oxygen consumption can at best give only long term averages. Mechanical tethers restrict free movement. Light ray systems, where the interruption of light beams to various photocells are counted, make it impossible to subject the fish to a normal day-night cycle. The utilization of infra-red light beams, to eliminate the effect of visible light on exogenous rhythms during darkness, has proved effective, but the nature of the system precludes its use with very small fish.

An ultrasonic method is free from most of the drawbacks of previous methods, but provides many challenging problems of its own.

Cummings (1963) has described a system operating on the Doppler effect. Sound waves, reflected from a moving object, are shifted in frequency by a fraction which is dependent on the ratio of the linear speed of the

object to the velocity of propagation of sound in the medium used. This effect is fairly easy to visualize if the object is moving along the direction of a sound beam either toward or away from an observer, but in an enclosed space with reflecting walls, like a tank, sound waves fall on the object from all directions and give rise to Doppler shifts of widely varying magnitude. For aural observation of sudden movements this may yield useful results. For a recording system designed to give a numerical value which is directly proportional to total activity a design philosophy based on phase shift as described below leads to a better understanding of the problems involved.

GENERAL DESCRIPTION

The system operated at 200 kHz which was assumed to be beyond the upper limit of hearing of the small sensor fish. A small piezo electric transducer used as transmitter sets up a continuous sound field within an enclosed chamber and an identical transducer oriented for a minimum direct signal pick-up operates as receiver (Plate 8).

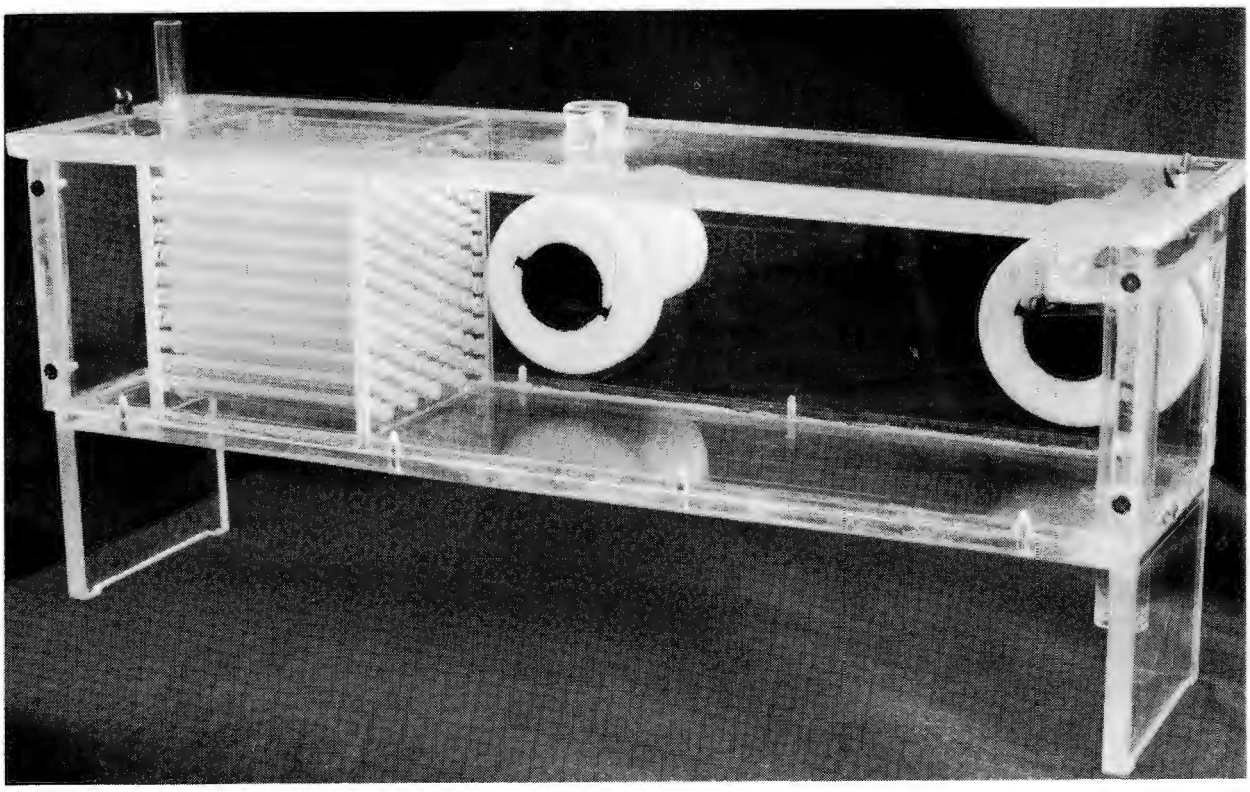

PLATE 8. Sensor fish activity detection chamber (500 mm long x 100 mm wide x 100 mm deep) incorporating one ultrasonic transmitting element and one ultrasonic receiving element.

As the walls and bottom of the chamber and also the water surface were highly reflective, part of the transmitted energy eventually reached the receiver. This signal may be regarded as a combination of numerous "sound beams" arriving from many directions, each with a definite amplitude and phase with respect to the original transmitted signal. If the sound field is stationary, the received signal will be constant in amplitude and phase; a change in any of these sound beams would, however, alter both amplitude and phase of the received signal relative to the transmitted signal. To extract the desired information the signal was amplified and limited so that only the phase changes remained. These were transformed into a linear phase detector (Figure 68) where a bi-stable circuit was switched ON by a pulse from the

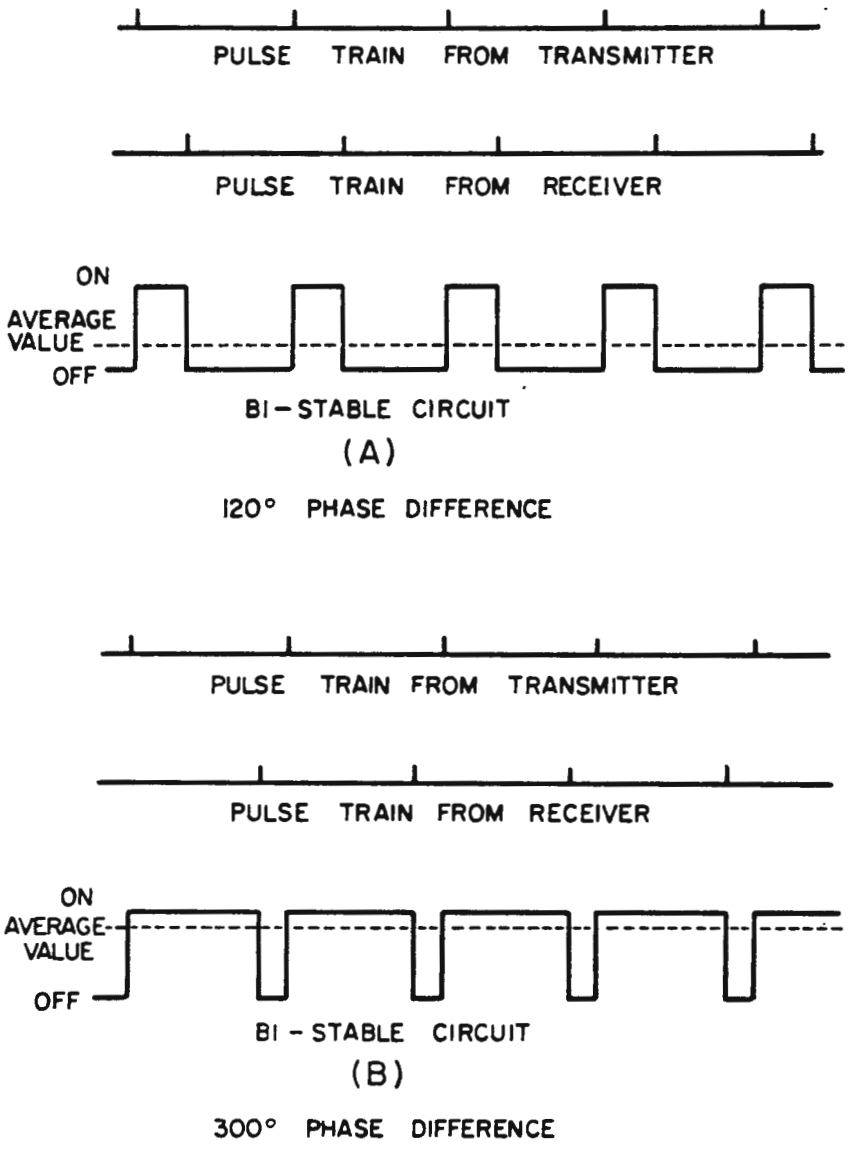

FIGURE 68. Operation of phase detector.

transmitter and OFF by a pulse derived from the received signal. The average value of the output obtained after integration was linearly proportional to phase difference (Figure 69, A).

Phase deviations around the average phase difference yielded an AC voltage component which was a measure of the disturbance in the sound field as illustrated in Figure 69,B.

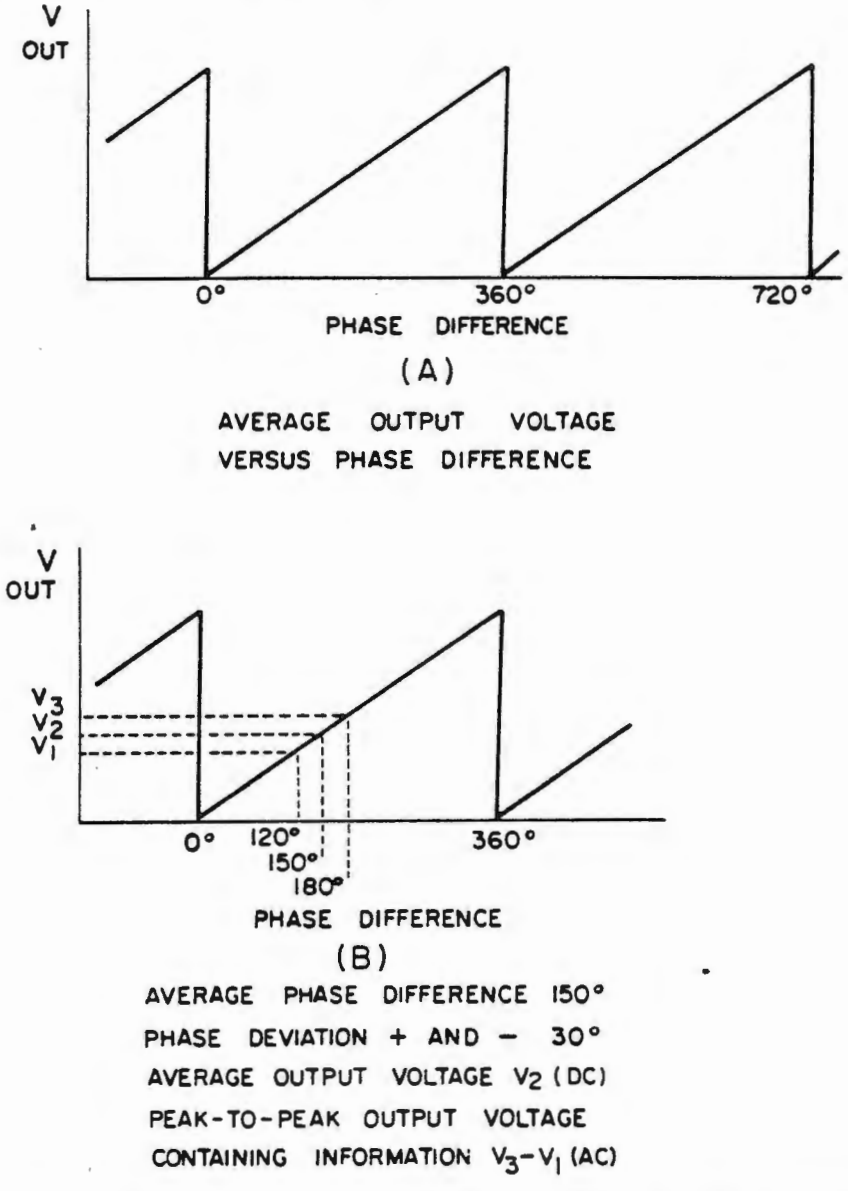

FIGURE 69. Phase detector output voltage versus phase difference.

This signal was AC coupled to the input of a counter which advanced every time the phase deviation caused the voltage applied to it to exceed both hysteresis limits of its input circuit.

The monitoring equipment (Plate 9) was constructed to provide a capacity for assessing the activity levels of six fish maintained in separate chambers. Each movement made by a fish was counted and stored in one of six counters. At the end of every hour the accumulated activity count for each fish was printed out. The count could also be read at any time from a digital display. In addition, each channel had the facility for a response threshold to be set. Whenever the accumulated count for a particular channel exceeded its set threshold, a response light was activated and the alarm condition was indicated on the next hourly print-out.

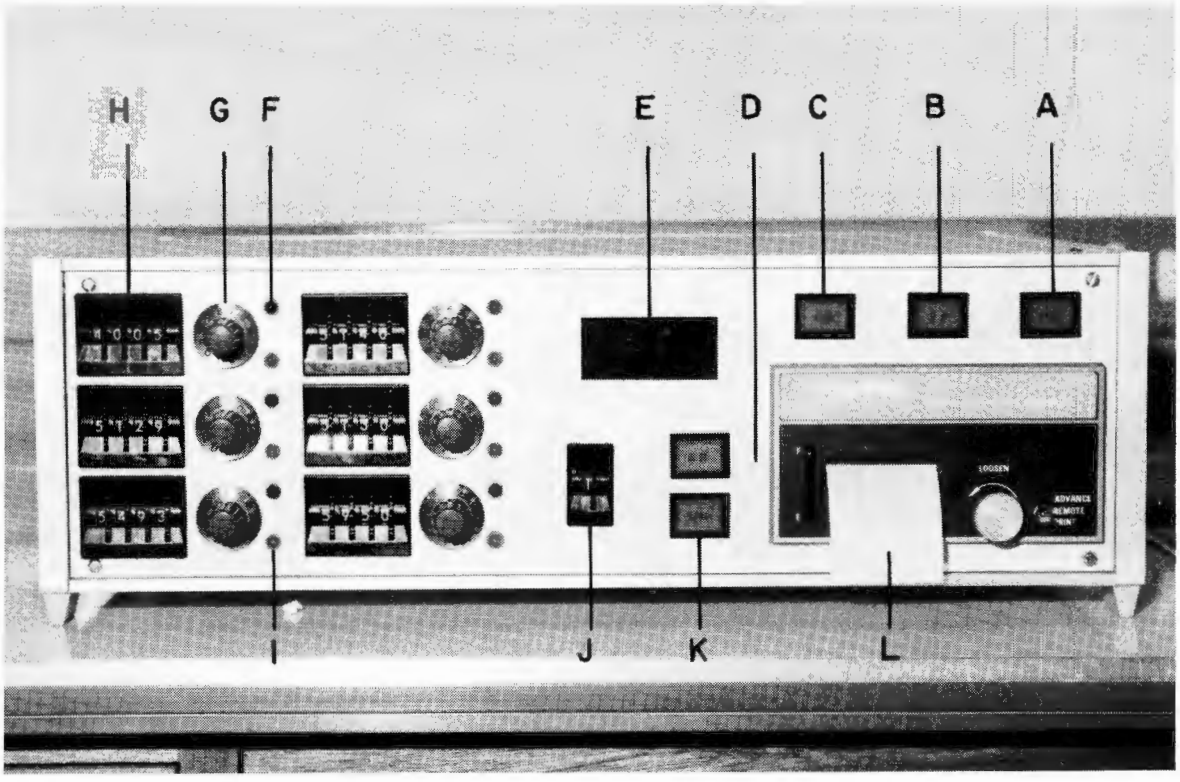

PLATE 9. Electronic monitoring unit. A - on/off power switch, B - printer on/off switch, C - manual printout control, D - alarm reset control, E - LED visual display of activity rate, F - alarm light, G - sensitivity control, H - 4 decade alarm threshold control, I - signal level indicator, J - channel selector switch, K - Master reset switch, L - printer.

CIRCUIT DESCRIPTION

BLOCK DIAGRAM

A transmitter driver circuit supplied each transmitter with a 200 kHz triangular wave (Figure 70). The signal from each receiver was amplified, filtered, detected and used to increment a counter. A magnitude comparator compared the content of the counter with a pre-set threshold. Whenever this threshold was exceeded, an alarm signal was generated. The content of the counter, the alarm status and a B.C.D. digit from one to six, identifying the channel, was held in a data buffer. When the data for any particular channel was required for display and print-out, the contents of the buffer were gated onto a data bus. The data then appeared on the display and was printed out. Any channel could be read, at any time, by selecting the appropriate number with the "channel select" thumbwheel switch, and the data printed out if so desired. Once every hour, the six channels were read and printed out sequentially. A timing and control circuit provided the timing pulses required to accomplish this.

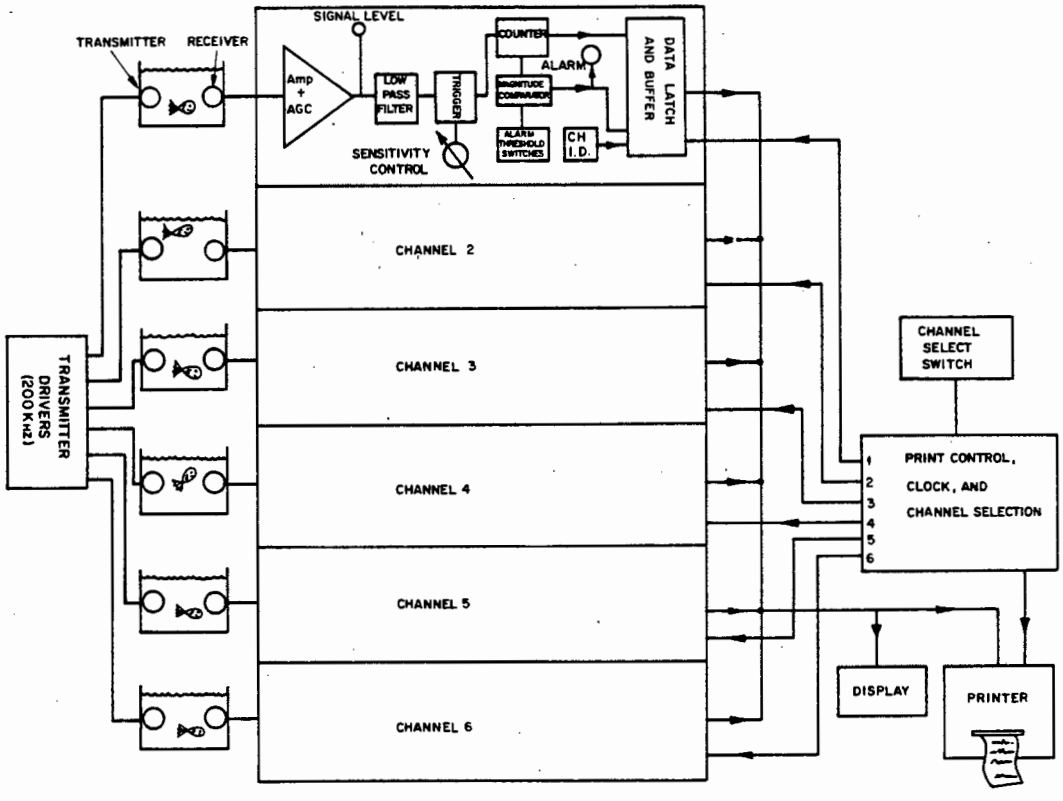

FIGURE 70. Block diagram.

SIGNAL PROCESSING BOARD

There were six of these boards, each one providing analogue processing, counting, alarm and identification facilities.

The analogue section is shown in Figure 71. IC15, Q1, D1 with associated components formed an amplifier, detector and automatic gain control. The a.g.c. compensated for any variations in signal level caused by differences in the ultrasonic transducers, temperature changes etc. The Doppler frequency, with the chambers and fish used, was found to be 10 hz or less. R10, R11, C5, C6 and part of IC14 comprised a low pass filter with a 10 hz cut-off frequency. This improved the signal to noise ratio by limiting the band width of the system to no more than was required. This filter was followed by a stage of amplification. Two sections of IC14, together with a sensitivity control on the front panel and associated components, formed a trigger circuit, which activated a monostable circuit, IC16, which provided a 1-s pulse every time a fish movement caused a signal which exceeded the sensitivity setting. The 1-s pulse from IC16 enabled the circuit to reject any further counts within that period, thus preventing erroneous counts due to minor tail movement or other sources. Q2 caused a yellow LED on the front panel to be illuminated as long as the carrier signal level of the respective channel was sufficient for reliable operation. The cables connecting the transducers to the monitor were fitted with co-axial plugs which mated with similar sockets at the rear panel of the monitor.

The digital section is shown in Figure 72, IC12 and IC5 formed a four digit B.C.D. counter. The pulses from the analogue section incremented this counter. The B.C.D. data was fed to latch/buffers IC3 and IC1. The counter outputs were also connected to magnitude comparators IC6, IC7, IC8 and IC9, where the accumulated count was compared with the alarm threshold for that particular channel, which was set using thumbwheel switches located on the front panel. IC10 and IC11 contained pull-down resistors for the thumbwheel switch data lines. Whenever an alarm condition was sensed, the signal from IC9 pin 13 set the latch formed by half of IC13. Thus the alarm condition remained active until reset from the front panel. Q3 activated the alarm LED on the front panel. The alarm signal was also taken to data latch/buffer IC1. A

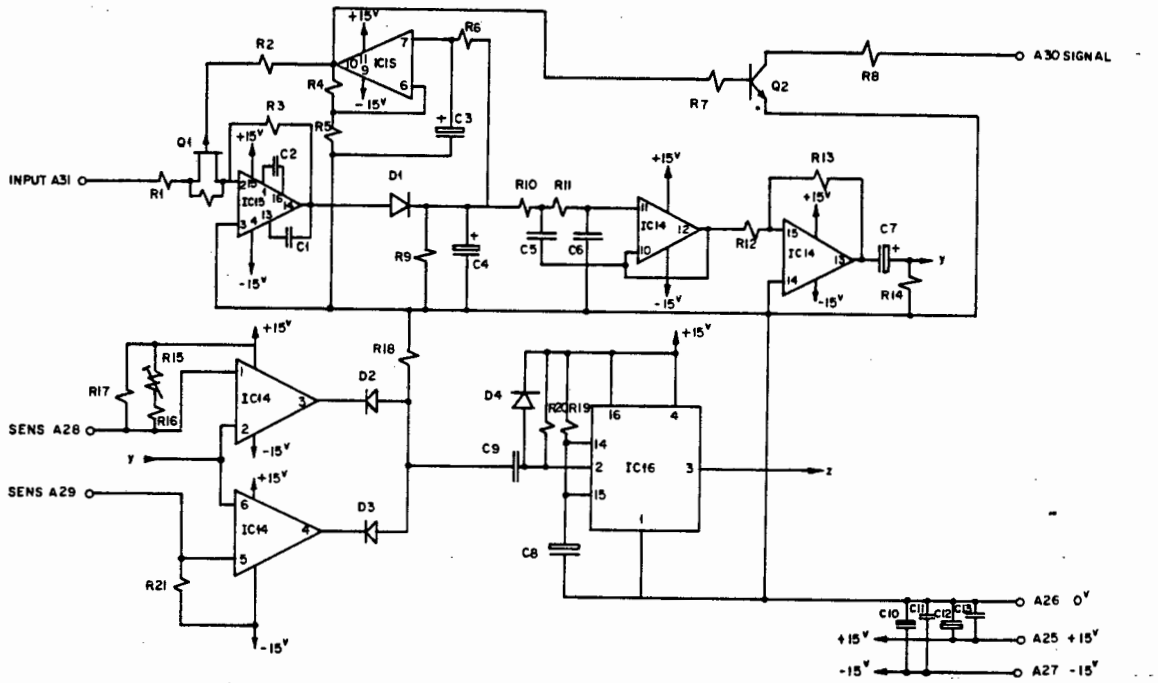

FIGURE 71. Signal amplifier and trigger.

B.C.D. digit from one to six, corresponding to the appropriate channel number, was also hard-wired to IC2. This digit identified the channel on the print-out. A master reset signal (MR) reset the counters every hour after the data had been printed out.

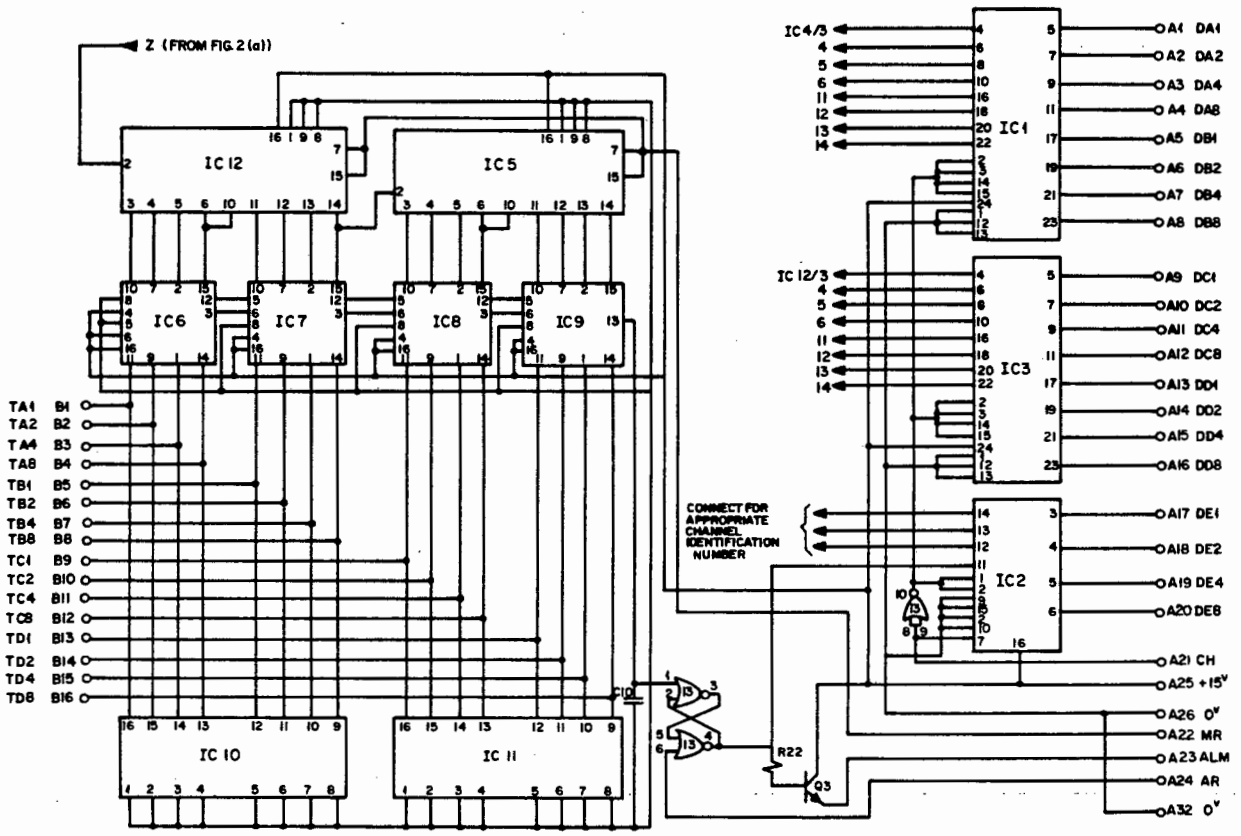

Figure 72. Counter, alarm and data buffer.

SEQUENCER BOARD

This board contained the clock, the print control circuitry, the display/printer interface and the transmitter drivers (Figure 73).

IC15a, IC8, IC9 and IC10 formed the clock. The 100 Hz input signal was derived from the power supply. IC15a was a monostable circuit which provided a squaring and holdoff function to eliminate any interference. A pulse was generated once an hour at IC10 pin 15 and two pulses per second at IC8 pin 12.

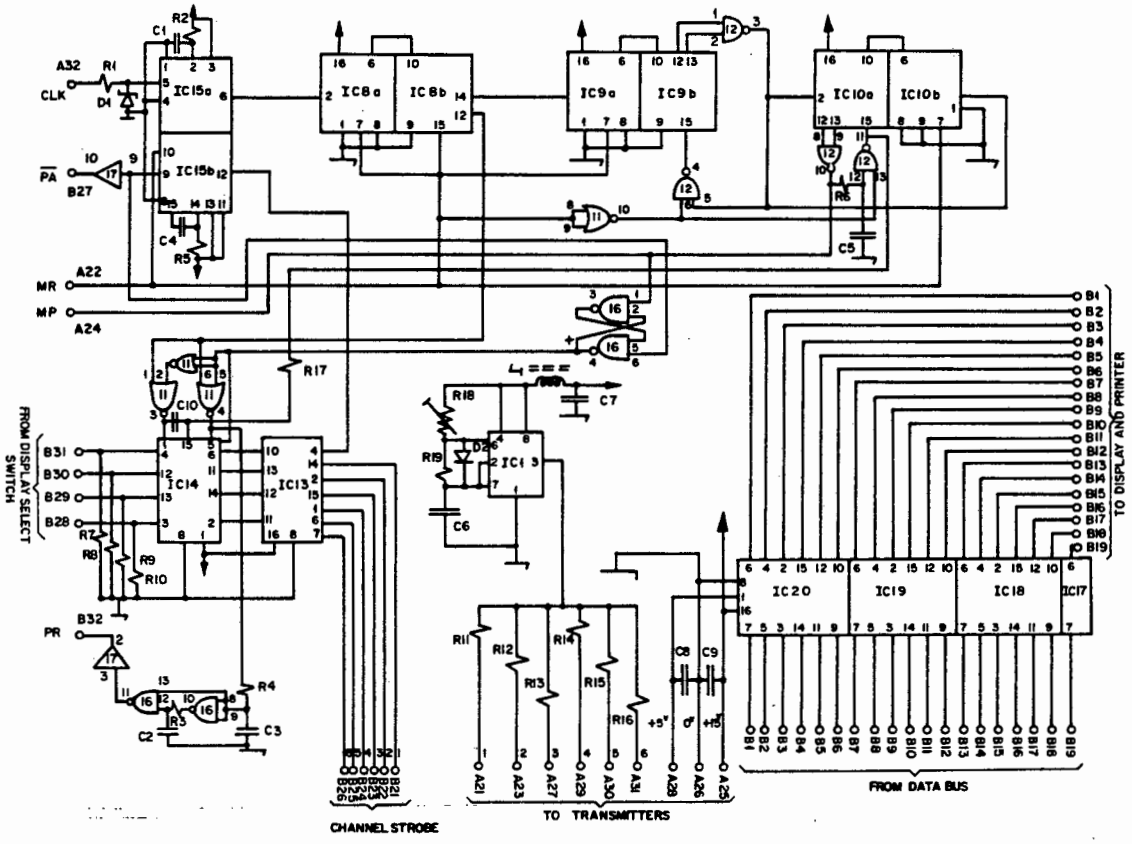

FIGURE 73. Sequencer board.

IC14 and IC13 caused the data of the various channels to be placed on the data bus at the correct time. Between the hourly printouts, the data present on the four lines coming from the "channel select" thumbwheel switch activated one of the channel strobe lines to cause the data of that channel to be placed on the data bus. When the hourly pulse occurred, the 2 hz pulses were gated to IC14 as well as a short reset pulse. IC14, therefore, counted up from zero, activating each channel strobe line in turn. Thus the data from the six channel boards was placed sequentially on the data bus.

Print (PR) and paper advance (PA) signals were also generated by the circuit to cause a sequential printout. A manual print (MP) input was also provided from a front panel switch, so that a full printout could be obtained without waiting for the next automatic hourly printout.

IC18, IC19 and IC20 were level shifters which converted the +15 volt CMOS levels from the data bus to +5 volt TTL levels for driving the digital display and printer.

IC1 and associated components formed a 200 kHz oscillator. This signal drove the six ultrasonic transmitters through current limiting resistors.

DISPLAY AND PRINTER

The TTL compatable data signals from the level-shifting interface were fed to the display board which was mounted behind the front panel. This board contained four decoder/drivers (IC1, IC2, IC3, IC4) and four seven segment LED display modules (Figure 74).

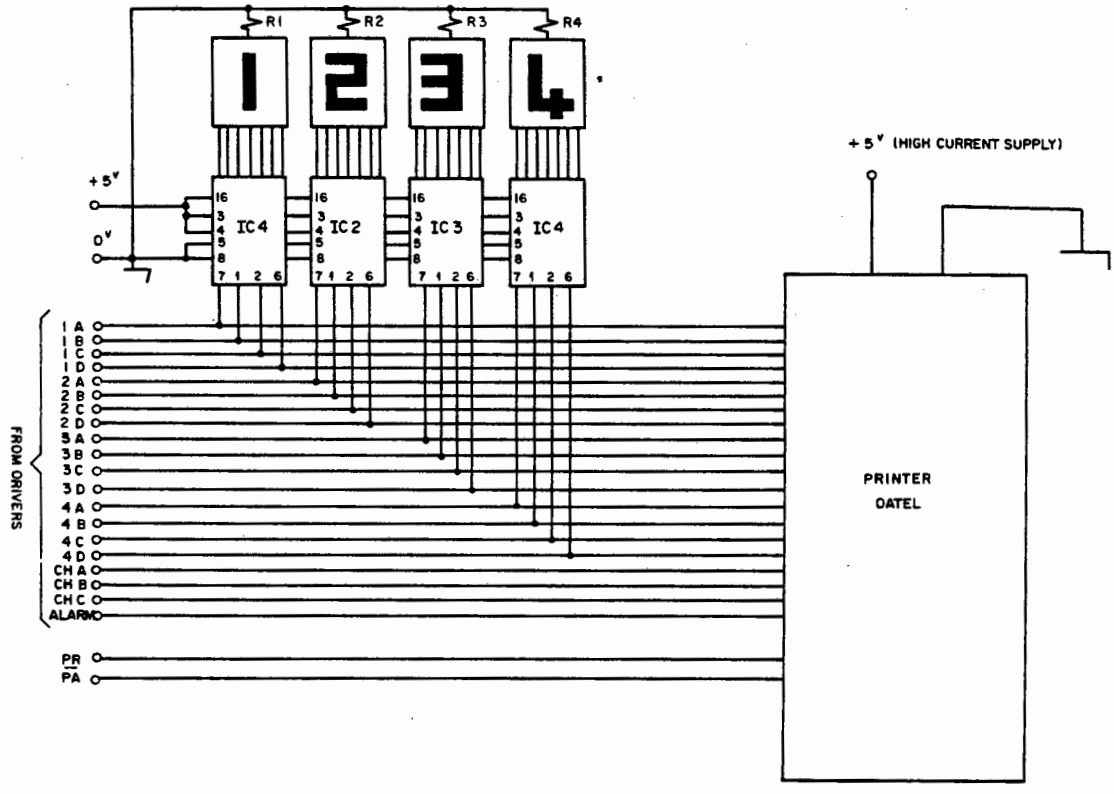

FIGURE 74. Display and printer.

The same data signals were also fed to the printer data inputs, together with the two printer control signals PR and PA.

POWER SUPPLY

The circuitry was straightforward, making use of integrated regulators for the +12 volt and -12 volt supplies (Figure 75).

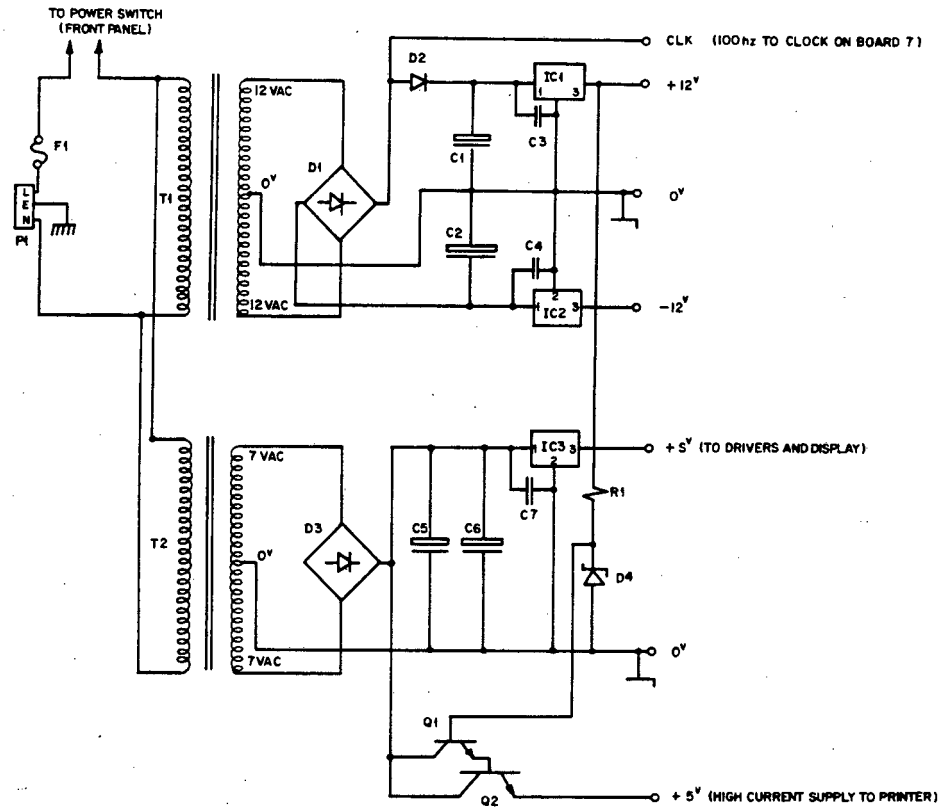

FIGURE 75. Power supplies.

The high current supply for the printer was regulated to +5 volts by a simple transistor regulator. The 100 Hz signal for the clock was also derived from the power supply, and was fed, via the "printer on" switch on the front panel, to the sequencer board.

The 230 volt power was connected to the power supply through an interface filter P1. This filter was an integral part of the mains socket at the rear of the equipment.

FRONT PANEL WIRING

Figure 76 details the wiring of the front panel controls and shows how they were connected to the rest of the circuitry.

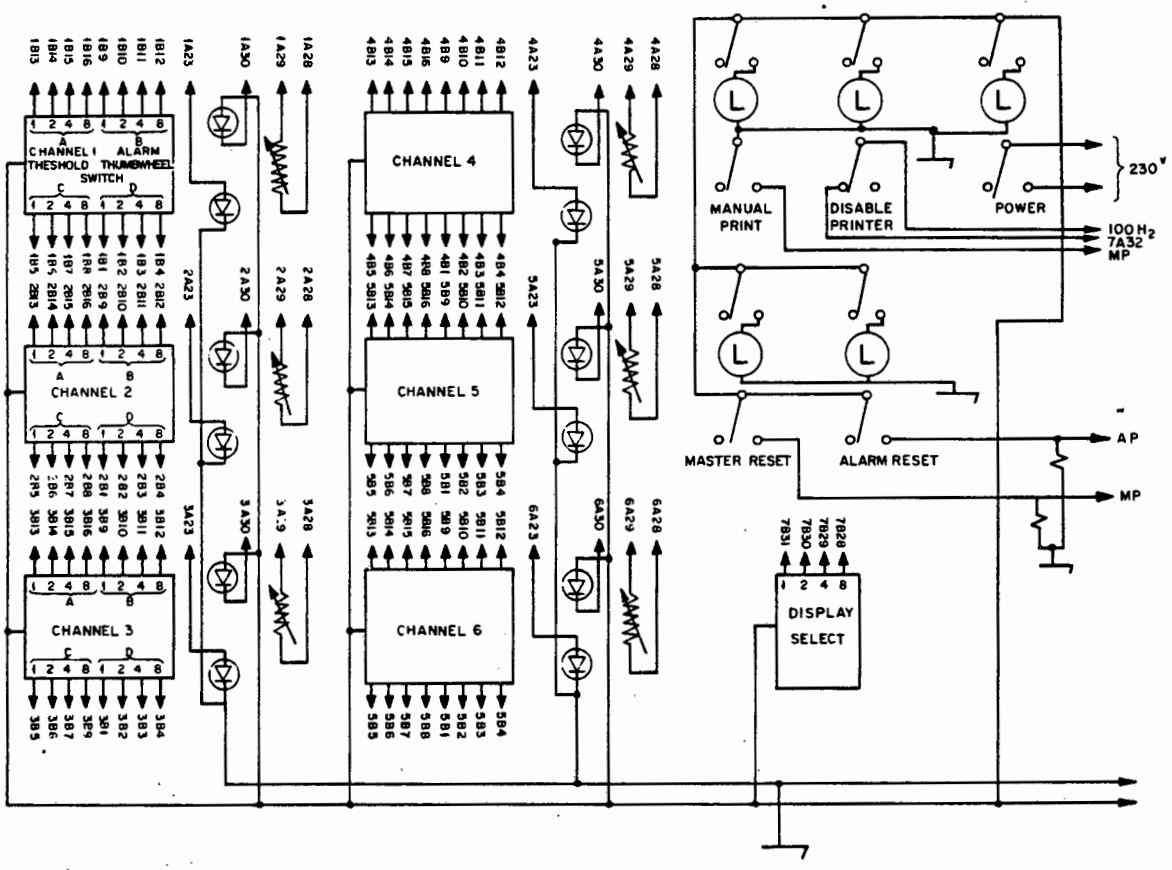

FIGURE 76. Front panel.

PRELIMINARY STUDIES ON THE USE OF THE ULTRASONIC FISH ACTIVITY MONITOR TO DETECT WATERBORNE TOXICANTS

Eighteen experimental analyses were conducted to determine the efficacy of the system in providing for an adequate advance warning of developing toxic conditions. The investigation was conducted at laboratories of the University of Cape Town, thus, providentially, providing an opportunity of confirming the fact that this system would perform as effectively in a marine environment as in freshwater. The external electrode technique for fish opercular rhythm measurement, previously described, is not effective in marine systems. The ultrasonic method for monitoring fish activity could, therefore, prove advantageous in this respect.

EXPERIMENTAL PROCEDURE

The sensor fish employed was the Silverside (*Atherina breviceps*), a species of mullet found only in South Africa from Port Nolloth round the Cape to Natal. The adult has an average length of 80 mm . The individuals used in these determinations were between 50 and 60 mm long and were netted from a single shoal trapped in a large rock pool, at Muizenberg, at low tide. Sensor fish were maintained in a stock holding laboratory for two weeks prior to experimentation, and only individuals showing no signs of ill health were exposed to intoxication.

Individual sensor fish were exposed to one of the three concentrations of the six toxicants studied, in order to determine the effects of both lethal and sub-lethal intoxication upon fish locomotor activity in sea water. An analysis of the data showed that the sensor fish portrayed similar sources of variation to those exhibited by freshwater species, being individual and diurnal variation. Therefore, the same statistical appreciation of locomotory behaviour was used as in previous experimentation with freshwater species. To allow for individual variation each fish served as its own control and to preclude diurnal variation it was decided to use the maximum 95% confidence limit of average hourly activity levels as the critical limits to provide response thresholds.

Sensor fish were established individually in the activity chambers, which were screened from other laboratory activities, in static sea water at a pH of 7,8 and a diurnal temperature which varied between 16 and 23 °C . They were allowed to acclimate to enclosure within the chambers for 48 h before any records were made. Normal locomotor activity levels were recorded, for each sensor fish, for five days after they had recovered from the initial stress of being enclosed within the chambers. Hourly records of activity levels were used to calculate mean and standard deviation values for each hour of the day, and utilized to establish maximum 95% confidence limits for activity. These values were regarded as being the response levels for the sensor fish concerned. The response levels were set independently for each channel, using the alarm threshold controls. During experimental toxicant addition each sensor was deemed to have responded to the changed environment as soon as its channel alarm circuit had triggered, indicated by a red warning light on a particular channel. Toxicant levels were established in the chambers by the addition of predetermined volumes of 1 g/dm^3 solutions, in such a way as to limit the amount of disturbance to the fish to a minimum. Each sensor was exposed for a period of 48 h or until death ensued.

DISCUSSION OF RESULTS

Figure 77 depicts the effect of exposure to a nominal concentration of 0,1 mg/dm^3 of cyanide upon the normal activity pattern of a sensor fish. As previously stated, locomotor activity differed in individual fish during different periods of the day and also between one fish and another. However, the normal activity pattern presented in Figure 77 may be regarded as a typical example of that shown by all sensors. A low level of activity was maintained throughout the night, rising sharply during the dawn period. A fairly high level was maintained throughout the day, peak activity occurring just before dusk. The locomotor activity response to lethal intoxication was also a fairly typical reaction. A considerable increase in activity was observed almost immediately. Thereafter a high level of activity was sustained until death.

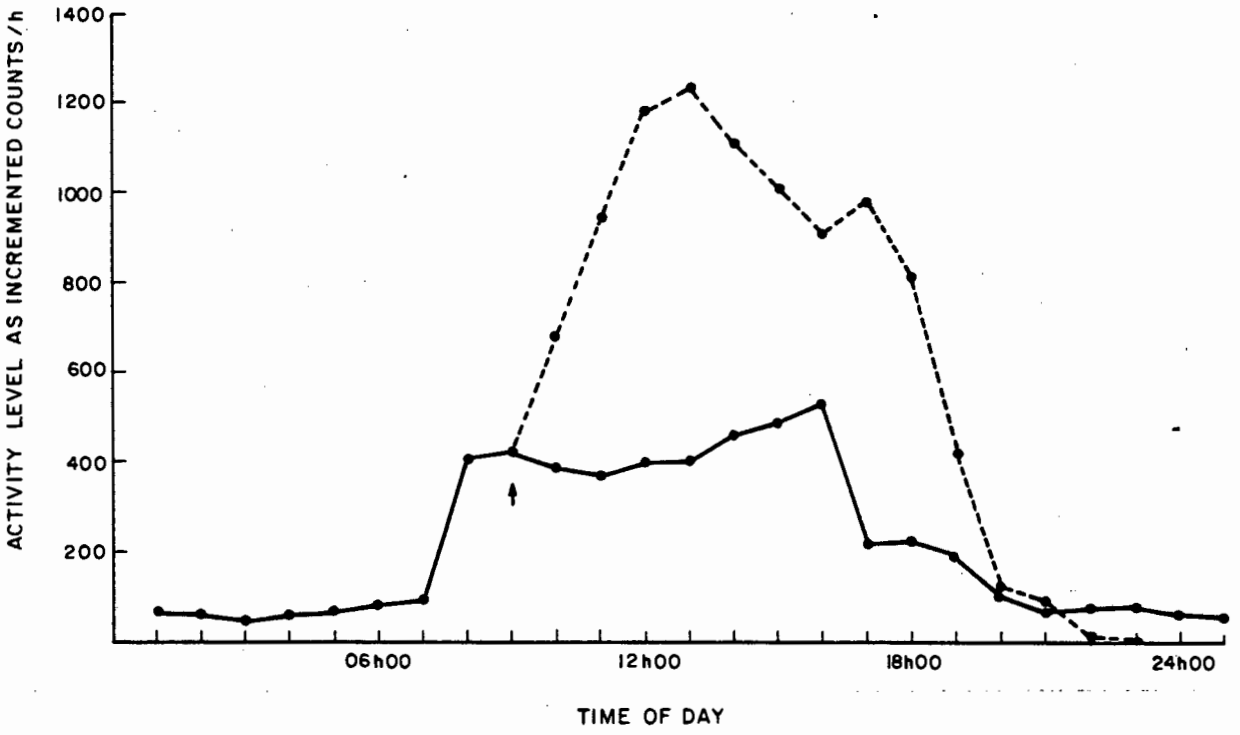

FIGURE 77. The effect of 0,1 mg CN^-/dm^3 (dotted line) on the normal activity pattern (continuous line) of *Atherina breviceps*.

Both 1,0 and 0,5 mg/dm^3 of *cadmium* proved lethal to the sensors. Exposure times to death being 24 h and 37 h respectively (Tables 65 and 66). Response to lethal cadmium exposure occurred within a very short time after initial exposure, two h and five h respectively. This species proved slightly more sensitive to lethal cadmium toxicity than *Micropterus salmoides*, although the time to response for the lower lethal concentration was similar. It is possible that the increased activity was due to a general escape reaction, on the part of the fish, on becoming aware of the physiological impairment of the respiratory apparatus.

A similar type of response occurred when sensor fish were exposed to lethal concentrations of *copper*. Sensor fish responded to both 5,0 and 1,0 mg/dm^3 within one h, and died after 33 h and 32 h exposure respectively (Tables 67 and 68). The slightly faster response to copper compared with cadmium may be due to the relatively quicker destructive effect of these copper concentrations on the gill apparatus. Cadmium is known to have a more insidious effect. The sensor fish exposed to the lower

TABLE 65. Hourly activity levels for a sensor fish before and after exposure to 1,0 mg/dm^3 of cadmium in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									870	1109	378	584	504	608	992	1896	1996	1188	1056	192	252	228	268	104
2	112	64	184	43	172	176	722	653	769	743	262	588	780	436	624	1812	1852	852	512	112	96	92	80	92
3	52	77	95	56	99	64	842	1505	1148	1101	296	540	496	448	632	1916	1868	516	516	44	136	132	140	120
4	129	270	189	111	267	159	593	1152	1268	1298	358	708	684	612	428	1496	1604	804	764	376	76	124	56	52
5	52	82	90	237	176	249	610	756	1221	1040	410	656	672	632	1128	1964	1332	776	684	296	108	96	68	48
6	60	60	146	262	189	189	834	894	*1964*	*2313*	*1812*	*1761*	*1990*	*1842*	*1666*	*2008*	*1412*	*1381*	*1368*	*942*	*809*	*408*	*643*	*304*
7	*198*	*94*	*206*	*81*	*74*	*23*	*4*	*8*																
a	81	110	141	142	181	167	720	992	1055	1058	341	615	627	547	761	1817	1730	827	706	204	139	134	122	83
b	37	90	47	102	59	67	118	342	222	201	61	66	124	97	289	188	264	240	224	134	65	55	88	32
c	154	286	233	342	297	298	951	1662	1490	1452	461	744	870	737	1327	2186	2247	1297	1145	467	266	242	295	146

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 66. Hourly activity levels for a sensor fish before and after exposure to 0,5 mg/dm^3 of cadmium in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									244	345	384	45	112	354	326	554	531	811	120	105	130	33	115	28
2	35	33	106	103	33	117	190	275	379	377	236	77	40	246	236	540	519	832	58	110	33	65	63	28
3	37	28	33	35	48	107	190	352	306	313	246	137	35	331	441	591	577	869	242	148	100	100	157	98
4	45	83	65	115	158	140	135	387	248	288	253	177	130	232	308	660	575	830	108	103	105	60	130	75
5	36	25	25	25	68	80	197	370	370	234	326	235	102	395	352	600	538	802	125	85	95	123	47	45
6	30	52	49	35	35	103	210	390	*669*	*514*	*714*	*816*	*941*	*1082*	*1173*	*1218*	*1106*	*997*	*989*	*1004*	*901*	*846*	*734*	*616*
7	*542*	*403*	*517*	*391*	*313*	*284*	*196*	*177*	*94*	*106*	*138*	*81*	*94*	*80*	*64*	*23*	*13*	*34*	*17*	*8*				
a	37	44	56	63	68	109	184	355	309	311	289	134	84	312	333	589	548	829	131	110	93	86	102	55
b	5	24	32	43	52	22	29	47	64	55	64	76	44	70	74	47	27	26	68	23	36	54	46	31
c	47	91	119	147	170	152	166	447	401	419	414	283	170	449	478	681	601	880	264	155	164	192	192	116

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 67. Hourly activity levels for a sensor fish before and after exposure to 5,0 mg/dm^3 of copper in sea water.

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									103	150	18	45	167	154	142	241	353	231	48	42	52	13	46	11
2	14	13	46	43	13	47	76	110	165	164	31	16	106	107	106	235	362	226	23	44	13	26	25	11
3	14	11	13	14	19	43	76	141	133	136	55	14	107	144	192	257	378	251	97	59	40	40	63	39
4	19	24	27	50	63	56	54	154	108	125	71	52	110	101	134	287	361	250	43	41	42	24	52	30
5	14	10	10	10	27	32	79	148	161	102	94	41	142	172	153	261	349	234	50	34	38	49	9	8
6	12	21	20	14	14	41	84	156	157	131	81	*423*	*856*	*1456*	*1224*	*53*	*41*	*78*	*47*	*64*	*34*	*214*	*188*	*272*
7	*99*	*154*	*126*	*27*	*107*	*125*	*228*	*113*	*87*	*188*	*10*	*39*	*31*	*127*	*247*	*294*	*652*	*328*	*3*					
a	15	16	23	27	27	44	73	142	138	135	58	34	126	136	145	256	361	238	52	44	36	30	39	20
b	3	6	14	19	21	9	12	19	28	21	29	17	27	31	31	20	11	11	27	9	17	14	22	14
c	21	28	50	64	68	62	97	179	193	176	115	67	179	197	206	295	383	260	105	62	69	57	82	47

a, b and c are, respectively, the mean, standard deviation of upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 68. Hourly activity levels for a sensor fish before and after exposure to 1,0 mg/dm^3 of copper in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									330	357	157	155	282	460	649	268	625	469	151	56	69	58	35	72
2	22	41	42	68	61	62	146	143	371	346	219	182	360	261	196	243	697	437	223	31	23	41	50	41
3	23	42	31	40	80	72	152	162	338	330	268	143	334	371	348	561	616	450	450	70	50	40	51	10
4	25	43	38	42	100	73	150	177	398	363	155	197	331	378	771	662	660	584	384	57	43	42	50	35
5	24	43	74	54	94	98	151	208	394	386	157	167	291	461	284	542	624	496	268	56	57	38	41	27
6	28	41	78	38	83	94	174	232	323	371	184	*962*	*771*	*959*	*815*	*373*	*140*	*121*	*22*	*33*	*27*	*119*	*73*	*91*
7	*30*	*45*	*12*	*58*	*2*	*0*	*42*	*1*	*0*	*2*	*18*	*99*	*3*	*25*	*26*	*16*	*42*	*10*	*10*					
a	24	42	53	48	84	80	155	184	359	359	190	169	320	386	450	455	644	487	295	54	48	44	45	37
b	2	1	22	13	15	16	11	36	33	20	46	21	32	82	248	188	34	59	121	14	17	8	7	23
c	28	44	96	74	113	111	177	255	424	398	280	210	383	547	936	823	711	603	532	81	81	60	59	82

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

concentration of copper was less resistant to intoxication than that exposed to the higher. No other explanation would serve for the exposure times to lethality being so alike.

The two lethal concentrations of *mercury* utilized in the study produced results very similar to those for copper (Tables 69 and 70). Sensor fish exposed to 1,0 and 0,1 mg/dm^3 elicited responses within one h and died at approximately the same time (25 and 27 h post-exposure). Although recognized as a cumulative toxicant mercury must have the same effect upon the gill apparatus as other heavy metals, when present at high concentrations. The escape reaction on the part of the fish when exposed to heavy metal toxicity may also, therefore, be due, in part, to neural recognition of oxygen lack and a method of searching for an area of higher oxygen content.

Only one of the *phenol* concentrations, employed in this study, proved lethal within the exposure time of 48 h (5,0 mg/dm^3). The behavioural response occurred after two h exposure (Table 71) and the fish died within 31 h . In this respect locomotor activity proved more effective as a detector parameter for phenol than respiratory impairment. However, a comparison between largemouth bass and this species of mullet would seem to indicate that *Atherina breviceps* is more sensitive to phenol intoxication.

A lethal level of *ammonia* (5,0 mg/dm^3) also provided a very much quicker response than was elicited by breathing rate in largemouth bass, five h compared to 17 h (Table 72). Once again, however, a direct comparison may be odious as the species proved more sensitive to intoxication by ammonia. A concentration of 5,0 mg/dm^3 did not prove lethal to *Micropterus salmoides* in previous experimentation, whereas in this case lethality occurred after 38 h exposure.

Response times to lethal concentrations of *cyanide*, 0,5 and 0,1 mg/dm^3, compared favourably with those elicited by largemouth bass opercular rhythm responses. Sensor fish responded to these concentrations within one h in both cases. Death occurred after six h and 14 h respectively (Tables 73 and 74).

TABLE 69. Hourly activity levels for a sensor fish before and after exposure to 1,0 mg/dm^3 of mercury in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									202	361	278	270	202	274	249	164	142	126	74	66	71	89	74	47
2	28	63	61	43	63	79	362	364	261	265	430	311	421	364	526	188	134	154	52	45	27	12	71	43
3	44	29	45	35	25	45	162	253	315	209	284	262	382	585	345	166	179	101	65	53	62	92	47	45
4	57	24	29	65	28	71	260	252	282	268	203	304	247	284	354	103	177	104	53	57	57	42	41	43
5	47	24	32	60	71	42	261	384	303	200	204	271	384	232	415	154	157	178	71	55	61	53	21	51
6	38	17	27	12	92	62	381	263	278	194	264	*1154*	*1561*	*1442*	*1008*	*51*	*43*	*1*	*0*	*0*	*0*	*5*	*47*	*10*
7	*19*	*46*	*36*	*42*	*32*	*23*	*41*	*20*	*7*	*2*	*22*	*29*												
a	43	31	39	43	56	60	285	303	274	250	277	284	327	348	376	155	158	133	63	55	56	58	51	46
b	11	18	14	21	29	16	89	65	40	64	83	22	96	141	103	32	20	33	10	8	17	34	22	3
c	65	66	66	84	113	91	459	430	352	375	440	327	515	603	578	218	197	198	83	71	89	125	94	52

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 70. Hourly activity levels for a sensor fish before and after exposure to 0,1 mg/dm^3 of mercury in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									366	453	305	243	299	303	358	376	267	318	46	90	64	65	71	26
2	43	45	15	44	21	66	270	454	453	442	185	140	289	235	250	254	231	138	50	27	63	19	48	25
3	44	26	33	14	10	16	128	441	317	322	230	184	206	254	251	360	260	244	98	84	78	52	81	18
4	13	14	15	24	18	32	145	357	447	105	178	281	286	301	388	306	277	201	91	77	64	41	40	18
5	14	21	17	10	19	31	134	282	317	310	214	297	306	284	351	294	247	194	82	70	71	37	28	9
6	18	14	10	13	19	43	101	231	311	424	194	*1297*	*955*	*122*	*44*	*46*	*1097*	*1364*	*1094*	*688*	*273*	*250*	*90*	*120*
7	*779*	*958*	*420*	*345*	*283*	*155*	*521*	*596*	*375*	*569*	*272*	*331*	*2*											
a	26	24	18	21	17	38	156	353	368	343	218	229	277	275	320	338	256	219	73	70	68	54	43	19
b	16	13	9	14	4	19	66	97	66	132	47	66	41	30	65	57	18	67	24	25	6	22	17	7
c	57	50	36	48	25	75	285	543	497	602	310	358	357	334	447	450	291	350	120	119	80	97	76	33

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 71. Hourly activity levels for a sensor fish before and after exposure to 5,0 mg/dm^3 phenol in sea water.

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									661	847	783	394	439	645	660	765	641	706	225	221	142	97	111	35
2	70	58	27	67	45	195	309	693	755	788	623	338	570	579	766	546	588	263	239	145	131	53	97	57
3	57	33	44	21	22	121	217	542	521	663	439	414	610	748	677	805	635	441	284	384	172	84	130	58
4	23	20	35	30	26	90	359	424	656	589	357	481	592	650	696	677	640	402	238	187	122	92	73	45
5	35	33	27	18	27	130	296	467	492	517	667	456	629	612	707	766	605	376	233	213	135	78	57	42
6	25	27	18	20	26	126	193	547	*913*	*1008*	*1461*	*1384*	*1231*	*1233*	*996*	*984*	*718*	*643*	*348*	*421*	*408*	*391*	*506*	*224*
7	*213*	*194*	*306*	*391*	*483*	*762*	*515*	*542*	*307*	*118*	*94*	*104*	*63*	*52*	*8*									
a	42	34	30	31	29	132	275	535	617	681	574	417	568	647	701	712	621	437	243	230	140	81	94	47
b	21	14	10	21	9	38	68	103	109	137	173	56	75	63	40	109	24	164	23	91	19	17	29	10
c	83	61	50	72	47	207	408	737	831	950	913	527	715	771	779	926	668	758	288	408	177	114	151	67

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 72. Hourly activity levels for a sensor fish before and after exposure to 5,0 mg/dm^3 of ammonia (as N) in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									223	302	236	96	119	253	287	514	503	554	192	77	97	45	91	27
2	31	24	75	57	37	79	179	214	279	275	247	112	118	178	196	497	461	523	93	69	29	44	42	25
3	25	23	28	24	36	61	193	351	287	285	154	136	80	222	300	515	522	499	186	80	67	67	96	64
4	38	73	55	71	110	89	137	328	272	295	161	177	151	193	208	517	488	516	150	99	62	46	72	44
5	24	23	23	40	55	69	170	273	327	238	249	200	135	277	317	421	436	498	148	80	61	74	32	29
6	22	33	42	48	40	85	202	299	*377*	*406*	*416*	*503*	*674*	*717*	*707*	*694*	*581*	*478*	*312*	*286*	*253*	*229*	*194*	*187*
7	*179*	*166*	*177*	*231*	*481*	*503*	*496*	*219*	*117*	*93*	*107*	*128*	*88*	*86*	*64*	*47*	*29*	*19*	*24*	*13*	*7*	*4*		
a	28	35	45	48	56	77	176	293	278	279	209	144	121	225	262	493	482	518	154	81	63	55	67	38
b	7	22	21	18	31	12	25	53	37	25	48	43	26	41	56	41	34	23	39	11	24	14	29	16
c	42	78	86	83	117	101	225	397	351	328	303	228	172	305	372	573	549	563	230	103	110	82	124	69

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 73. Hourly activity levels for a sensor fish before and after exposure to 0,5 mg/dm^3 of cyanide in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									665	953	640	510	628	636	751	789	507	572	96	189	134	136	149	55
2	88	94	32	92	44	138	268	951	749	926	388	294	607	493	525	533	439	248	105	57	132	40	101	53
3	92	55	69	29	21	33	304	928	951	749	483	386	432	533	527	756	494	439	206	176	164	109	170	38
4	27	29	32	50	38	67	567	676	665	529	373	590	600	632	814	642	526	362	191	161	134	86	84	37
5	29	44	36	21	40	65	281	665	938	485	449	623	643	596	737	827	469	349	172	147	149	78	59	40
6	39	29	21	27	40	90	212	890	*1381*	*1542*	*1889*	*904*	*74*											
a	55	50	38	44	37	79	326	822	794	741	467	481	582	578	671	709	487	394	154	146	143	90	113	45
b	32	27	18	29	9	39	139	140	142	204	107	139	86	63	135	120	34	121	50	52	14	36	46	9
c	118	103	73	101	55	155	598	1096	1072	1140	677	753	751	702	936	944	554	631	252	247	170	161	203	63

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 74. Hourly activity levels for a sensor fish before and after exposure to 0,1 mg/dm^3 of cyanide in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									284	509	392	381	285	386	351	231	200	178	104	93	100	126	104	66
2	40	89	86	61	90	111	510	513	368	374	606	439	594	513	742	265	190	217	73	63	38	17	100	61
3	62	41	63	49	35	63	228	357	444	295	400	369	539	824	486	234	252	142	91	74	87	130	66	64
4	80	34	41	92	39	100	367	355	398	378	286	429	348	400	499	145	250	147	75	80	82	59	55	60
5	66	34	45	85	100	59	368	541	427	282	288	382	541	327	585	217	221	251	100	77	86	75	30	72
6	54	24	38	14	130	87	537	371	*672*	*942*	*1181*	*1232*	*1104*	*1009*	*908*	*974*	*801*	*421*	*94*	*80*	*14*	*6*		
a	60	44	55	60	79	84	402	427	384	368	394	400	461	490	532	218	223	187	87	77	79	81	71	65
b	15	26	20	31	41	23	125	92	63	91	130	32	136	199	144	45	28	47	14	11	24	48	31	5
c	89	95	94	121	159	129	647	607	507	546	648	462	727	880	814	306	278	279	114	99	126	175	132	75

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

Although a direct comparison between opercular rhythm response in the freshwater fish, *Micropterus salmoides*, and locomotory response in the marine fish, *Altherina breviceps*, to lethal levels of intoxication, would be spurious, it would seem that locomotory behavioural response, to intoxication, is a better parameter to use for phenol and ammonia detection. Further, it would not be disadvantageous to use activity aberrations for heavy metal and cyanide detection.

Responses were also elicited by all sub-lethal concentrations tested. Sensor fish responded to the introduction of 0,1 mg/dm^3 of cadmium within 18 h (Table 75); 0,1 mg/dm^3 of copper within 31 h (Table 76); 0,05 mg/dm^3 of mercury within 25 h (Table 77); 1,0 and 0,5 mg/dm^3 of phenol within 10 h and 16 h respectively (Tables 78 and 79); 1,0 and 0,5 mg/dm^3 of ammonia within 14 h and 31 h respectively (Tables 80 and 81) and 0,05 mg/dm^3 of cyanide within five h (Table 82).

Exposure to sub-lethal levels of cadmium (Figure 78), copper (Figure 79), phenol (Figures 80 and 81), ammonia (Figure 82) and cyanide (Figure 83) induced a marked increase in activity for the first 24 h of exposure. The lower sub-lethal concentration of ammonia (Figure 84) produced high activity levels during the second 24 h of exposure. This aberration was not sustained and in all of the above cases the normal locomotory behaviour pattern was quickly re-established. Exposure to 0,1 mg/dm^3 of copper resulted in a single unprolonged peak of activity, which may be considered as being a "false" response in this case. On the other hand, the sensor fish exposed to mercury at a concentration of 0,05 mg/dm^3 (Figure 85) performed normally over the first 24 h exposure period after which time the activity rate increased exceptionally and was sustained at a very high level throughout the second 24 h period of exposure. It is possible that this concentration of mercury would, therefore, have proved lethal over an exposure period longer than 48 h.

The resumption of normal activity levels, in the majority of cases, after a short period of heightened activity in sub-lethal toxicant concentrations would indicate a reasonable degree of adaptability on the part of the species of fish used as sensors to changed environmental

TABLE 75. Hourly activity levels for a sensor fish before and after exposure to 0,1 mg/dm^3 of cadmium in sea water

Day.	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									1142	1235	543	536	975	1591	2245	927	1622	1512	522	193	238	202	141	78
2	76	142	142	145	209	210	505	495	1283	1197	757	563	1245	1595	2062	877	1718	1557	771	201	79	141	173	142
3	78	142	107	131	211	249	526	560	1169	1141	927	520	1155	903	1896	1081	1532	1622	1601	247	173	102	176	34
4	83	134	133	235	346	250	516	612	1377	1255	536	681	1006	1283	2321	1341	981	1371	1328	191	149	163	173	121
5	81	161	256	138	277	316	518	719	1363	1335	543	578	1145	1307	2020	1268	1003	1486	927	172	197	131	142	93
6	94	152	268	186	325	298	602	801	*1249*	*1416*	*604*	*908*	*918*	*1501*	*2004*	*2161*	*2331*	*1968*	*1817*	*946*	*1942*	*2009*	*2213*	*2162*
7	*2198*	*2816*	*2727*	*2613*	*2589*	*1949*	*1887*	*1641*	*1443*	*1334*	*908*	*918*	*1614*	*1921*	*2213*	*1949*	*1771*	*1631*	*1423*	*1117*	*946*	*978*	*872*	*774*
8	*748*	*753*	*616*	*423*	*501*	*517*	*614*	*1009*																
a	82	146	181	167	274	265	533	637	1267	1232	661	576	1105	1336	2109	1099	1371	1510	1030	201	167	112	161	94
b	7	11	75	44	63	42	39	123	108	72	176	63	112	284	172	204	352	93	443	28	59	55	. 18	42
c	96	168	328	253	398	347	609	878	1479	1373	1006	700	1325	1893	2446	1499	2061	1692	1879	256	283	220	196	176

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 76. Hourly activity levels for a sensor fish before and after exposure to 0,1 mg/dm^3 of copper in seawater

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									202	258	88	146	126	152	248	474	474	297	264	48	63	57	67	26
2	26	15	43	10	40	41	168	152	179	173	257	147	195	109	156	453	403	213	128	28	24	23	20	23
3	12	18	22	13	23	15	196	350	267	256	61	135	124	112	158	479	467	129	129	11	34	33	35	30
4	30	63	44	26	62	37	138	268	295	302	69	177	171	153	107	374	401	201	191	94	19	31	14	13
5	12	19	21	55	41	58	142	176	284	242	171	164	168	158	282	241	333	194	171	74	27	24	17	12
6	14	14	34	61	44	67	194	208	304	322	254	*379*	*457*	*352*	*282*	*155*	*379*	*399*	*337*	*209*	*155*	*54*	*68*	*0*
7	*53*	*44*	*142*	*135*	*0*	*48*	*146*	*101*	*51*	*26*	*11*	*43*	*106*	*54*	*59*	*421*	*53*	*889*	*371*	*235*	*150*	*154*	*115*	*70*
8	*141*	*75*	*228*	*147*	*150*	*111*	*191*	*236*	*116*	*221*	*90*													
a	18	26	33	33	42	44	168	231	255	259	150	154	157	137	190	404	416	207	177	51	33	34	31	21
b	9	21	11	24	14	20	28	80	52	52	91	17	31	24	72	101	58	60	56	34	17	14	22	8
c	36	47	55	80	69	83	223	388	357	361	328	187	218	184	278	602	530	325	287	118	66	61	74	37

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 77. Hourly activity levels for a sensor fish before and after exposure to 0,05 mg/dm^3 of mercury in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									573	740	925	278	250	654	569	740	774	840	355	252	150	57	72	14
2	52	22	21	41	45	252	349	435	559	649	858	382	533	665	1007	559	736	278	373	232	130	66	92	61
3	21	10	18	13	22	209	131	156	377	577	394	441	787	963	826	853	776	442	362	592	180	58	89	78
4	18	10	37	10	13	113	151	172	374	586	340	372	584	668	578	712	753	441	285	212	110	97	61	52
5	41	21	17	14	13	194	311	268	319	549	884	289	614	628	677	704	741	402	294	279	121	77	54	43
6	11	23	14	13	11	161	174	203	402	581	712	*105*	*40*	*959*	*0*	*43*	*40*	*2*	*3*	*13*	*36*	*56*	*58*	*62*
7	*52*	*67*	*34*	*53*	*63*	*58*	*36*	*128*	*379*	*490*	*832*	*1749*	*1819*	*1815*	*1855*	*1800*	*1839*	*1729*	*1807*	*1859*	*1866*	*1816*	*1718*	*1858*
8	*1733*	*1726*	*1690*	*1601*	*1485*	*1575*	*1728*	*1651*	*1609*	*1699*	*1701*													
a	29	17	21	18	21	186	223	247	434	614	686	352	554	716	731	714	756	481	334	313	138	71	74	50
b	17	7	9	13	14	52	100	114	106	70	257	68	195	139	186	105	18	212	41	158	28	17	16	24
c	62	31	39	44	48	288	419	470	642	751	1190	485	936	988	1096	920	791	896	414	623	193	104	105	97

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 78. Hourly activity levels for a sensor fish before and after exposure to 1,0 mg/dm^3 of phenol in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									618	781	342	413	402	456	675	1136	1132	753	551	141	158	147	170	65
2	78	55	100	44	97	121	496	554	611	593	224	364	535	336	437	1033	1042	495	281	70	80	56	64	59
3	48	52	64	35	49	40	485	973	732	712	263	362	351	351	442	1138	1064	380	307	64	107	92	111	69
4	71	142	102	68	143	96	369	755	858	702	268	495	485	457	408	901	941	503	428	227	70	83	48	35
5	37	42	82	136	104	110	484	588	769	675	312	477	489	458	740	1179	790	485	383	183	90	67	48	29
6	39	37	78	138	104	116	467	563	*801*	*749*	*534*	*604*	*673*	*746*	*914*	*1009*	*1212*	*1342*	*1468*	*1311*	*1204*	*1117*	*1094*	*914*
7	*704*	*538*	*312*	*294*	*387*	*401*	*642*	*712*	*914*	*973*	*1004*	*1153*	*946*	*809*	*631*	*646*	*718*	*856*	*830*	*700*	*638*	*513*	*444*	*312*
8	*104*	*341*	*98*	*126*	*271*	*209*	*683*	*734*																
a	55	66	85	84	99	97	460	686	718	693	282	422	452	411	540	1077	993	523	390	137	101	89	88	51
b	19	43	16	50	33	33	52	180	105	68	46	62	74	62	155	112	133	138	107	71	35	35	53	18
c	92	150	116	182	164	162	562	1039	924	826	372	544	597	533	844	1297	1254	794	600	276	170	158	192	86

a, b, and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 79. Hourly activity levels for a sensor fish before and after exposure to 0,5 mg/dm^3 of phenol in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									736	796	350	346	629	1026	1447	598	1124	991	337	125	154	130	88	75
2	49	92	92	107	135	138	326	319	827	772	488	373	803	928	1129	560	1208	994	497	116	51	91	112	92
3	51	92	69	86	146	161	339	361	754	736	598	334	746	637	1122	821	1074	1036	1026	159	112	71	114	22
4	54	89	86	139	223	162	333	395	888	809	346	439	669	831	1546	1002	821	978	856	124	96	103	112	78
5	56	102	165	96	186	208	335	464	879	861	350	373	718	884	1153	905	816	991	598	114	127	85	92	60
6	61	97	173	112	204	196	388	517	*904*	*832*	*516*	*473*	*937*	*956*	*1481*	*1342*	*1146*	*1238*	*1379*	*1515*	*1534*	*1573*	*1604*	*1703*
7	*1649*	*1333*	*941*	*733*	*412*	*286*	*484*	*518*	*912*	*806*	*744*	*681*	*777*	*981*	*1462*	*1103*	*1184*	*1004*	*931*	*546*	*214*	*231*	*203*	*192*
8	*186*	*131*	*124*	*117*	*203*	*317*	*463*	*497*																
a	54	94	117	108	179	173	344	411	816	794	426	373	713	861	1279	777	1008	998	662	128	108	96	104	65
b	5	5	48	20	38	28	25	79	70	46	113	41	67	144	202	192	180	22	277	18	38	22	13	27
c	64	104	211	147	254	228	393	566	953	884	648	453	844	1143	1675	1153	1361	1041	1205	163	183	139	130	118

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 80. Hourly activity levels for a sensor fish before and after exposure to 1,0 mg/dm^3 of ammonia (as N) in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									250	334	166	120	171	306	270	380	447	410	148	109	79	44	70	15
2	41	21	37	48	37	187	265	338	273	309	167	142	240	286	406	313	439	210	142	103	52	56	72	45
3	21	12	17	16	24	157	123	172	198	270	203	161	329	409	385	427	461	280	175	237	83	58	89	70
4	22	19	37	32	42	101	125	190	185	268	185	156	260	284	270	393	444	280	122	95	60	75	66	48
5	39	19	17	15	22	142	241	248	192	243	200	165	286	306	314	377	434	259	128	115	62	75	39	32
6	16	26	28	16	14	125	155	210	*312*	*261*	*243*	*314*	*368*	*381*	*264*	*394*	*462*	*405*	*416*	*411*	*408*	*508*	*576*	*603*
7	*594*	*581*	*667*	*701*	*742*	*603*	*708*	*712*	*642*	*603*	*591*	*497*	*468*	*471*	*452*	*399*	*501*	*304*	*241*	*200*	*193*	*190*	*164*	*134*
8	*127*	*204*	*104*	*99*	*64*	*78*	*104*	*206*																
a	27	19	27	25	28	142	182	232	220	285	184	149	257	318	334	378	445	287	143	132	67	62	67	42
b	11	10	5	14	11	32	67	66	39	36	18	18	59	52	63	41	10	74	21	59	13	13	18	20
c	49	29	47	52	50	205	313	361	296	356	219	184	373	420	458	458	465	432	184	248	93	88	102	81

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 81. Hourly activity levels for a sensor fish before and after exposure to 0,5 mg/dm^3 of ammonia (as N) in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									243	435	335	326	244	330	300	198	171	152	89	80	86	108	89	57
2	34	76	71	52	77	95	436	439	315	320	518	375	508	441	634	226	162	186	63	54	33	15	86	52
3	54	35	54	42	30	55	195	305	380	252	342	316	461	705	416	200	216	122	78	64	75	111	57	55
4	69	29	35	79	34	86	314	304	340	323	245	367	298	342	427	124	214	126	64	69	70	51	48	52
5	57	29	39	73	86	51	315	463	365	241	246	327	463	279	500	186	189	215	86	66	74	64	26	62
6	46	21	33	13	111	75	459	317	*351*	*373*	*406*	*431*	*447*	*503*	*500*	*404*	*286*	*215*	*194*	*134*	*123*	*98*	*106*	*97*
7	*81*	*67*	*104*	*136*	*168*	*274*	*433*	*506*	*574*	*604*	*681*	*713*	*741*	*736*	*779*	*842*	*800*	*716*	*649*	*532*	*414*	*484*	*336*	*324*
8	*247*	*197*	*113*	*217*	*235*	*286*	*506*	*631*																
a	52	38	46	52	68	72	344	366	329	314	337	342	395	419	455	187	190	160	76	67	68	70	61	56
b	13	22	16	26	35	19	107	79	54	77	111	27	116	170	123	38	24	40	12	9	20	40	27	4
c	78	81	77	103	137	109	554	521	435	465	555	395	622	752	696	262	237	238	100	85	107	148	114	64

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

TABLE 82. Hourly activity levels for a sensor fish before and after exposure to 0,05 mg/dm^3 of cyanide in sea water

Day	Time of day																							
	am												pm											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1									335	518	314	194	175	457	398	518	541	588	248	176	105	74	94	18
2	67	28	27	52	60	327	453	565	442	454	302	267	373	465	705	391	515	194	261	162	91	86	119	79
3	27	13	21	17	29	271	170	202	263	403	351	308	551	674	578	597	543	309	253	414	126	75	115	101
4	24	13	46	13	20	146	196	224	261	410	298	260	409	467	405	498	527	309	200	148	77	126	79	67
5	60	27	23	20	17	252	404	348	223	384	306	289	430	440	474	492	519	281	206	195	85	100	70	56
6	18	30	17	17	14	209	226	264	*476*	*584*	*514*	*647*	*812*	*946*	*1062*	*1141*	*1347*	*1212*	*1081*	*946*	*958*	*823*	*727*	*263*
7	*149*	*252*	*303*	*194*	*97*	*384*	*617*	*753*	*712*	*699*	*608*	*549*	*634*	*421*	*347*	*376*	*401*	*384*	*268*	*319*	*328*	*204*	*198*	*188*
8	*163*	*151*	*109*	*204*	*208*	*273*	*114*	*236*																
a	39	22	27	24	28	241	290	285	305	434	314	264	388	501	512	499	529	336	234	219	97	92	95	64
b	23	9	11	16	19	68	129	197	87	54	21	43	136	98	130	74	13	149	28	110	19	22	22	31
c	84	40	49	55	65	374	543	671	476	540	355	348	654	693	767	644	555	628	289	435	134	135	138	125

a, b and c are, respectively, the mean, standard deviation and upper 95 % confidence limit.

Figures in italics represent post-exposure activity levels.

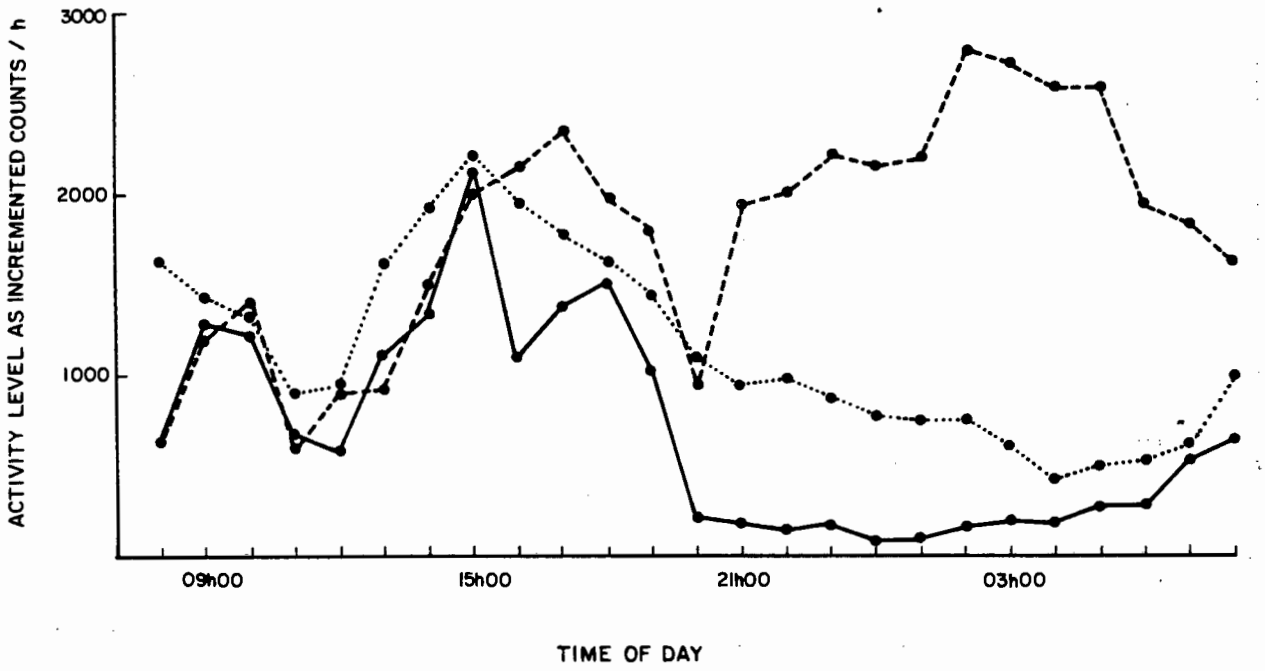

FIGURE 78. The effect of 0,1 mg Cd^{2+}/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. Broken and dotted lines represent, respectively, activity during the first and second days after exposure.

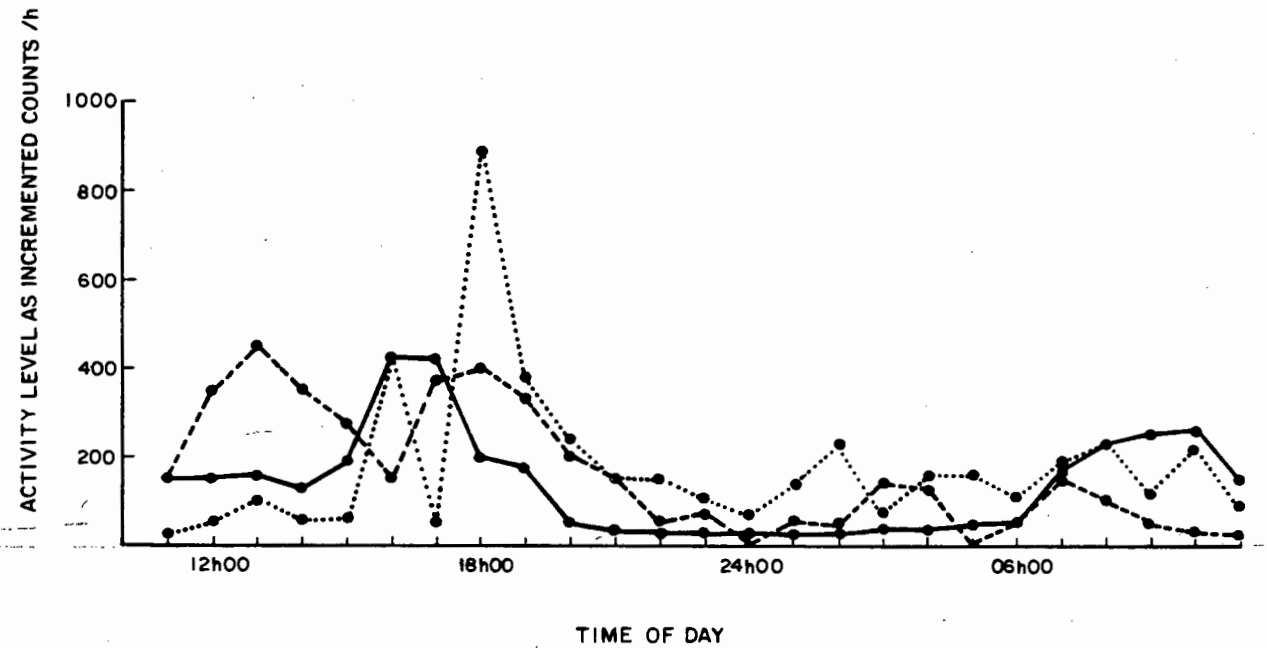

FIGURE 79. The effect of 0,1 mg Cu^{2+}/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. broken and dotted lines represent, respectively, activity during the first and second days after exposure.

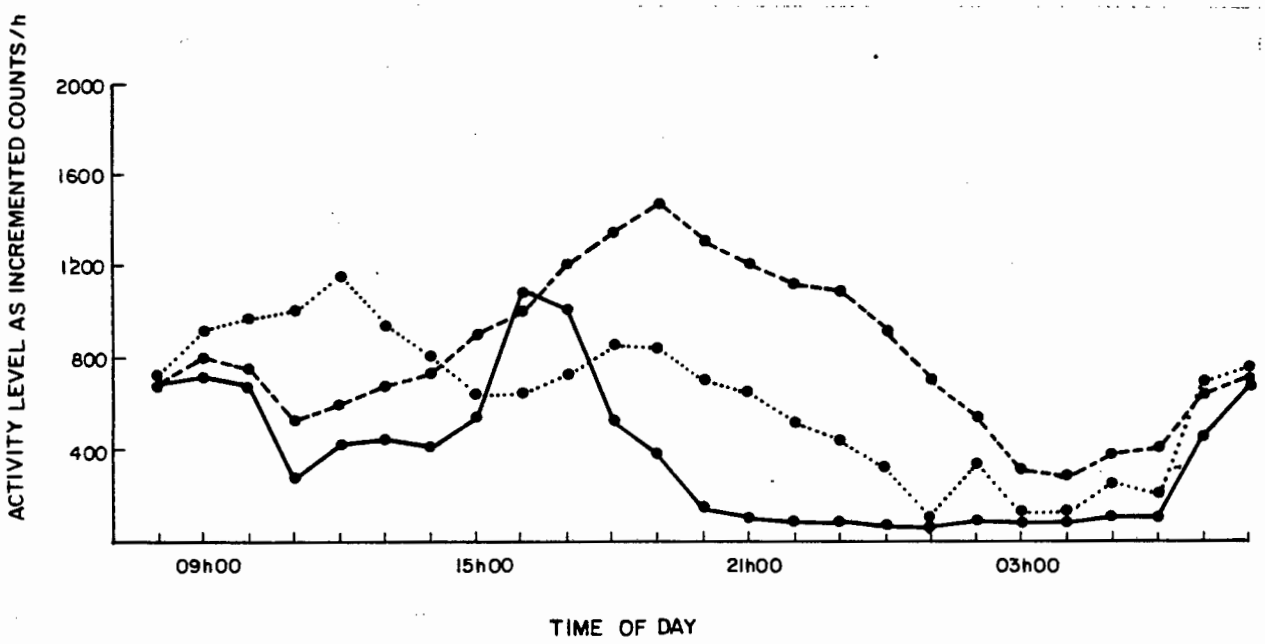

FIGURE 80. The effect of 1,0 mg C_6H_5OH/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. broken and dotted lines represent, respectively, activity during the first and second days after exposure.

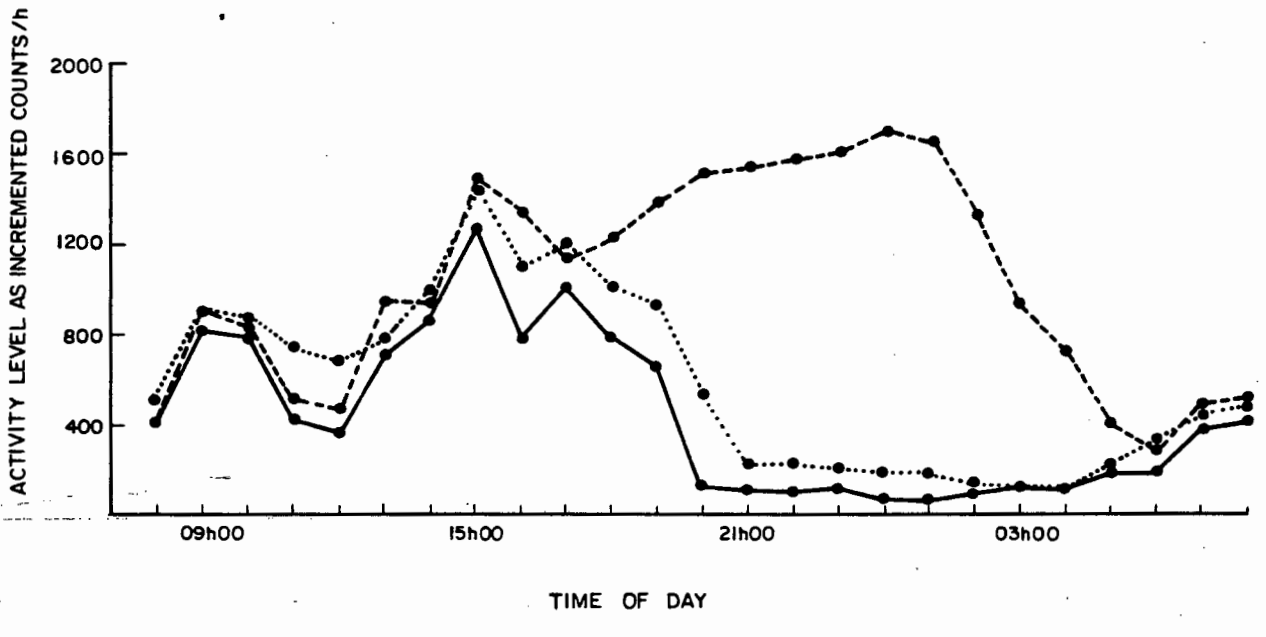

FIGURE 81. The effect of 0,5 mg C_6H_5OH/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. Broken and dotted lines represent, respectively, activity during the first and second days after exposure.

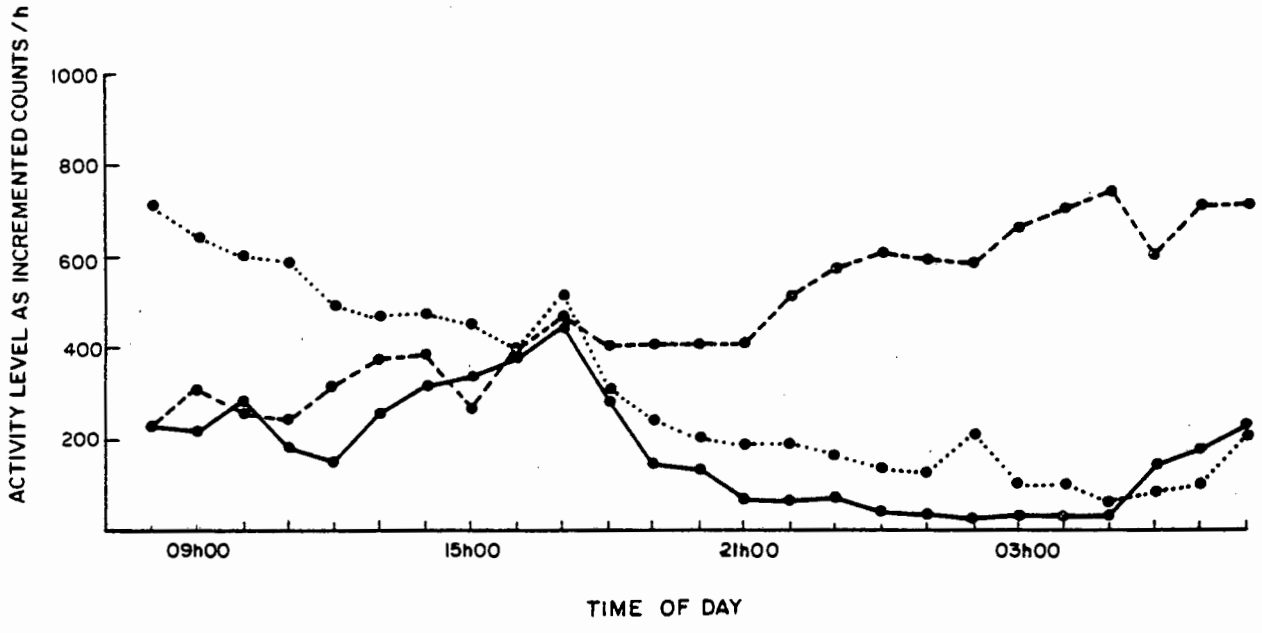

FIGURE 82. The effect of 1,0 mg NH_3-N/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. Broken and dotted lines represent, respectively, activity during the first and second days after exposure.

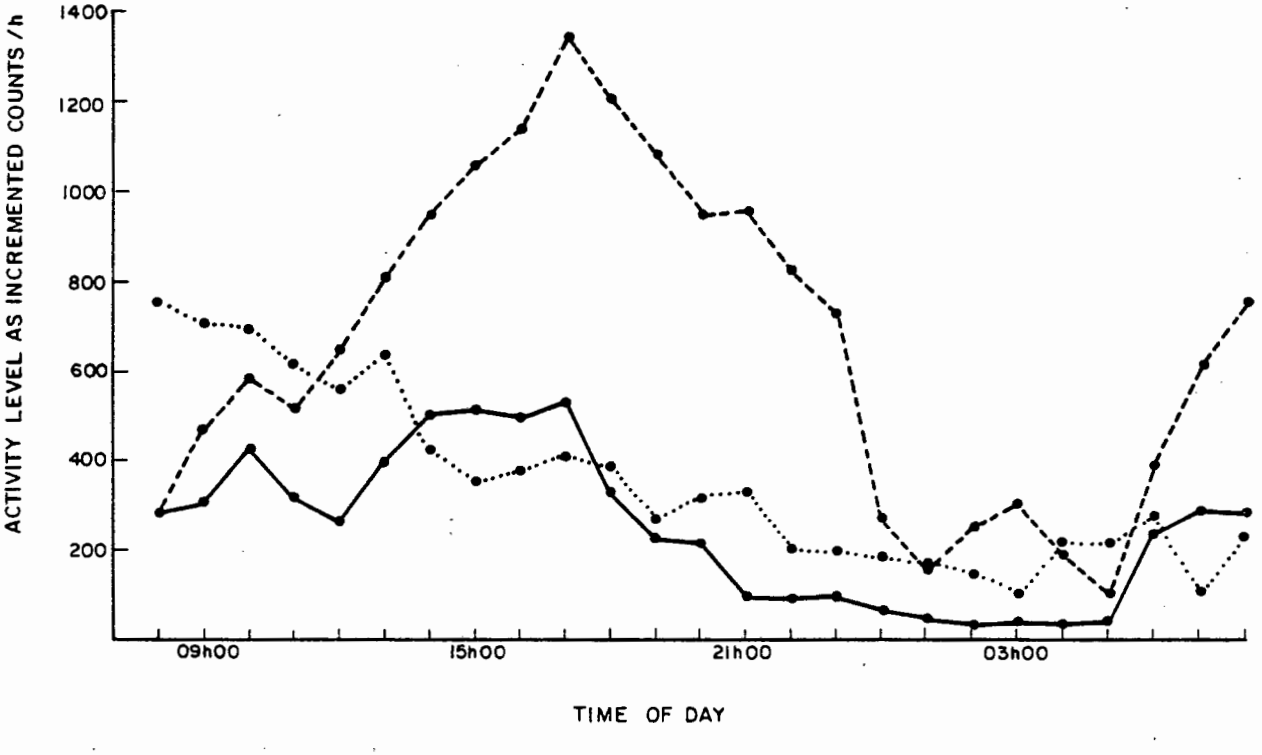

FIGURE 83. The effect of 0,05 mg CN^-/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. Broken and dotted lines represent, respectively, activity during the first and second days after exposure.

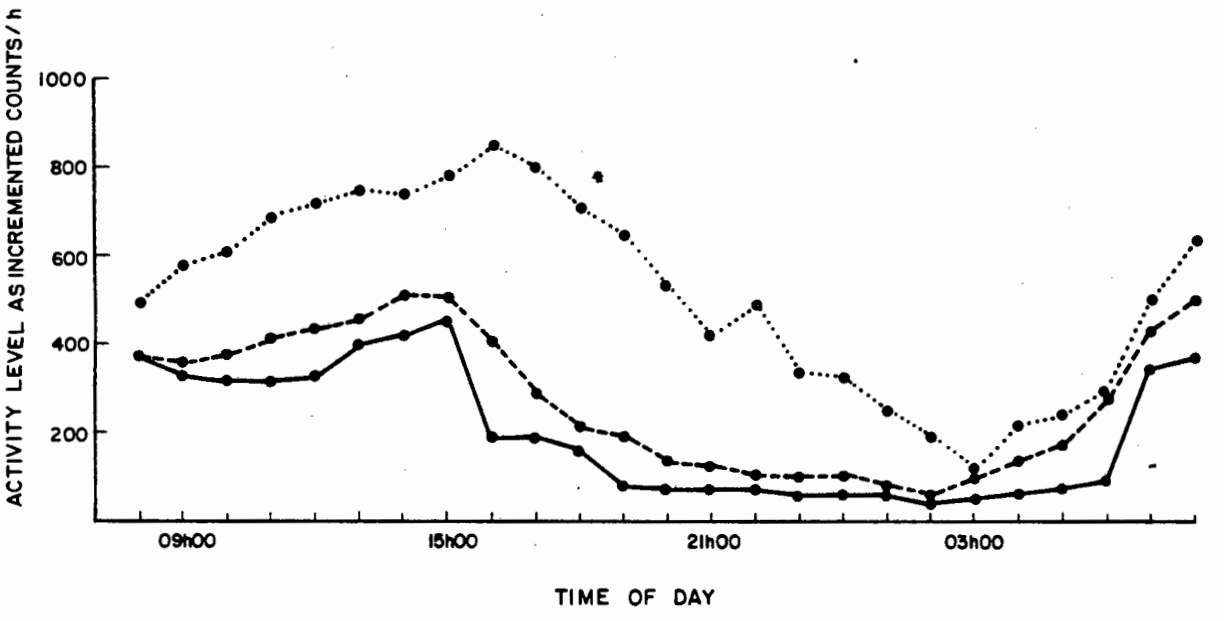

FIGURE 84. The effect of 0,5 NH_3-N/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. Broken and dotted lines represent, respectively, activity during the first and second days after exposure.

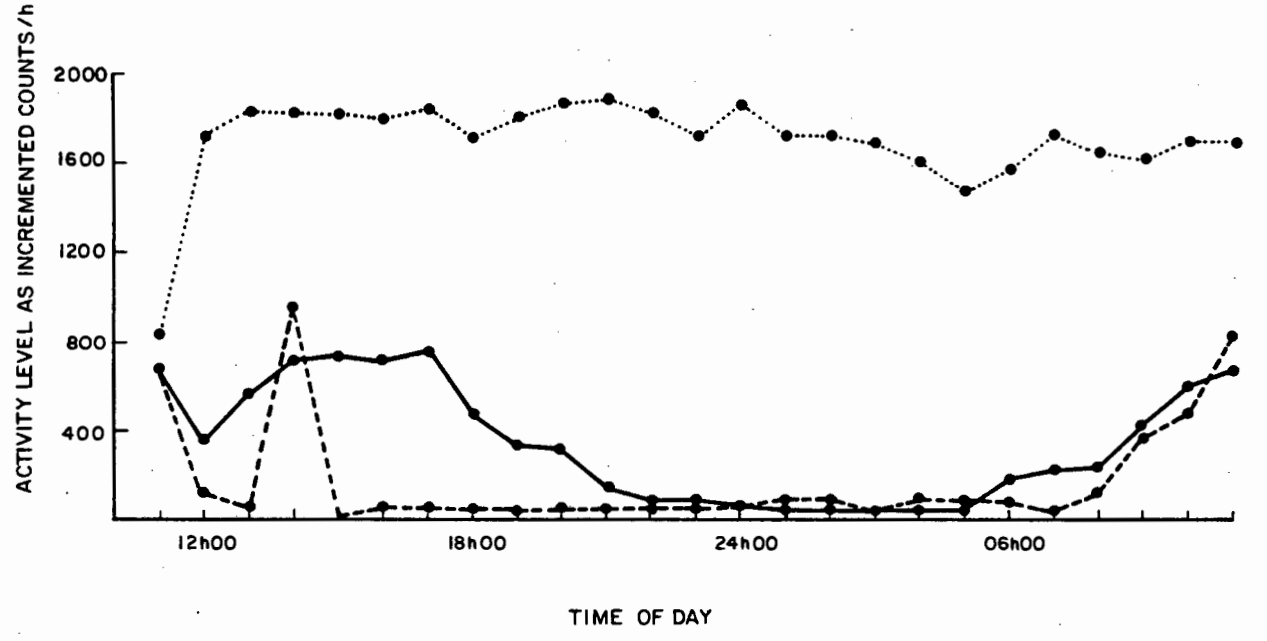

FIGURE 85. The effect of 0,05 mg Hg^{2+}/dm^3 on the normal activity pattern (continuous line) of *Atherina breviceps*. Broken and dotted lines respresent, respectively, activity during the first and second days after exposure.

conditions. In the case of phenol and ammonia, however, the destruction of the olfactory apparatus may have been a factor, as the fish then would not be able to respond to the presence of toxicants which they were not able to sense.

CONCLUSION

Normal and aberrant locomotor behaviour patterns of small fish can be detected and monitored using a system based upon ultrasonic sound wave interference. The system, as described, operates effectively in the marine environment. The system has certain advantages over previous monitoring techniques in being more compact and, therefore, easily transportable. It is constructed in such a way as to employ small fish including those at a stage of development purported to be the most sensitive to intoxication.

Using the monitoring system to assess the effect of a number of toxicants on locomotory behaviour patterns of *Atherina breviceps* has indicated the parameter to be comparable in efficiency to monitoring fish respiratory responses to intoxication, and, in certain cases, to be superior.

However, further development of the prototype electronic system was deemed necessary to enable the unit to conform to its possible use in industial on-site situations.

AN AUTOMATIC BIOLOGICAL MONITORING SYSTEM, BASED ON THE DETECTION OF FISH LOCOMOTOR BEHAVIOUR PATTERNS BY ULTRASONIC MEANS, DESIGNED TO OPERATE AT ON-SITE INDUSTRIAL LOCATIONS

Preliminary investigations into the effects of intoxication upon locomotory behaviour in marine and freshwater fish have shown this parameter to be a feasible pre-mortal indication of toxic stress. Aberrations in activity, processed by means of ultrasonic echoes, were found suitable for automatic and continuous measurement. Thus it may be used, in conjunction with appropriate electronic monitoring apparatus, to operate an early warning device to indicate the appearance of toxic conditions.

The monitoring system, described below, was designed to operate within an industrial complex in order to assess and control effluent quality. As well as being constructed to be electronically stable under these conditions, and to operate continuously and automatically, the system was built in such a way as to minimize the necessity for maintenance and operation by highly trained personnel. Further, the possibility that a slug dose of toxic material may pass through the system at a concentration that would kill fish immediately necessitated the introduction of a feature to cope with this eventuality. Thus an alarm condition was introduced whereby zero activity on the part of sensor fish over a period of a few hours would be indicated visually and audibly.

Successful laboratory simulations of the monitoring system induced two industrial concerns in South Africa, African Explosives and Chemical Industries (Modderfontein, Transvaal) and South African Paper and Pulp Industries (Mandini, Natal), to incorporate the system into their effluent control complexes. The description of the monitoring system presented below is that provided for the operating personnel at the industries concerned.

Prior to on-site installation the monitoring systems were tested in the laboratory, and the opportunity was taken to determine whether the system would provide long-term protection against chronic toxicity. The system was also compared to that based on opercular rhythm reponses by presenting it with similar challanges.

ULTRASONIC FISH ACTIVITY MONITOR CHARACTERISTICS

GENERAL INTRODUCTION AND INFORMATION

The equipment was developed as an on-line, continuous, biomonitoring system for detecting potentially harmful substances in flowing water. The system can monitor either five or ten channels (Plates 10 and 11), depending upon whether one or two monitoring chassis are fitted. Each channel consists of a tank, containing one fish, through which a sample of the water to be checked is passed. The system operates by monitoring and recording the activity level of each free-swimming fish. The activity of each channel is also continuously checked against an adjustable threshold level. Should the activity exceed this level a response is elicited for that channel. If more than six out of ten responses are elicited, an external audible alarm signal is generated.

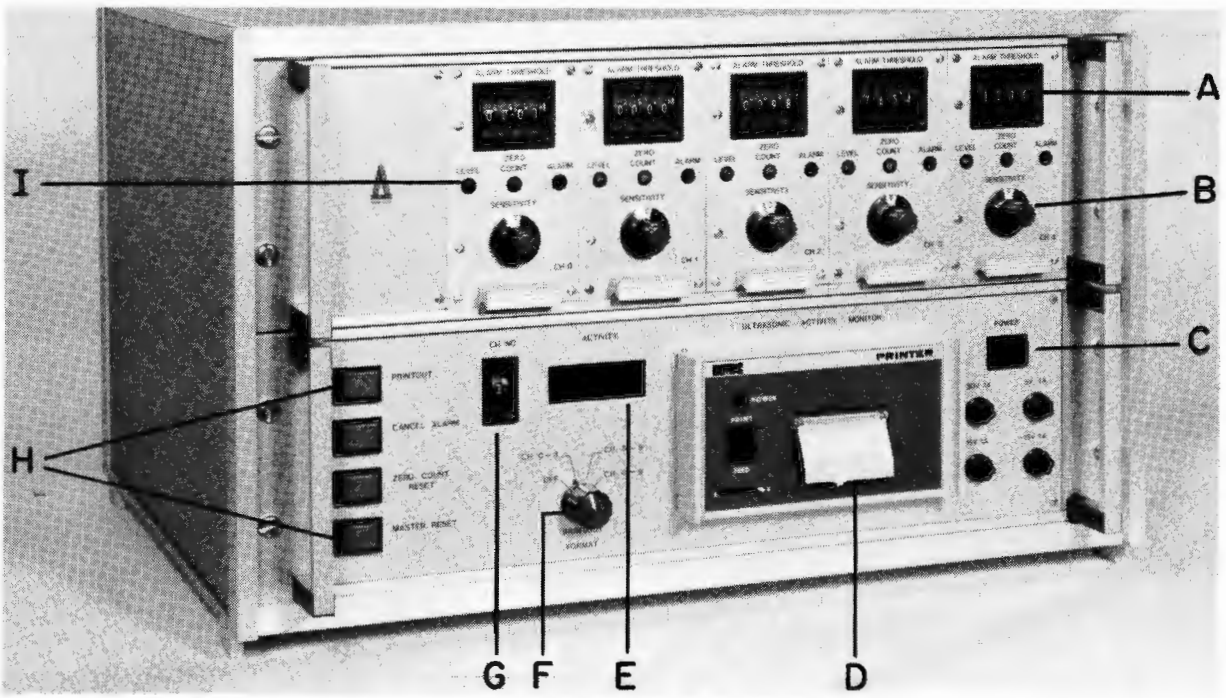

PLATE 10. Electronic fish activity monitoring unit (5 channel). A - 4 decade activity threshold control, B - sensitivity control, C - power on/off switch, D - printer, E - 4 digit LED activity count display, F - channel printout selector, G - channel select control, H - manual printout, alarm and master reset controls, I - signal level and alarm indicator lights.

The data for each channel is printed out once per hour or on demand.

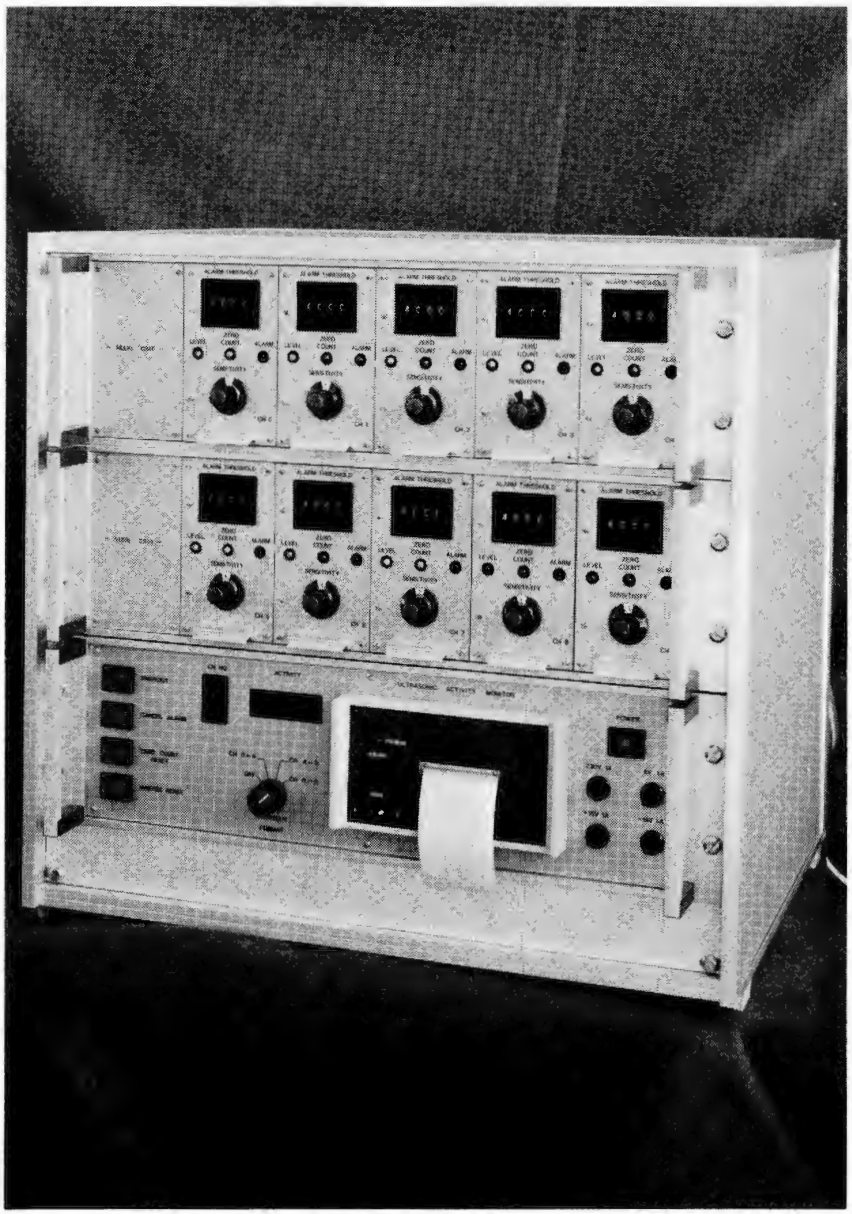

PLATE 11. Electronic fish activity monitoring unit (10 channel).

The system described refers to a 10 channel model with two monitoring chassis fitted. The five channel model is identical in all respects except that only one monitoring chassis is fitted.

The system senses the activity of the fish by means of ultrasonic echoes. Each tank contains one ultrasonic transmitting element and one receiver (Plate 12). The received signal consists of the sum of the various reflected sound waves. Whenever the fish moves, those reflec-

tions coming from the body of the fish exhibit a Doppler shift in frequency. This frequency shift, after mixing in the receiver with the other reflections, shows up as an amplitude modulation of the total signal. This modulation is processed and causes the activity counters to advance each time the fish moves.

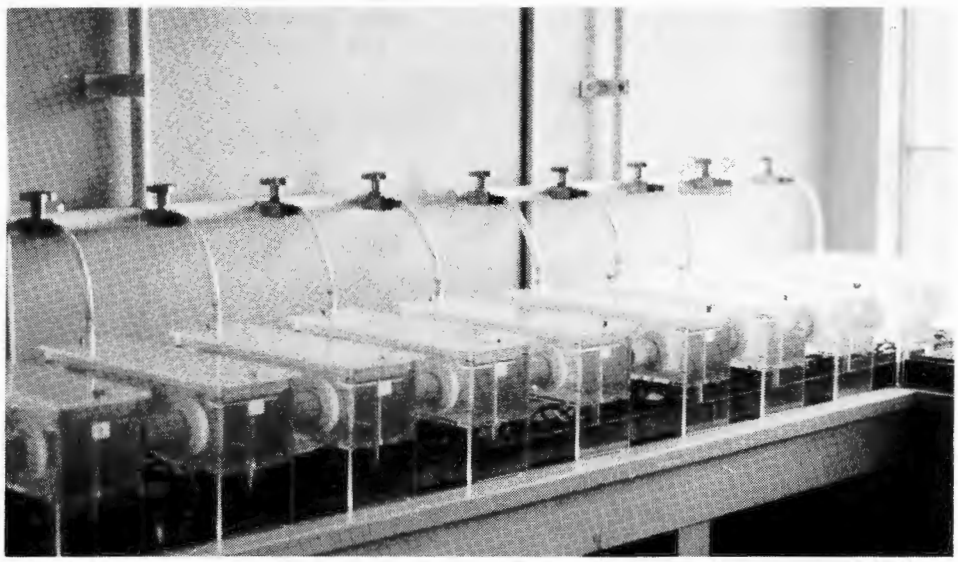

PLATE 12. Sensor fish activity detection chambers in one-site industrial biomonitoring facility.

TECHNICAL SPECIFICATIONS

Number of monitoring channels	-	10
Transmitter frequency	-	100 kHz
Activity range	-	0 to 3 600 counts/h
Range of alarm threshold switches	-	0 to 3 600 counts/h
ZERO COUNT function	-	Indicator is illuminated if no activity is registered for two consecutive hours.
External alarm	-	Activated when 6 out of 10 channels exhibit response status.

Printout	-	Once per hour, or on demand. Data for each channel are accompanied by channel identification and response status.
Printout format	-	May be selected to allow printout of channels 0 - 4; 5 - 9; or 0 - 9, or to inhibit printout altogether.
Power requirements	-	230 V ±10% 50 Hz (consumption ±150 Watt).

OPERATING INSTRUCTIONS

INTRODUCTION

Front Panel Controls

Monitoring Chassis

SENSITIVITY CONTROL - Adjusts the sensitivity of a particular channel to a convenient level on a scale of 0 to 10 depending upon fish size, type, etc.

ALARM THRESHOLD - This 4 decade thumbwheel switch is used for setting the alarm threshold of each channel. It is calibrated in units of activity per hour.

LEVEL INDICATOR - A green light emitting diode (LED) should glow permanently, showing that the signal from the associated tank is sufficient to enable the system to operate reliably.

ZERO COUNT INDICATOR - This orange LED on each channel will glow if no activity has been registered for that channel for a period of two consecutive hours.

ALARM INDICATOR - A red LED on each channel will glow if the activity for that channel exceeds the ALARM THRESHOLD setting in any given hour.

Control Chassis

POWER SWITCH - Switches the 230 V power on and off for the entire system.

PRINTOUT - This illuminated push switch is used to obtain a complete data printout at any time.

CANCEL ALARM - This switch resets the alarm registers of all channels and is used to clear the alarm condition when desired.

ZERO COUNT RESET - Used to clear the zero count condition of all channels.

MASTER RESET - A switch used to reset the entire system, to initiate the hourly printout and to reset all counters.

PRINTOUT FORMAT - A rotary switch which is used to determine how many channels are printed during the printout sequence. Channels 0 - 4, channels 5 - 9 or channels 0 - 9 may be selected. In the OFF position the printout is completely disabled.

PRINTER - The printer is located on the front panel of the control chassis. There is a Print/Advance switch on the printer itself. The PRINT function enables just one channel to be printed out and the ADVANCE function causes blank paper to be fed out as long as the switch is depressed. The printout of each channel's data is made up as follows:

- The first column indicates the alarm status. A plus sign (+) indicates a response condition for that channel, while a blank indicates that no response condition exists.

- The second column identifies the channel which is being printed out by means of a digit from 0 to 9.

- The third column is blank and is used as a space.

- The fourth, fifth, sixth and seventh columns contain the four digit activity count for that particular channel.

DISPLAY AND CHANNEL NO. SWITCH - The four digit, seven segment LED display indicates the activity of any one of the channels, which has been selected by the CH NO. thumbwheel switch. Thus the activity of any one of the channels can be continuously observed by selecting that channel. It is also this selected channel whose data is printed out when the PRINT switch on the printer is activated for a single channel printout.

FUSEHOLDERS - There are four fuseholders on the front panel below the POWER switch. They contain the fuses for the 230 V, +15 V, -15 V and +5 V lines.

Rear Panels

Monitoring Chassis

This panel carries a 37 pin connector for connecting the data output, supply voltages and control signals between the monitoring chassis and the control chassis. There are also 20 co-axial cables for connecting the tanks to the system. Ten connectors are labelled A 0 to 9 and ten B 0 to 9. The B connectors are for connection to the transmitters and the A connectors to the receiver transducers.

Control Chassis

This panel carries two 37 pin connectors for connecting the data output, supply voltages and control signals between the monitoring chassis and the control chassis. It also carries a Bulgin 3 pin socket for connecting the system to the 230 V power line. A 2 pin output socket is provided for connecting an external hooter as an audible alarm.

OPERATING PROCEDURE

- Connect the tanks to the system by plugging all the cables into their respective sockets, following the identification on the cables.
- Connect the two flat ribbon cables at the rear of the equipment between the two monitoring chassis and the control chassis.
- Connect the power lead to the mains supply.
- Make sure that the water level in each tank at least covers the ultrasonic transducers completely.
- Switch the power switch ON.
- Depress the MASTER RESET, CANCEL ALARM and ZERO COUNT RESET switches. All red and orange lights should now be extinguished.
- Ensure that all green lights come on (this may take up to several minutes).
- All the sensitivity controls can be initially set at 5. This may be altered later to optimize each channel's sensitivity according to the size of the particular fish.
- Switch the display to monitor the desired channel by means of the CH. NO. switch. Note that the displayed count advances for each separate movement of the fish in the corresponding tank.
- Set the PRINTOUT FORMAT switch to the appropriate position depending upon how many channels are to be printed. The first printout will occur one hour after the MASTER RESET switch was last activated and will continue at hourly intervals thereafter. After each printout has been completed, the activity counters are all reset to zero automatically. A printout will also occur if the PRINTOUT switch is activated. If the PRINT switch on the printer itself is activated, only the data of the channel corresponding to the setting of the CH. NO. switch will be printed out.
- The response threshold for each channel may be set by means of the thumbwheel switches, according to the activity pattern of each fish. If the activity of any channel exceeds the set threshold during any one hour, the red LED on that channel module will be illuminated and will remain on until cleared by means of the CANCEL ALARM switch. If six out of ten channels show a response condition, a 15 V signal will appear at the socket mounted on the rear panel of the instrument. A solid state hooter may be connected to this socket to act as an audible alarm.

CIRCUIT DESCRIPTION

INTRODUCTION

Figure 86 depicts the various major parts of the system. The two monitoring chassis with plug-in modules for channels 0 - 4 and 5 - 9 are shown with the fish tanks connected to them. Each group of five modules has its own 100 kHz oscillator. Each module contains the amplifier, filter and detector for processing the ultrasonic signal as well as the activity counters, alarm and zero count circuits and tri-state data buffers for placing the data on the common bus at the right time. The data from each group of five modules is fed to the control unit which contains the display, printer, control functions and power supplies. Control signals are also fed from the control unit to the monitoring chassis to carry out the Reset, Cancel Alarm and Zero Count functions, as well as to control the data output of each channel.

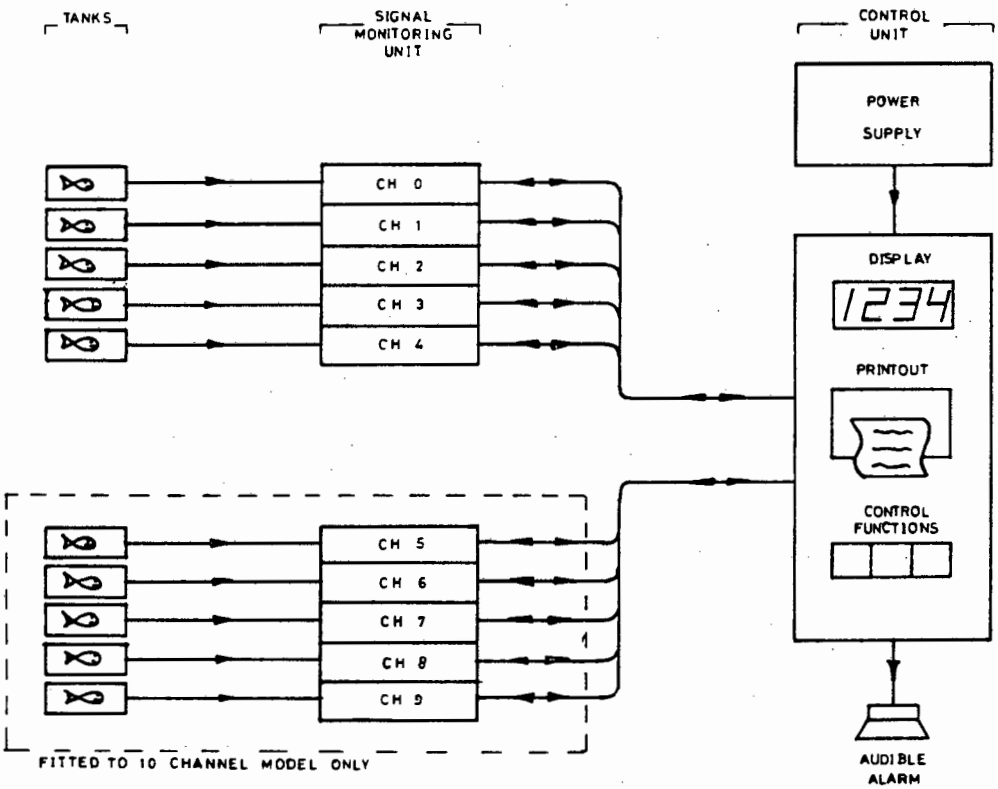

FIGURE 86. Block diagram.

CHANNEL MODULES

Figures 87, 88, 89 and 90 refer.

Analogue Circuitry

The ultrasonic signal containing the activity information is connected to the terminal marked INPUT (Figure 88). IC14, together with R1, R22 and R3 form a signal amplifier. D1, R9 and C4 rectify the signal and remove the 100 kHz carrier. The signal amplitude, after being integrated by R6 and C3, is amplified by IC15 and used to control the gain of IC14 by means of Q1. This automatic gain control function maintains the average value of the output voltage at a constant to compensate for differences between various ultrasonic transducers, temperature fluctuations etc. The signal across R9 is, therefore, a d.c. voltage which fluctuates when the fish moves. This voltage is filtered by a section of IC16, R10, R11, C5 and C6 which form a low-pass filter with a cutoff frequency of about 10 Hz . One of the four amplifiers in IC16 further amplifies the signal after which the d.c. component is removed by C7. The remaining two sections of IC16, together with their associated components, form a window comparator. The two terminals marked SENS are connected to the SENSITIVTY potentiometer shown on Figure 90. The setting of this potentiometer determines the levels at which the comparator switches. When the signal input at Y fluctuates outside these levels, one of the two outputs of the comparator will switch negative. This in turn triggers IC17, a monostable which produces a pulse of one second in length. One such pulse represents one movement of the fish or one ACTIVITY. A continuously moving fish would, therefore, record an activity level of 3 600 counts per hour.

While the automatic gain control circuitry maintains control, the output of IC15 is above 0,7 V . This keeps transistor Q2 switched on and the LEVEL indicator shown on Figure 90 is illuminated. This indicates that there is sufficient signal amplitude from the tank to enable the equipment to operate correctly. If the a.g.c. loop loses control, the output of IC15 falls below 0,7 V and Q2 switches off, thus causing the LEVEL indicator to be extinguished, indicating that there is insufficient signal amplitude from the tank for reliable operation.

Digital Circuitry

The pulses generated by IC17 are connected to a four decade BCD counter consisting of IC12 and IC5 (Figure 89). The BCD outputs of this

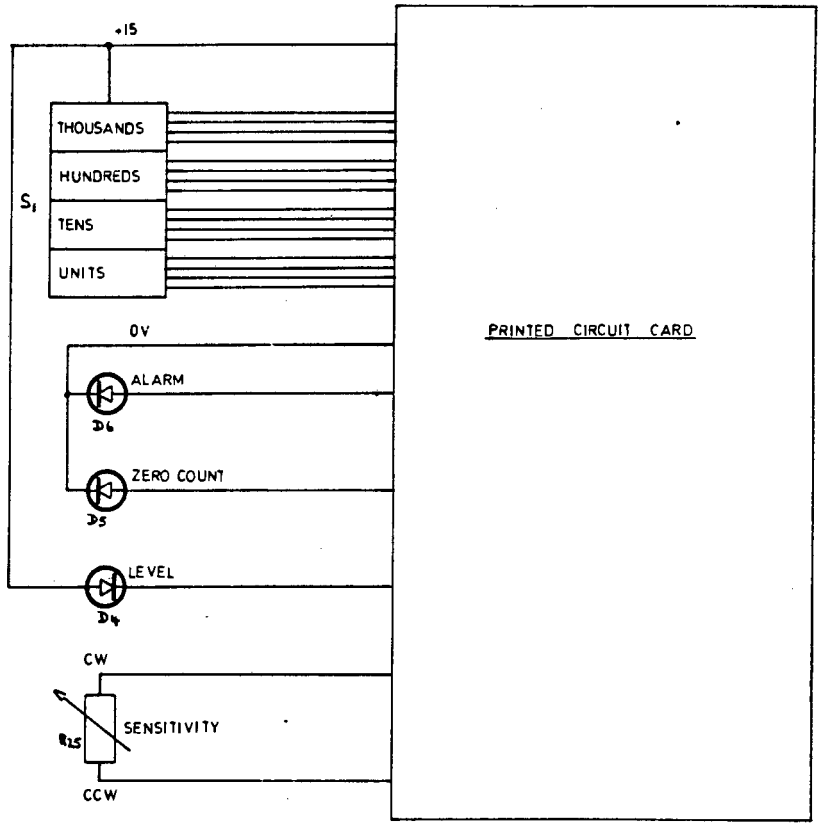

FIGURE 87. Channel module.

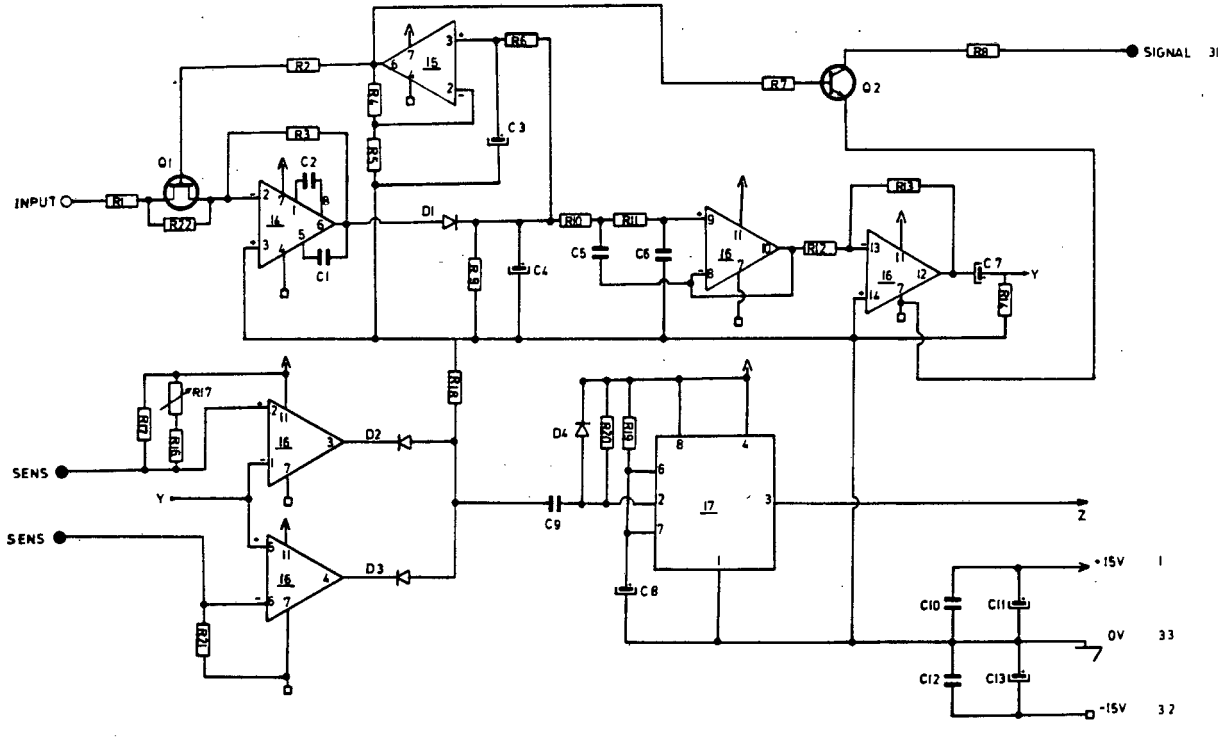

FIGURE 88. Channel module - analogue circuitry.

counter are connected to IC1 and IC3, which are eight bit data latches with tri-state outputs. The outputs of all these latches in the various channel modules are connected to a common data bus. The data is then switched onto this bus from each module in turn by enabling the output of its tri-state data latches.

ICs 6, 7, 8 and 9 form a four decade magnitude comparator. This compares the output of the four digit thumbwheel switch (SI on Figure 90), which is connected via TA1 to TD8, with the contents of the four digit counter. When the counter contents exceed the tumbwheel setting, the output of the comparator will go high, thus setting flip-flop IC13. An alarm status signal from this flip-flop is connected via the data latch IC2 to the common data bus. The output of this flip-flop is also used to switch driver transistor Q3, which in turn illuminates the red alarm indicator on the front panel of each module. The emitter of Q3 is also taken out to the "6 out of 10" decoder on the transmitter oscillator board.

Four lines of IC2 are used as channel identification. A four line BCD code is hardwired by means of wire links to correspond to the channel number of the module. This number is then printed out together with the activity count and alarm status.

The dual flip-flop, IC4, forms the ZERO COUNT alarm circuit. After the MASTER RESET switch has been operated, both sections of IC4 are in the reset condition. Any activity pulse appearing at pin 3 of IC4 will set this flip-flop. It will remain set, irrespective of whether any further pulses appear at pin 3 or not. Pin 2, the $\bar{Q}$ output is, therefore, low. After two hours, a pulse appears on the ZERO COUNT CLOCK line, which clocks this logic low into the second flip-flop. Pin 13, the Q output will therefore remain low and driver transistor remains off. If, however, no activity pulses appear at Pin 3 during the two hours, then Pin 2 ($\bar{Q}$) will be high when the ZERO COUNT CLOCK pulse appears and this high will be clocked into the second flip-flop. Pin 13 will then go high and Q4 will be switched on, illuminating the yellow ZERO COUNT indicator, which shows that no activity has been recorded for a period of two consecutive hours.

Transmitter Oscillator Board

This board is located behind the blank panel on the left of the modules in the monitoring chassis.

The 100 kHz oscillator, as well as the "6 out of 10" decoder are located on this board.

IC1 is connected as an astable multivibrator, running at 100 kHz . Frequency can be adjusted by R12 to the exact resonant frequency of the ultrasonic transducers. The oscillator drives five channels through current limiting resistors R20 through R24.

IC2 and IC3 form the 6 out of 10 decoder. The alarm outputs of 10 channels are connected to pins 21 through 25. IC2 sums the inputs and feeds the output to IC3, a Schmidt trigger. R16 is adjusted so that when six or more alarm signals are present, the output of IC2 exceeds the trigger level of IC3 and the output at pin 15 switches from -0,6 V to +15 V .

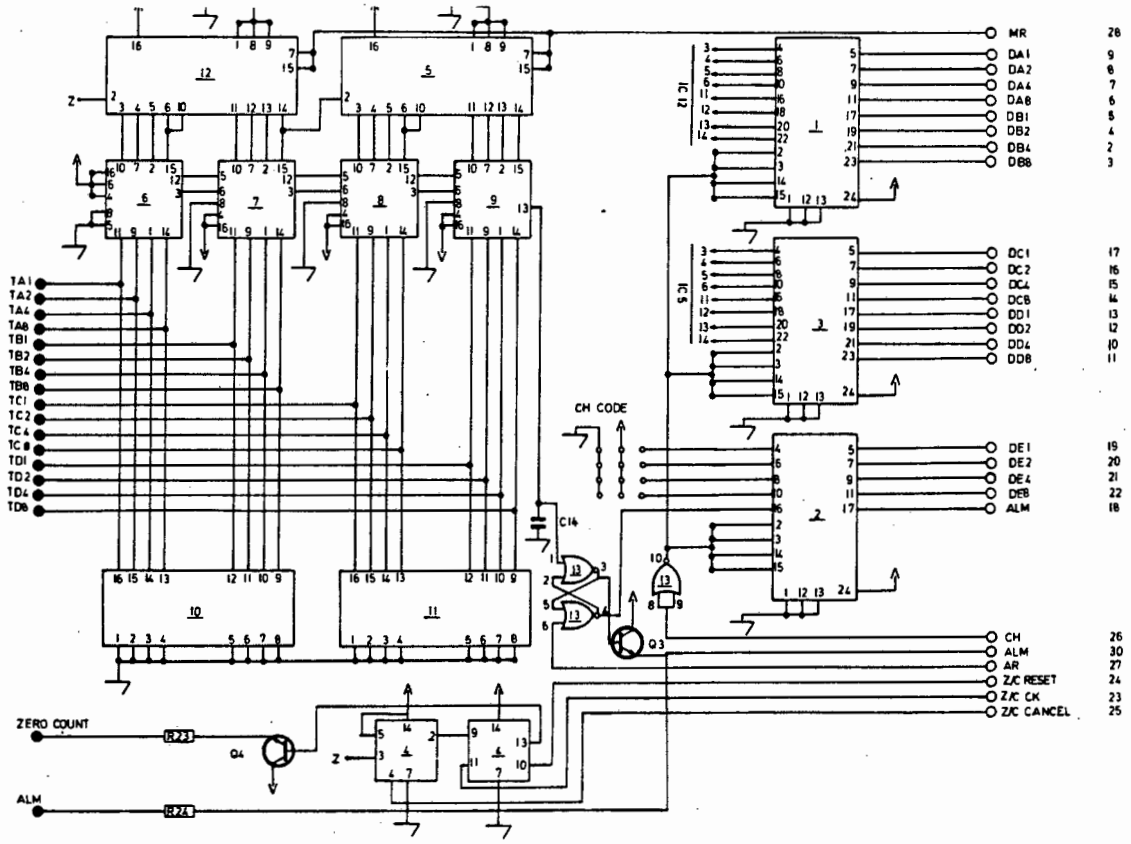

FIGURE 89. Channel module - digital circuitry.

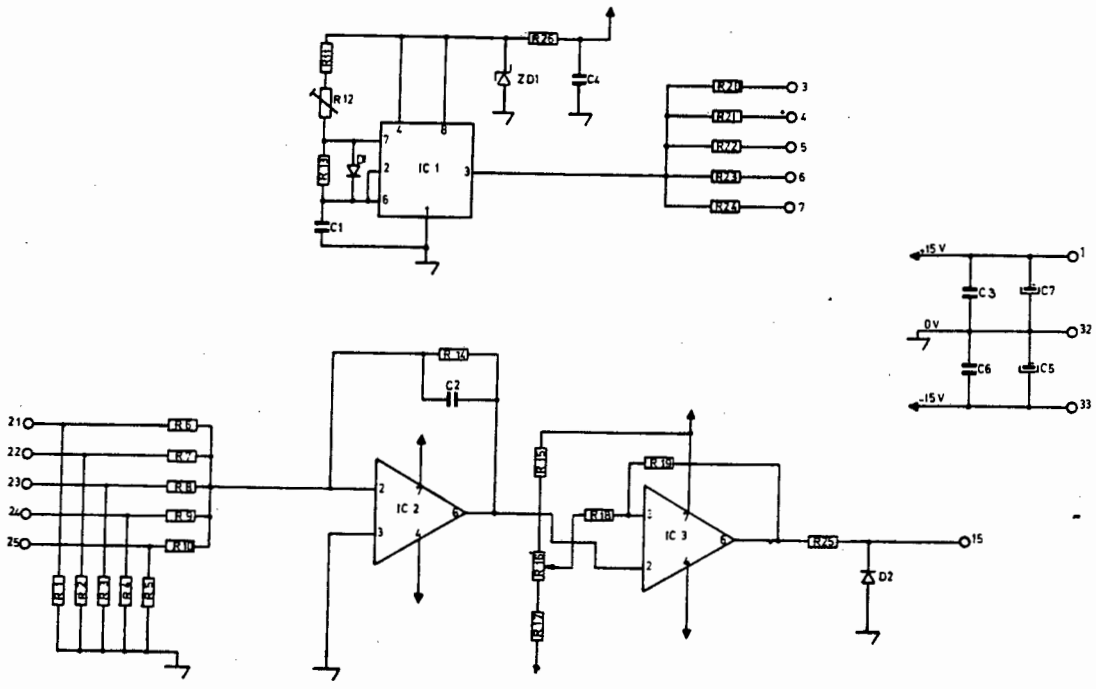

FIGURE 90. Transmitter oscillator board.

DATA BUFFER BOARD

This board (Figure 91), located in the control unit, contains level shifters/buffers to change the voltage levels of all the data lines from the +15 V levels provided by the CMOS circuitry to +5 V levels which are compatible with the TTL circuitry of the printer and seven segment display.

PRINT SEQUENCER BOARD

This board (Figure 92) contains all the circuitry necessary for the timing and sequencing of the various functions of the system.

The full wave rectified 100 Hz pulses from the power supply unit are connected to the board at pin 2. These pulses provide the timing for the whole system. One of the two monostables in IC1 is triggered by these rectified pulses, and provides the 15 V, 100 Hz square pulse required by the remainder of the circuitry.

IC2, IC3 and IC4 are all dual BCD counters and together they form a modulus 3 600 counter. The outputs of IC4 are decoded by a section of

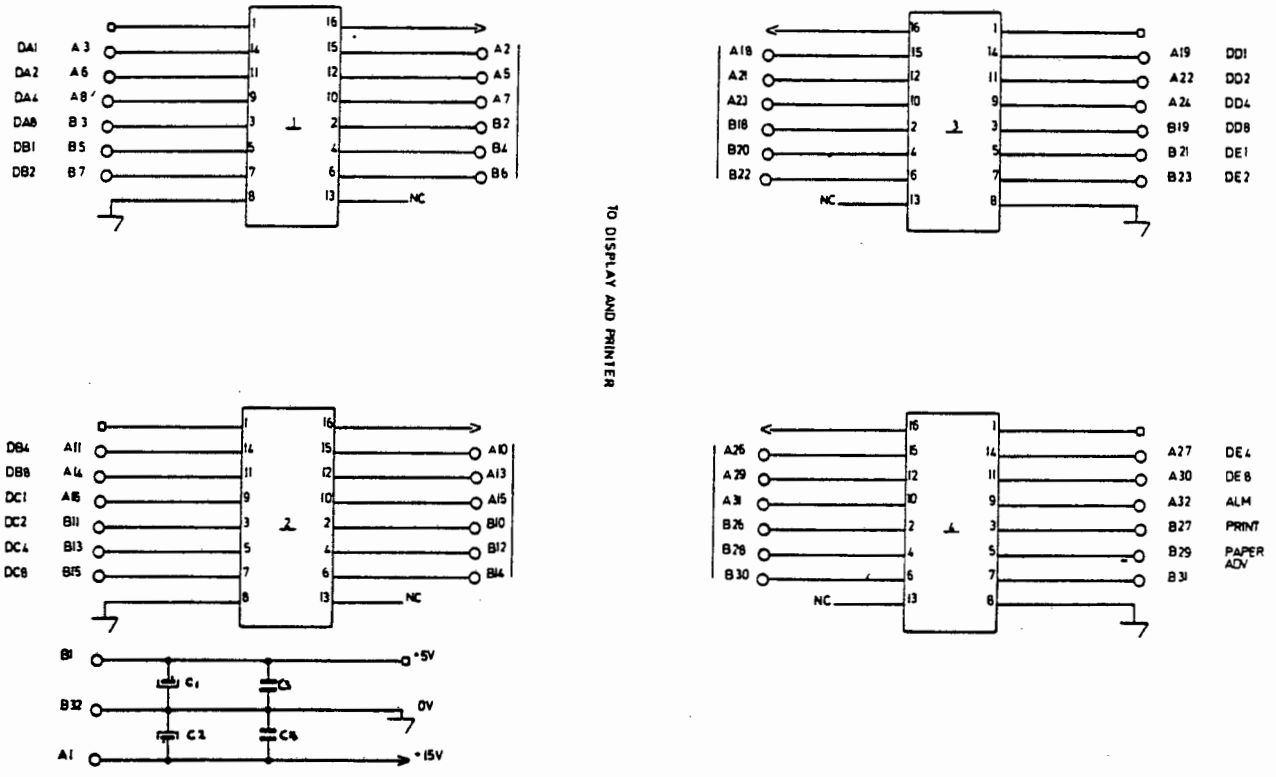

FIGURE 91. Data buffer board.

IC10 to provide one pulse every hour. Pulses at a rate of two per second appear at pin 12 of IC2. These pulses control the flow of data on the common bus as follows: IC6 is a four bit binary counter with parallel load facility. In between the hourly pulses, when no printing is taking place, the flip-flop comprised of two sections of IC12 is in the reset condition, with pin 10 high. This line from pin 10 of IC12 directs the 2 Hz clock pulse from IC2 to either the parallel load or serial input pin of IC6.

With pin 10 of IC12 high, the clock signal is directed to pin 1 (parallel load) of IC6. This repeatedly loads the BCD code of the digit set on the CHANNEL SELECT thumbwheel switch into IC6. This code is then fed to the decoder IC7 and the corresponding "Channel strobe" output is pulsed high. Each "Channel strobe" signal is connected to one of the channel modules and enables the data output of that module when pulsed high. This places the data of that channel onto the common bus from where it is routed to the display and printer.

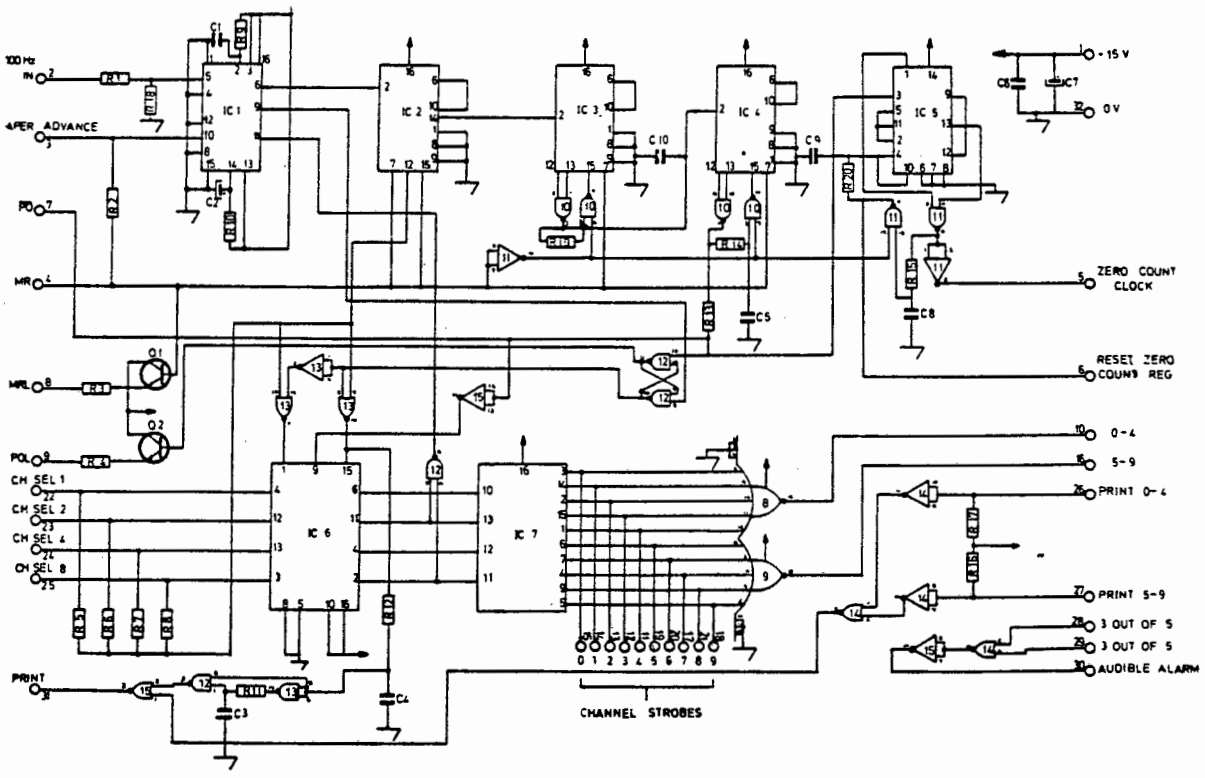

FIGURE 92. Print sequencer board.

With pin 10 of IC12 low (when a printout is initiated) the 1 Hz clock signal is directed to pin 15 (serial input) of IC6. The pulse which sets pin 10 of IC12 low also resets IC6 at pin 9. This ensures that the printout always begins at channel 0. The 2 Hz clock pulses coming at pin 15 now step IC6 through its count sequence from 0 to 9. The count is continuously decoded by IC7 and gates the data of channels 0 to 9 onto the common bus in the correct sequence. For each clock pulse, a PRINT pulse is generated at pin 31 of the edge connector. This causes each line of data to be printed after a short delay determined by R12 and C4. This delay allows the data to settle before printing. Some or all of these PRINT pulses may be inhibited depending on the setting of PRINT FORMAT switch. This is achieved by means of the two NOR gates IC8 and IC9, and three sections of IC14.

When IC6 has stepped through all 10 channels, the eleventh count is decoded by a section of IC12 (pins 4, 5 and 6). The output of this gate then triggers the second half of dual monostable IC1. This provides the "Paper advance" pulse at pin 3, to advance the printer after completion of a printout, thus separating one block of data from the next. This

pulse also resets the activity counts after the printout. IC5 and associated components form the ZERO COUNT clock circuit, comprising a modulus two counter. IC5 receives a clock pulse at pin 3 once every hour when the data is printed out. After two hours, a ZERO COUNT clock pulse is produced at pin 5, which is connected to all channel modules, and clocks the status of the ZERO COUNT register through to the ZERO COUNT indicators. Immediately after this clock pulse, a "Reset zero count register" pulse is produced at pin 6 of IC5. This is also connected to all the channel modules and reset all the ZERO COUNT registers in readiness for the next two hour cycle.

The decoded 6 out of 10 signals from the two monitoring chassis are OR'ed and are used as an "Audible alarm signal" to drive an external alarm (hooter) via a switching transistor on the power supply board.

DISPLAY BOARD

This board (Figure 93), mounted against the front panel, contains the 4 digit, seven segment LED display, and the four decoder/driver integrated circuits which convert the BCD data into seven segment format to drive the displays. The display inputs are driven in parallel with the printer from the output of the Data Buffer Board (Figure 91).

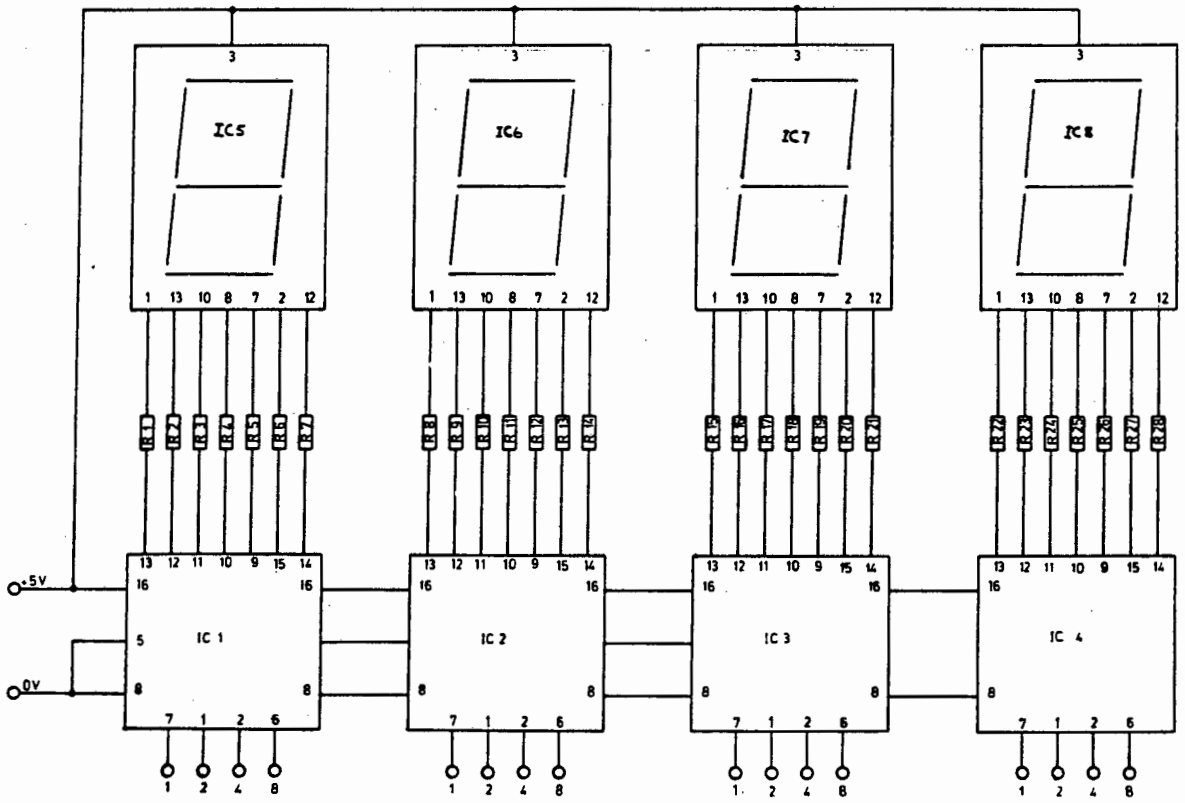

FIGURE 93. Display board.

POWER SUPPLY

The power supply for the entire system is contained in a module situated in the control chassis (Figure 94). It is a standard ±15 V supply capable of delivering 1A. Regulation is achieved by means of terminal integrated regulators. The supplies are protected by 1A fuses situated on the front panel. D3 is a blocking diode which enables a 100 Hz full wave rectified signal to the taken off as a clock signal for the Print sequencer board. There is also a 5 V supply capable of producing 1A. This supply drives the display and is also used on the Data Buffer board.

Q1, Q2 and R1 form a driver circuit which, when activated by the "Audible alarm" signal from the Print sequence board, can drive an external solid state hooter. "S" is an optional solid state beeper which can be mounted in the equipment as an additional feature.

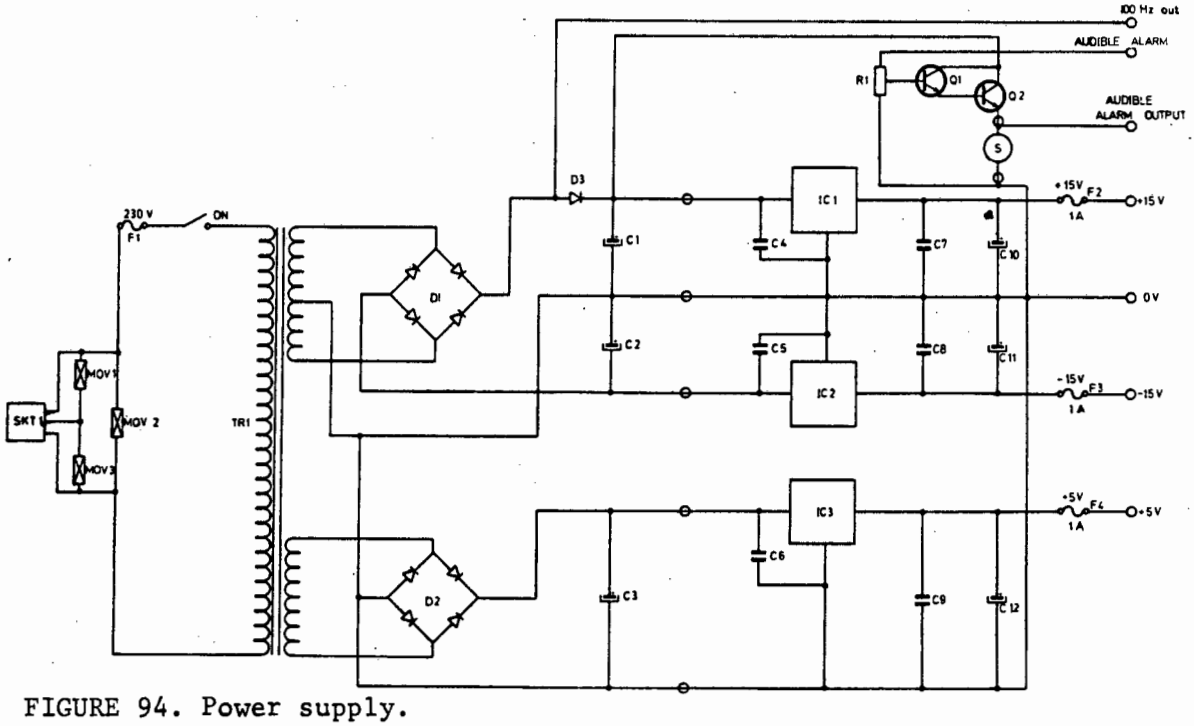

FIGURE 94. Power supply.

CALIBRATION

INTRODUCTION

This system has been so designed as to reduce the number of calibration adjustments to a minimum. The points which require adjustment are: transducer operating frequency, comparator symmetry, six-out-of-ten decoder sensitivity and the amplitude of the audible alarm signal.

EQUIPMENT REQUIRED

Oscilloscope	-	1 MHz bandwidth or better, input sensitivity 10 mV/cm or better
Digital Voltameter	-	3½ digit, 10 V DC range.

ADJUSTMENT PROCEDURE

(a) Connect transducers and fill all tanks with water. Do not introduce fish at this stage.

(b) Switch on equipment and allow a half-hour warming up period.

(c) Remove the four screws holding one of the monitoring chassis in place and slide this out of the cabinet to obtain access to the channel module boards and the transmitter oscillator board.

(d) Connect the oscilloscope probe to pin 6 of IC14 on the channel 0 module (Figure 88). Check that a 100 kHz signal is present at this point, having an amplitude of approximately 2 V p/p.

(e) Move the oscilloscope probe to pin 6 of IC15 on the same board. Check that a d.c. voltage of between 1 V and 2,5 V is present at this point. If no signal is measured in step (d) or if the d.c. voltage measured is less than 1 V, adjust R16 on the transmitter oscillator board (Figure 89) until these conditions are satisfied.

(f) Repeat steps (d) and (e) for channels one to four, readjusting R16 so that the voltages on all five boards are within the stated limits.

(g) Turn the SENSITIVITY control fully clockwise on channel module 0. Connect the digital voltameter between any one of the pins of the SENSITIVITY potentiometer and OV. Adjust R17 so that the reading is less than 10 mV .

(h) Repeat steps (d), (e), (f) and (g) for the second monitoring chassis containing channel modules five to nine and the second transmitter oscillator board.

(i) The final adjustment step consists of setting the amplitude of the audible alarm signal. This is accomplished by adjusting R1 on the power supply board (Figure 94) until the volume of the external hooter reaches the desired level.

COMPONENTS LIST

CHANNEL MODULE (Figures 87, 88, 89 and 90)

Resistors

R1	2 K 2	1/4 W	
R2, R7, R12	3 K 3	1/4 W	
R3, R19	560 K	1/4 W	
R4, R17	18 K	1/4 W	
R5	2 K 7	1/4 W	
R6	150 K	1/4 W	
R8, R23, R24	1 K	1/4 W	
R9, R18, R21	12 K	1/4 W	
R10, R11	820 K	1/4 W	
R13	27 K	1/4 W	
R14	100 K	1/4 W	
R20	270 K	1/4 W	
R16, R22	22 K	1/4 W	
R15	50 K	1/2 W	15 turn trimmer
R25	1 K	1/4 W	
Potentiometer			

Capacitors

C1	100 pf	50 V	Ceramic
C2	10 pf	50 V	Ceramic
C3, C7	100 µf	16 V	Tantalum
C4	0,01 µf	200 V	Polycarbonate
C5	0,047 µf	200 V	Polycarbonate
C6	0,1 µf	200 V	Polycarbonate
C8	2,2 µf	35 V	Tantalum
C9	0,039 µf	200 V	Polycarbonate
C10, C12	22 µf	35 V	Tantalum
C11, C13	10 nf	50 V	Ceramic
C14	22 nf	50 V	Ceramic

Semiconductors

Q1	2N3820	Transistor
D1, D2, D3	IN3070	Diode
D4, D5, D6		Light emitting diode

Integrated Circuit Packages

IC1 to IC3	SCL4508B	Data latch
IC4	SCL4013B	Dual flip-flop.
IC5, IC12	SCL4518B	Dual counter
IC6 to IC9	SCL4585B	4 bit counter
IC10, IC11	8 x 100 K	Resistors
IC13	SCL4518B	Quad nor gate
IC14	SN709P	Op-Amp
IC15	SN741P	Op-Amp
IC16	RC4136DB	Quad Op-Amp
IC17	NE555V	Timer
S1	44010 S	4 decade BCD thumbwheel switch

TRANSMITTER OSCILLATOR BOARD

Resistors

R1 to R10	10 K	1/4 W	
R11	1 K	1/4 W	
R12, R16	10 K		15 Turn Trimmer
R13, R18, R25	10 K	1/4 W	
R14, R17	1 K 5	1/4 W	
R15	4 K 7	1/4 W	
R19	100 K	1/4 W	
R20 to R24	2 K 2	1/4 W	
R26	560 Ω	1/4 W	

Capacitors

C1	560 pf	50 V	Styroflex
C2	1 nf	50 V	Ceramic
C3, C6	10 nf	50 V	Ceramic

C4	100 nf	50 V	Ceramic
C5, C7	10 µf	35 V	Tantalum

Semiconductors

D1, D2	IN3070	Diode
ZD1	8,2 V 400 mW	Zener diode

Integrated Circuit Packages

IC1	NE555V	Timer
IC2, IC3	NS741P	Op-Amp

PRINT SEQUENCER (Figure 92)

Resistors

R1, R2, R13, R14	10 K	1/4 W
R3, R4	180 Ω	1/4 W
R5 to R9, R16, R17	100 K	1/4 W
R10, R11	1 M	1/4 W
R12	22 K	1/4 W
R14, R15, R19, R20	47 K	1/4 W
R21	1 K	1/4 W

Capacitors

C1, C3, C4	0,047 µf	200 V	Polycarbonate
C2	0,47 µf	35 V	Tantalum
C5, C6, C8 to C11	10 nf	50 V	Ceramic
C7	10 µf	35 V	Tantalum

Semiconductors

Q1, Q2	BC107	Transistor

Integrated Circuit Packages

IC1	SCL4528B	Dual monostable
IC2, IC3, IC4	SCL4518B	Dual counter
IC5	SCL4013B	Dual flip-flop

IC5	SCL4013B	Dual flip-flop
IC6	SCL4516B	Binary counter
IC7	SCL4028B	Decoder
IC8, IC9	SCL4078B	8 input NOR gate
IC10, IC11, IC12	SCL4011B	Quad Nand gate
IC13, IC14, IC15	SCL4001B	Quad Nor gate

DATA BUFFER BOARD (Figure 91)

Capacitors

C1, C2	10 µf	35 V	Tantalum
C3, C4	10 nf	50 V	Ceramic

Integrated Circuit Packages

IC1 to IC4	SCL 4010B	Hex buffer

DISPLAY BOARD (Figure 93)

Resistors

R1 to R28	180 Ω	1/4 W

Integrated Circuit Packages

IC1 to IC4	SN7447N	Decoder
IC5 to IC8	MAN71	Display

POWER SUPPLY (Figure 94)

Resistors

R1	10 kΩ	Potentiometer

Capacitors

C1, C2	3300 µf	40 V	Electrolytic
C3	4700 µf	25 V	Electrolytic
C4 to C9	10 nf	50 V	Ceramic
C10 to C12	10 µf	35 V	Tantalum

Semiconductors

D1, D2	VH448	6A	Bridge rectifier
D3	IN4007		Diode
Q1	BC107		Transistor
Q2	BD437		Transistor

Integrated Circuit Packages

IC1	MC7815	+15 V	Regulator
IC2	MC7915	-15 V	Regulator
IC3	MC7805	+5 V	Rgulator

SKT 1	Bulgin mains connector		
MOV 1 to MOV 3	250 V	250LA40B	Varistor
S1	Push switch		
F1 to F4	1A fuse in holder		
TR1	50 VA	Transformer	
S	Sonalert		

CONTROL CHASSIS FRONT PANEL

Resistors

R1, R2	220 Ω	1/2 W
S1 to S4	Push switch	
S5	BCD Thumbwheel switch	
S6	Rotary switch	
L1 to L4	6 V indicator lamp	
P1	NP7A Printer	

Semiconductors

D1, D2	VH448	6A	Bridge rectifier
D3	IN4007		Diode
Q1	BC107		Transistor
Q2	BD437		Transistor

Integrated Circuit Packages

IC1	MC7815	+15 V	Regulator
IC2	MC7915	-15 V	Regulator
IC3	MC7805	+5 V	Rgulator

SKT 1	Bulgin mains connector		
MOV 1 to MOV 3	250 V	250LA40B	Varistor
S1	Push switch		
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CONTROL CHASSIS FRONT PANEL

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R1, R2	220 Ω	1/2 W
S1 to S4	Push switch	
S5	BCD Thumbwheel switch	
S6	Rotary switch	
L1 to L4	6 V indicator lamp	
P1	NP7A Printer	

LABORATORY EVALUATIONS OF THE EFFICACY OF THE AUTOMATIC BIOMONITORING SYSTEM BASED UPON ULTRASONIC DETECTION OF FISH LOCOMOTOR BEHAVIOUR PATTERNS

As water pollution control becomes a reality, discharges of high concentrations of toxicants are more and more being replaced by long-term discharges of low levels. Thus, fish are being increasingly exposed to low levels of pollution for long periods of time, and fish toxicologists are becoming more and more concerned about the effects of long-term low-level exposure.

Long-term (chronic) flow-through bioassays extending over the entire life cycle of the test organism are used to determine the highest (safe) concentration of a pollutant at which no adverse biological effects are observed. Information from such studies has been used to derive "application factors" that permit the calculation of "safe" concentrations of pollutants from short-term acute toxicity.

Continuous monitoring of behavioural responses of fish may permit rapid detection of pollutants at stressing levels as low as those found by chronic tests to be biologically safe. These techniques, may, therefore, prove useful for in-plant monitoring of the toxicity of industrial effluents and may protect aquatic organisms in receiving waters from sudden exposure to adverse conditions. However, behavioural studies have not achieved importance in pollution control work because experimental evidence of their biological significance is lacking.

The research described in this section was designed, therefore, to establish whether the biomonitoring system would be as effective in protecting aquatic ecosystems against long-term low-level toxic concentrations as it is in safeguarding the environment from acute lethal effects. The system was also presented with similar challanges as that based upon fish respiratory response, in order to provide performance comparisons.

EXPERIMENTAL PROCEDURE

Some 42 experimental series, employing 330 sensor fish, were conducted in order to analyze system efficiency. Only adult male guppies were

used so that metabolic functions associated with reproduction would not interfere with the results. The propensity of adults to eat their young may also have provided an uncontrolled parameter. The adult males were, therefore, separated from the females for the duration of the monitoring procedures.

For each experimental series, sensor fish were established, individually, in 10 chambers. The chambers were supplied with continuously flowing dechlorinated municipal tap water (approximate hardness 85 mg/dm^3 as $CaCO_3$), the pH being adjusted to 7,0 and the temperature to 22 ±1 °C . All experiments were carried out under conditions of natural photoperiod. The sensor fish were allowed to recover from the stress of enclosure within the experimental chambers for a period of 48 h before any activity levels were measured. Activity levels were established for five days, post-acclimation, for each hour of the day and night and used to establish critical response limits for each individual fish using a statistical method of maximum variance, as previously described. The response levels were set, independently for each channel, using the alarm threshold controls.

Each sensor fish was deemed to have responded as soon as the channel alarm circuit triggered, indicated by a red warning light on a particular channel and on the hourly printout. A toxicant was defined as being detected when a majority of the sensor fish (60%) had elicited a response.

To be effective in an industrial in-plant situation, the system would need to detect the onset of a deleterious condition within a day. All experiments, except one, were, therefore, curtailed after a toxicant exposure period of 24 h . The series of experiments conducted to establish the degree of response to chronic intoxication were sustained through an exposure period of seven days.

RESPONSE TO CHRONIC INTOXICATION

For each experimental series, 10 fish were exposed to that toxicant concentration which had been established, in previous experiments, as being the minimum level which deleteriously affected growth and/or

reproduction. The toxicant concentrations used were 0,1 mg Cu^{2+}/dm^3; 0,02 mg Cd^{2+}/dm^3; 0,001 mg Hg^{2+}/dm^3; 1,5 mg C_6H_5OH/dm^3; 3,0 mg NH_3-N/dm^3 and 0,015 mg CN^-/dm^3. These levels were set up in the chambers by the addition of pre-determined volumes of 1 g/dm^3 solutions, being maintained thereafter using the dosing units previously described.

Sensor fish were exposed for a period of seven days, whether responses occurred or not. Subsequently, fish which had elicited a response were individually mated with virgin females that had also been exposed to the same toxicant concentrations for a similar period of time.

DEFINING THRESHOLD DETECTION LEVELS

The response of male guppies to increasing concentrations of a number of toxicants was utilized to determine the threshold levels of detection. The initial toxicant concentrations, established in the chambers, were based upon results obtained in previous experimentation where opercular rhythms were monitored. The toxicant concentrations were set up as above being maintained in the chambers for a period of 24 h . Subsequent to this period of exposure, sensor fish were allowed to resume a normal activity level in continuously flowing freshwater before being exposed to successively higher concentrations. Each experimental series was concluded when 60% or more of the sensor fish had elicited a response to a particular toxicant concentration. For all determinations, a separate group of fish were directly exposed to the final toxicant concentration in order to confirm the defined threshold.

THE EFFECT OF DIURNAL ACTIVITY RHYTHMS UPON SENSOR FISH RESPONSE TO INTOXICATION

The utilization of a fixed level of activity as a critical response limit, based upon maximum variance, may result in a belated response to intoxication, by sensor fish, when the activity level is naturally depressed. This factor could result in the elapsed time to response being artificially lengthened to an extent where adequate protection against lethal intoxication is precluded. Sensor fish were, therefore, exposed to selected acute levels of a number of toxicants, where exposure was initiated at different times of day.

For each toxicant concentration, chosen to provide responses in all sensor fish within 24 h, four independent groups of five fish were employed. Exposure was initiated at 06h00, 12h00, 18h00 or 24h00. The average response times for the groups were compared using Student's 't' test at two levels of significance ($P<0,05$, $P<0,01$). Toxicant addition, to individual chambers, was discontinued as soon as its sensor fish had elicited a response. Each group of twenty fish was maintained independently in stock tanks, supplied with continuously flowing freshwater, for a period of three months after experimentation.

EFFECTS OF ACCLIMATION TO INTOXICATION UPON SENSOR FISH RESPONSE

Six experiments were designed to establish whether sensor fish, exposed for long periods to low levels of intoxication, would provide adequate advance warning against acute conditions. Groups of five fish were maintained in the highest toxicant concentrations which elicited no response in the experiment to define threshold limits for a period of three months. On transference to the activity detection chambers, the sensors were exposed to those acute toxicant concentrations employed in the previous experiment. Exposure was initiated at 12h00 so that a direct comparison could be made between the results achieved. Toxicant exposure was discontinued on receipt of a response and the sensor fish were maintained for a further three months in freshwater in order to establish survival potential.

DISCUSSION OF RESULTS

All sensor fish exposed to chronic levels of copper, phenol and ammonia responded within 24 h of initial exposure, indicating an olfactory recognition of the changed environment (Table 83). It is improbable that any acute pathological effects would have been evinced, within this time period, at such low toxicant concentrations. On the other hand, the response to a chronic level of cyanide by all 10 sensor fish, occurring as it did within 72 h of intitial exposure, may well have been due to an alarm reaction to progressive physiological stress. Sensor fish did not respond to chronic levels of either cadmium or mercury and it is, therefore, doubtful whether adequate protection against chronic intoxication, by these metals, is afforded using the system. The fish exposed to cadmium and mercury were maintained for a further three weeks in the chronic solutions without any apparent effect on activity rates.

TABLE 83. Elapsed times to response by *Poecilia reticulata* exposed to chronic levels of intoxication

Toxicant	Concentration (mg/dm^3)	Elapsed times to response (h) for sensor fish									
		1	2	3	4	5	6	7	8	9	10
Copper	0,1	9	12	8	14	13	7	9	13	15	11
Cadmium	0,02	-	-	-	-	142	-	-	-	-	-
Mercury	0,001	-	-	-	-	-	-	-	-	-	-
Phenol	1,5	3	5	10	6	7	4	6	6	5	8
Ammonia	3,0	11	9	6	6	6	3	7	9	6	4
Cyanide	0,015	50	64	69	48	71	70	51	58	64	53

Sensor fish exposed to chronic intoxication by copper, phenol, ammonia and cyanide, for seven days, were mated with virgin females exposed to the same toxicant levels for a similar period of time. No adverse effects upon reproductive potential were realized. The system, therefore, affords adequate protection against chronic intoxication by these pollutants.

Threshold levels of toxicant detection for industrial effluent control, *viz.* responses by a majority of sensor fish within 24 h of initial exposure, were established as 0,05 mg Cu^{2+}/dm^3; 0,1 mg Cd^{2+}/dm^3; 0,025 mg Hg^{2+}/dm^3; 0,5 mg C_6H_5OH/dm^3; 1,0 mg NH_3-N/dm^3 and 0,05 mg CN^-/dm^3 (Table 84). Thus the system would effectively protect receiving waters against immediate hazard. Sensor fish used in the tests were transferred to freshwater on their completion and exhibited 100% survival.

The efficiency of the system, in protecting aquatic environments from deleterious toxic effects, was not adversely influenced by the time of day an effect may be initiated. The elapsed time to response by sensor fish was affected in two cases only, mercury and cyanide, when exposure was initiated at periods of low activity (Table 85). In both cases, however, the increased exposure period, caused by the delayed response, did not seriously affect the fish. All fish survived when toxicant addition was discontinued on the receipt of a response.

Loss of sensitivity to acute intoxication occurred following long-term exposure to very low levels of cadmium, mercury, phenol and ammonia (Table 86). Long-term exposure of fish to phenol and ammonia probably resulted in a loss of olfactory sensitivity, whereas pre-exposure to cadmium and mercury may result in a degree of resistance to acute stress being induced. Response to intoxication, nevertheless, occurred swiftly enough to provide adequate protection against lethal effects. No fish died when transferred to freshwater on the receipt of a response.

The experiments described above show that it is probably feasible to use fish in continuous monitoring systems at industrial sites to warn of developing acutely toxic conditions in time to prevent acute damage to the fish populations present in the receiving system. The movements

TABLE 84. Elapsed times to response by *Poecilia reticulata* exposed to various degrees of intoxication

Toxicant	Concentration (mg/dm^3)	Elapsed times to response (h) for sensor fish									
		1	2	3	4	5	6	7	8	9	10
Copper	0,01	–	–	–	–	–	21	–	–	–	–
	0,025	–	14	–	–	–	18	–	–	–	–
	0,05	–	7	18	16	15	9	–	18	–	21
	0,05	*18*	*20*	–	*21*	*16*	*17*	–	–	*13*	–
Cadmium	0,05	–	19	13	–	–	–	–	–	–	–
	0,1	21	11	9	–	18	15	–	13	12	10
	0,1	*23*	*16*	*15*	*18*	*13*	*10*	*23*	*23*	–	*21*
Mercury	0,01	18	–	–	16	–	23	–	–	–	19
	0,025	9	11	13	11	9	14	13	11	10	17
	0,025	*17*	*21*	*15*	–	–	*16*	*12*	*18*	–	*14*
Phenol	0,25	23	–	–	–	19	–	–	–	–	–
	0,5	21	13	18	–	14	19	–	20	21	–
	0,5	*18*	*16*	*13*	*19*	*17*	*10*	–	*19*	*17*	*15*
Ammonia	0,5	15	–	–	17	–	–	20	–	–	–
	1,0	7	11	13	14	8	14	18	16	17	17
	1,0	*7*	*10*	*8*	*7*	*9*	*5*	*9*	*13*	*18*	*9*
Cyanide	0,025	–	17	–	–	18	–	–	21	–	–
	0,05	13	18	11	9	11	11	11	8	15	8
	0,05	*9*	*12*	*14*	*8*	*10*	*17*	*11*	*15*	*11*	*10*

Only those toxicant concentrations in which any sensor fish response was observed, within 24h of exposure, are represented.
Figures in italics represent responses by separate groups of fish utilized for confirmation of threshold levels.

TABLE 85. The effect of time of initiation of intoxication upon response for *Poecilia reticulata* exposed to different toxicants

Nominal toxicant concentration (mg/dm^3)	Exposure initiation time	Times to response (h) for sensor fish 1	2	3	4	5	Average response time(h)
0,1 mg Cu^{2+}/dm^3	06h00	4	6	9	3	4	5,2
	12h00	3	5	7	9	5	5,8
	18h00	6	4	7	10	9	7,2
	24h00	5	10	12	7	8	8,4
0,2 mg Cd^{2+}/dm^3	06h00	10	8	11	9	9	9,4
	12h00	9	7	12	9	8	9,0
	18h00	10	10	13	14	9	11,2
	24h00	12	9	15	7	13	13,2
0,05 mg Hg^{2+}/dm^3	06h00	11	8	10	10	10	9,8
	12h00	12	14	9	10	12	11,4
	18h00	13	18	16	19	17	16,6††
	24h00	21	13	17	11	15	15,4†
1,0 mg Phenol/dm^3	06h00	8	7	5	4	9	6,6
	12h00	10	6	4	3	3	5,2
	18h00	7	11	6	5	3	6,4
	24h00	8	8	7	3	4	6,0
1,0 mg Ammonia/dm^3	06h00	6	7	10	10	11	8,8
	12h00	7	9	14	9	11	10,0
	18h00	4	11	10	7	8	8,0
	24h00	11	5	5	12	9	8,4
0,05 mg CN^-/dm^3	06h00	14	11	8	7	13	10,6
	12h00	13	10	9	9	7	9,6
	18h00	15	12	11	13	16	13,4†
	24h00	17	10	9	11	10	11,4

† and †† indicate that the average response times were significantly different at significance levels of $P<0,05$ and $P<0,01$ respectively.

TABLE 86. Elapsed times to response by *Poecilia reticulata* to acute intoxication* subsequent to long-term exposures to sub-chronic toxicant concentrations

Nominal toxicant concentration (mg/dm^3)	Times to response (h) for sensor fish					Average response time(h)
	1	2	3	4	5	
0,1 mg Cu^{2+}/dm^3	4	8	8	9	7	7,2
0,2 mg Cd^{2+}/dm^3	12	16	13	18	20	15,8†
0,05 mg Hg^{2+}/dm^3	21	18	22	19	19	19,8††
1,0 mg Phenol/dm^3	19	23	17	16	12	17,4††
1,0 mg Ammonia/dm^3	20	19	16	-	17	18,0††
0,05 mg CN^-/dm^3	12	10	9	8	10	9,8

* Exposure was initiated at 12h00 (see Table 85)

† and †† indicate that the average response times were significantly different from those obtained in previous experimentation at significance levels of $P<0,05$ and $P<0,01$ respectively.

of guppies can be continuously monitored and used to detect lethal and sub-lethal concentrations of a wide range of pollutants. The criterion for detection would be a certain number of fish showing an arbitrarily defined response in activity during a particular time period. In conjunction with stream water quality standards for chronic exposure, such biological monitoring systems should make it possible for healthy fish populations to co-exist with industrial water use.

In choosing a specific criterion for detection, the risk of not detecting stress quickly enough must be weighed against the risk of false detections and the choice would probably be determined by the nature of the pollutant. If a pollutant is easily detected by the biological monitoring system, and if the toxic effects are reversible, the criterion for detection might be responses by 75% of the test fish, in order to avoid the false detections that would necessitate expensive remedial action or a temporary shut-down. On the other hand, an effluent containing a fast-acting toxicant whose effects are irreversible would require a criterion that leads to rapid detection (responses by 25% to 50% of the test fish), and would necessitate the expense of installing holding ponds or recycling facilities to accommodate a relatively higher number of false detections. Alternatively, the elapsed time to response could be reduced by presenting test fish with a higher effluent concentration than is delivered to the stream. The higher a toxicant concentration, the faster the toxicant is taken up by the fish and the sooner the response appears.

The detection limits of the monitoring system could be related to standards for chronic exposure by additional growth and reproduction experiments. The growth and reproduction experiments in the present study indicated that $^{2}/_{5}$ of the lowest cadmium concentration detected and $^{1}/_{10}$ of the lowest mercury concentration detected were almost certainly not safe levels for chronic exposure. In an industrial in-plant situation this may be alleviated by using a much more sensitive test fish than the most sensitive species present in the receiving ecosystem, whenever possible.

Whether or not the detection limits of the monitoring technique are related to stream standards for chronic exposure, the technique could be useful in the prevention of accidental spills or environmental changes that produce acutely toxic conditions, detection occurs soon enough for fish to recover if toxicant addition is promptly discontinued.

In an actual industrial situation, water and waste qualities vary unpredictably, and it would certainly be desirable to monitor both breathing and activity. It is conceivable that some harmful combination of environmental conditions and waste quality would be detected by monitoring one biological function but not by monitoring another.

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SECTION V

THE ACCEPTABILITY, APPLICATION AND ADMINISTRATION OF CONTINUOUS AUTOMATIC BIOLOGICAL MONITORING SYSTEMS

INTRODUCTION

"The public interest requires doing today those things that men of intelligence and good will would wish, five or ten years hence, had been done."

Edmund Burke (1729-1797)

The quality of human life depends upon progressive industrial technological advancement; human survival upon the maintenance of a relatively stable natural environment. The two are not necessarilly compatible. Industry creates waste in relation to the prevailing market demand for its products rather than to the capacity of the environment to accommodate that waste.

In the past, industry has operated on the premise that natural aquatic ecosystems have an infinite capacity for waste assimilation, without the ecosystem being significantly spoiled. However, we now realise that an unmanaged environment is not capable of absorbing waste material without being seriously impaired at certain times and places. During the past five years the extent of the areas deleteriously affected, as well as the duration of the effect, have markedly increased, and it has become all too evident that exceeding environmental assimilative capacity has unpleasant consequences.

Increasing public concern over the impact of pollution on the environment has, therefore, resulted in industry being compelled to evaluate meticulously the influence its wastewaters have on biological systems, and to improve its effluent quality where necessary.

A fundamental question for contaminated aquatic ecosystems is how to establish that any amelioration of effluent quality has, in fact, resulted in biological and ecological benefits. For uncontaminated ecosystems a crucial question is whether quality can be maintained in such a way that industrial effluent disposal will not cause significant deterioration, whilst permitting the industry to produce efficiently and economically.

Because many chemical compounds produce adverse biological effects at concentrations below present analytical capabilities, biological evidence is required to answer both of the above questions. Further, effluents do not contain chemical entities in isolation, and chemical analysis alone cannot predict the toxicity of such complex wastes where the components may act synergistically or antagonistically with one another. It is also a well known fact that water quality has a decided influence on the degree of chemical toxicity. Whether an effluent may act deleteriously upon an aquatic ecosystem depends, therefore, upon the interaction between the effluent, receiving water quality and the organisms present.

Simply knowing the concentration of a potential pollutant is not likely to produce useful management information. This does not mean to say that there is not a need, also, for adequate physical-chemical data. Without knowing the concentration of a pollutant, a correlation between concentration and response cannot be assessed. Industry is, understandably, concerned that the biological techniques employed will provide valid assessments at minimum cost. Research toxicologists, therefore, bear a responsibility to provide techniques which will satisfy both industrial and environmental requirements.

Both receiving water and industrial effluent quality vary unpredictably. Industrial effluents also vary in quantity according to production schedules which are controlled by market demand rather than environmental considerations. The receiving capacity, therefore, of aquatic ecosystems, will not harmonize with fluctuations in industrial effluent quality or quantity. Thus, a critical management requirement is a means of assessing ecosystem assimilative capacity on a site specific basis. The biological methodology for accomplishing this systematically constitutes the developing field of biological monitoring.

One aspect of biological monitoring is the utilization of aquatic organisms to provide an early warning of the presence of toxic materials in water. The development of automatic continuous systems, which detect toxicant induced responses of fish, has been the object of this study. Possible applications of the techniques in an industrial situation are

to assist in the prevention of hazardous waste spills or in a water treatment plant as a check on potable water supplies. This section examines whether the systems meet the operational requirements necessary for approval by both the academic and commercial communities. The possible future exploitation of such systems in a watershed management complex, together with their acceptance as legislative elements by regulatory agencies, is also discussed.

BIOMONITORING : SCIENTIFIC MERIT

The utilization of aquatic organisms for continuously monitoring industrial effluents is not a new concept. The early types of monitoring systems employed fish in flowing wastewater (Henderson and Pickering, 1963; Jackson and Brungs, 1966). The fish were observed for mortality. Fish, exposed to diluted waste from a cellulose plant in Sweden (Hasselrot, 1975), have assisted in determining the source of toxic effects since 1965.

Visual assessment of lethality has the obvious limitation in that it requires the continual presence of personnel to observe the test organisms condition. Further, the fact that death may occur days after the onset of toxicity provides a considerable delay for hazard appreciation. Consequently, the emphasis, in this study, has been placed on automated devices which measure pre-mortal symptoms of toxicity, such as abnormal respiration and aberrant locomotory behaviour, allowing for toxicant-induced responses to be detected timeously and with greater sensitivity.

Although the potential number of early warning systems is quite large, research has indicated that they should have certain characteristics in common and that they must meet certain conditions to be useful. These characteristics and conditions must be taken into account when reviewing the performance of current systems or in designing new ones.

CHARACTERISTICS OF EARLY WARNING BIOMONITORING SYSTEMS

An early warning toxicity monitoring system is considered to have the following characteristics:

1. Sensor organisms are maintained in a laboratory situation at an industrial site, under controlled conditions, being exposed to a continuous flow of the test wastewater diluted to duplicate conditions in the receiving ecosystem.
2. Monitoring of physiological or behavioural parameters is accomplished using a recording device which has the capability of detecting abnormal conditions indicated by the response of the sensor organism.

3. The monitoring system functions primarily as a detector for short term acute changes in toxicity, only secondarily as a detector of chronic or cummulative effects.

REQUIREMENTS FOR AUTOMATIC BIOMONITORING SYSTEMS

The field of biological monitoring was developed in order to assist in the control and effective maintenance of environmental quality at socially and biologically desirable levels. In order to function successfully, as an intergral part of an environmental control framework, automatic early warning systems need to fulfil the following requirements:

1. *The operation of the system should be both continuous and automatic.* This is accomplished by the physiological or behavioural parameter of the organism selected for monitoring being quantifyable through appropriate interface techniques for analysis by electronic recording equipment. The basic design of the early warning biological monitoring units developed during the present study include electronic data transducers feeding into a simple data analysis system with alarm systems to provide notice of developing toxic conditions. The type of transducer used has been determined by the biological parameter being monitored, e.g. an amplifier which magnified the microvolt signal generated by fish as they ventilated their gills. In future more sophisticated methods may be employed by interfacing the electrical signals to a small computer. Commercial multichannel data acquisition systems are already available for this purpose.

2. *The method should not result in undue stress on the sensor organism.* It has been recognized that techniques requiring restraint of the sensor organism or the attachment of devices to it are not desirable. Even the use of light beams to monitor activity levels in fish were found to disturb normal diurnal locomotory patterns.

3. *Rapid, reliable detection of developing toxic conditions is crucial.* The speed with which a sensor organism may respond to a

toxic effect will be influenced by a number of variables. These include the species of sensor as well as the particular behavioural or physiological response being assessed. The particular mode of action of the toxicant as well as its concentration in relation to lethal levels will also modify reaction time. Behavioural and physiological responses of fish to intoxication does depend upon the toxic load, and, at sub-lethal levels, the manifestation of the response may take some time. This may not allow total prevention of a toxic waste spill unless there is a built-in delay between exposure of the sensor organism and the release of the toxicant.

The reliability of the monitoring method should be such that the system will respond repeatedly to the presence of a variety of toxic influences. It has been shown that it is possible to select a sensor organism which will posses an almost universal response to toxicants found in industrial waste waters. Protection of aquatic ecosystems would be enhanced by selecting a sensor organism which is more sensitive to intoxication than residents of the receiving waters.

Long-term exposure to very low levels of intoxication does produce a loss of sensitivity in biological organisms. However, over a period of between three and six months, this does not affect the ability of sensor organisms to respond in sufficient time to provide adequate ecosystem protection against lethal toxic loads. This type of problem would be minimized by replacement of the sensor organisms being monitored at regular intervals in such a way that continuity of effluent assessment is not interrupted.

4. *Biomonitoring systems should not exhibit alarm situations to non-harmful variations in water quality.* Certain characteristics of water, or effluents, such as temperature (Cairns *et al.*, 1974) and pH (Hargis, 1976) may cause responses from sensor organisms in the absence of toxicity. Water hardness modifies toxic influences even though non-toxic calcium concentrations have been found not to influence system performance (Cairns *et al.*, 1973a, b). Fluctua-

tions in oxygen concentration would, most definitely, have an effect on the operation of pollution monitoring systems measuring respiratory (opercular) rhythms. Precise regulation of these parameters, or at least a knowledge of their values, is necessary in order to evaluate correctly the cause of any abnormal reaction from sensor organisms.

5. *Data analysis must be simple and provide effective criteria for the recognition of abnormal responses by sensor organisms.* Living organisms, used as sensors in biomonitoring systems, elicit normal ranges of variation in behavioural and physiological parameters. This normal variation requires to be statistically determined so that reliable criteria can be established for abnormal responses. Variation between individuals of the same species makes it advisable to have each individual sensor serve as its own control. In the present study, control data, obtained after acclimation to test conditions, have been used to establish confidence intervals by which abnormal responses may be detected. Where the behavioural or physiological parameter being monitored has a diurnal periodicity, it may be advisable to establish separate normal values for different periods during the day. The use of a single statistical criterion for response assessment is, however, the simplest and easiest means of control.

6. *Operation of the system should be relatively simple.* The monitoring systems, developed during the course of the present study, have evolved from strip charts and other visually examined data recording systems to electronic systems in which all data analysis and most control functions are carried out automatically. The relatively simple electronic devices assess aberrant sensor responses and turn on alarm systems accordingly. The systems may also, through signals transmitted to appropriate valves in industrial waste lines, directly control effluent discharge. Highly trained personnel would not be needed to run such a system.

7. *Sensor organisms must be easily obtained, bred and maintained under laboratory conditions.* The organisms used in monitoring systems

should be fairly inexpensive and easily acquired. Although this limits the selection of species, since few are commercially available, the use of such standard species as the guppy (*Poecilia reticulata*) or zebra fish (*Brachydanio rerio*) provides advantages such as availability of toxicity literature and culture techniques with regard to the species. Using a standard species would not involve the continuous modification of monitoring systems. However, it is desirable to use an organism which adequately protects receiving systems from a wide variety of toxic effects.

8. *The monitoring system should require as little maintenance as possible.* Monitoring systems evolved during the course of this investigation have been built on a modular basis for easy replacement of electronic parts. Signal lights have been incorporated which inform operative personnel of part malfunction. Parts are easily interchanged and spare modules can be employed whilst any electronic fault is corrected. Mechanical arrangements have been kept to a minimum. Thus the systems compare favourably in cost and operation with physical-chemical monitoring systems.

The sensitivy to toxic substances of many aquatic organisms and the availability of different types of monitoring equipment indicates that automatic biological monitoring can develop further, as long as the basic requirements, presented above, can be met.

SCIENTIFIC VALIDITY

A major objective of the research inherent to this study has been to provide answers to the many questions which have arisen regarding the conceptual soundness of biological monitoring methods. A primary objection to biological monitoring is that there is no strong body of evidence to indicate that it will function successfully in the way supposed.

Concerning continuous automatic systems, it is envisaged that their use will fully permit non-degrading utilization of the receiving or assimilative capacity of ecosystems for industrial waste discharges, at the same time protecting them from deleterious effects.

For in-plant biological monitoring systems the most important questions, relating to scientific justifiability, pertain to their sensitivity, reliability, utility and cost, when compared with proven, well established methods.

The lag time between the onset and detection of toxic conditions, due to the inherent delay in biological processes, has resulted in critics questioning the ability of biomonitoring systems to detect emissions of lethal toxic concentrations from industry before they reach receiving systems. The elapsed time to sensor organism response depends, to a large degree, upon toxicant concentration. At high concentrations the response is swift, but at sub-lethal concentrations the response may take up to between 15 and 20 h to become apparent. This may require the introduction of holding facilities in industrial waste discharge systems to allow for biomonitor response delay. However, deleterious effects of toxic discharges upon receiving ecosystems arise as a result of the combined effect of concentration and exposure time, and laboratory investigations have shown that fish removed to freshwater at the time of response were unharmed for that particular time period. Accordingly, it may be reasonably assumed that should delivery of effluent to ecosystems be curtailed at time of response, fish and other aquatic organisms would be adequately protected. Conversely, the fact that biological material exhibits natural wide fluctuations in physiological and behavioural functions, has led to the suggestion that systems based upon such parameters would be over-sensitive in portraying adverse conditions, leading to false signals causing undue expenditures of time and effort by waste control personnel. It is true that individual fish, employed as sensors in biomonitoring systems, do occasionally elicit aberrant responses in the absence of any toxic influence. However, it has been shown that the chance of a number of sensors exhibiting such false responses, in non-harmful situations, at one and the same time, is virtually zero. Biomonitoring systems installed at industrial sites should, therefore, consist of a number of independent channels, whereby alarm situations would be established only upon the receipt of responses from a majority of sensors.

The suggested use of an "all-purpose" organism as a sensor in biomonitoring systems, for the reasons outlined previously, has been faulted in that the selected organism may not be sensitive enough for all situations. Furthermore, it has been shown that sensor fish may not adequately protect the receiving ecosystem from long-term damage. However, the use of the most sensitive organism indigenous to each receiving system would require an extensive site-specific developmental period whereas it would be a simple procedure to compare the sensitivity of a standard fish with the most sensitive indigenous organism and proceed accordingly. The effluent/diluent water characteristics of test samples passing through biomonitoring systems could be adjusted so that sensor fish would respond to toxic levels proportional to those causing long-term harm to the receiving ecosystem.

One continually encounters the question: Why use biological monitoring when physical-chemical monitoring has been *successfully* (italics mine) used in the past? Early detection of developing toxic waste conditions implies the need for automatic and frequent or continuous sampling and determination of critical waste parameters. This requirement immediately places a limitation on the physical-chemical parameters that can be monitored, because some analytical methods, e.g. the standard BOD test, require a great deal of time to complete (Ripley, 1972). Also, some wet-chemical methods may be difficult to use in a field situation (Klein *et al.*, 1968). Continuous monitoring is, however, possible with many parameters, including temperature, pH, conductivity, chloride, dissolved oxygen concentration, suspended solids, total carbon, ammonia and others. Specific ion electrodes further extend the list, but there remains a large number of toxic substances that cannot yet be monitored in a continuous manner.

Despite advancements in waste-water monitoring, predictions of toxicity from analyses of several individual physical or chemical factors are not entirely satisfactory. Most industrial wastes are complex mixtures varying continuously in composition and volume; knowledge of a fixed number of single parameters may not adequately predict the toxicity of the whole effluent because of interactive effects or the presence of an unsuspected material. The most important use of biological organisms as

real-time monitors of water quality involves the ability of the organism to respond to the totality of its environment, thus integrating the effects of all the various chemical and physical parameters into an overall indication of toxicity. The fact that biological monitoring assesses certain water quality characteristics which physical-chemical monitoring cannot is not to be interpreted as a proposal for the complete abandonment of the latter. Rather the two should be used in concert, as biological monitoring alone will not identify the particular toxicant causing a response but only indicate that some deleterious material is present. It should be possible to link biomonitoring systems to sampling instruments in such a way that effluent samples are automatically taken for analysis on receipt of alarm situations. This would relieve the industrial analytical chemist of the monotony of regular sampling at precise intervals, a procedure which does not often detect a pollution incident, and also expedite the identification of the particular deleterious component causing the warning response.

Biomonitoring systems have been installed at two industrial sites in South Africa so there is a little evidence available regarding the costs of implementation. A detailed evaluation of the cost effectiveness of the method is, however, difficult at such an early stage in development. Nevertheless, the costs are not so high that only large industries with sizable waste control staffs can afford them. The degree of automation inherent in the latest generation of biomonitoring systems means that industries do not require additional personnel for their operation or maintenance. Further, as stated, the use of continuous automatic biomonitoring systems for effluent control would relieve personnel from the time and effort of repetitive physical-chemical monitoring, with a concomitant saving in running costs.

It is hoped that the research conducted during this study will provide sufficient evidence for the academic community to make a tentative favourable judgement upon the efficacy of biological monitoring, without which industrial acceptance may not be forthcoming.

BIOMONITORING : INDUSTRIAL UTILITY

As long as there are discharges, industry will be required to undertake increasingly detailed assessments of the biological impacts of its wastewaters. Each discharger has a primary responsibility for understanding the nature of his effluent as well as its impact upon receiving systems. Industry also has a justifiable concern that sound techniques must be used and that meaningful assessments be made so that the greatest gain can be derived for a minimum cost. It is expected that biomonitoring, having been scientifically proved feasible, will grow. It is further expected that industry will participate in its growth as it has an understandable interest that the use of biomonitoring systems solve, rather than create, problems.

INDUSTRIAL APPLICATION

The purpose of a continuous automatic biomonitoring system is to provide a rapid indication of an unacceptable water quality and enable steps to be taken immediately to minimize its effects. Therefore the monitoring system must be linked to a control center where action can be initiated instantaneously. On an industrial site, providing there are control staff available, such an arrangement would be relatively straightforward.

It would certainly be desirable to have a redundant detection system in an industrial situation, where water and waste qualities are liable to vary unpredictably. It is conceivable that some harmful combination of environmental conditions and waste quality would be detected by monitoring one biological function but not by monitoring another. The systems measuring breathing rate and activity could be combined. It should be possible to feed test fish and use them for as long as they do not become incapable of response after long exposure to toxicants, due to impairment of sensory mechanisms or development of resistance.

For installations to monitor effluents it would be more appropriate for the sensor fish to be exposed to a dilution of the effluent rather than to the undiluted effluent, thereby simulating more closely the situation in the river downstream of discharge (Figure 95). The dilution water

used for this purpose should ideally be obtained from the watercourse upstream of the discharge, but this in itself introduces difficulties. A response from such a monitor could be the result of either an adverse effluent quality or an adverse quality in the watercourse upstream. It would, therefore, be desirable to have a control unit where sensor fish are exposed to upstream water containing no waste from the plant, in order to evaluate the effects of upstream conditions on the fish, and to detect extraneous effects such as floor vibrations or noises that continue for several hours and disturb the fish.

If a monitoring unit were placed on each waste stream in an industry as well as the combined waste stream, the source of toxicity could be more easily located. If the toxic waste were diverted to an emergency holding facility, chemical analysis on the waste or a check of the manufacturing process could determine precisely the cause of trouble. A deterioration in effluent quality would be indicated if any of the effluent monitors showed a response in the absence of a response in the upstream river monitor.

Installing such in-plant systems in a single industry would give management four options when a signal indicating deleterious effects appears:

(a) divert the waste to a holding pond, a procedure which could be accomplished by the monitor itself;

(b) recycle the waste for further treatment;

(c) scale down operations until the sensors indicate a moderation of the toxic effect; or

(d) pump upstream dilution water into the combined waste stream in order to minimize the impact upon the receiving system.

These actions are designed to protect the receiving system until the emergency is over. However, the degree of protection afforded by in-plant monitoring units should be evaluated by regular monitoring of downstream biological community structure and function. The cost to industry for maintaining these systems should be more than offset by the economic benefits of correct utilization of assimilative capacity.

INDUSTRIAL RESPONSIBILITY

The premise that, in future, biological monitoring will receive increased application, presupposes that many industries do not, at present, have a complete understanding of the effect of their effluents upon aquatic life. Manufacturing industries have not, for the greater part, provided technical personnel and scientific leadership in the development of biological methods. It is assumed, therefore, that where industry wishes to become involved in such developments, specialized leadership will come from outside. In order to demonstrate the industrial utility of continuous automatic biomonitoring systems, an extended developmental period will be required where academic and industrial communities collaborate to provide demonstration situations.

Although the industries that enter into this type of co-operative development will acquire a staff both knowledgable and experienced with such systems, they will face more difficulties than the enterprises which follow. Any explorative exercise has its advantages and disadvantages, and biomonitoring is no exception.

There is an understandable reluctance on the part of industry to become involved in biomonitoring developmental programmes, because they may result in increased governmental restraints or more required biomonitoring on their effluents as well as more stipulated government access to the information generated by demonstration projects during their development. Overcoming this situation, which actually retards the development of biomonitoring, has been possible through the conviction of a few South African industrialists of the value of such systems that they were either willing to take the risk or were acting in anticipation of future legislation.

Industries entering into co-operative biomonitoring developmental projects must, however, be prepared to release information to the public on the various industrial spills that have been identified and documented. In other words, industry should acknowledge and disclose its delivery of deleterious materials into the environment if there is to be a general conviction that they can be reliably detected by biological monitoring systems. Without such disclosures, although painful, biolo-

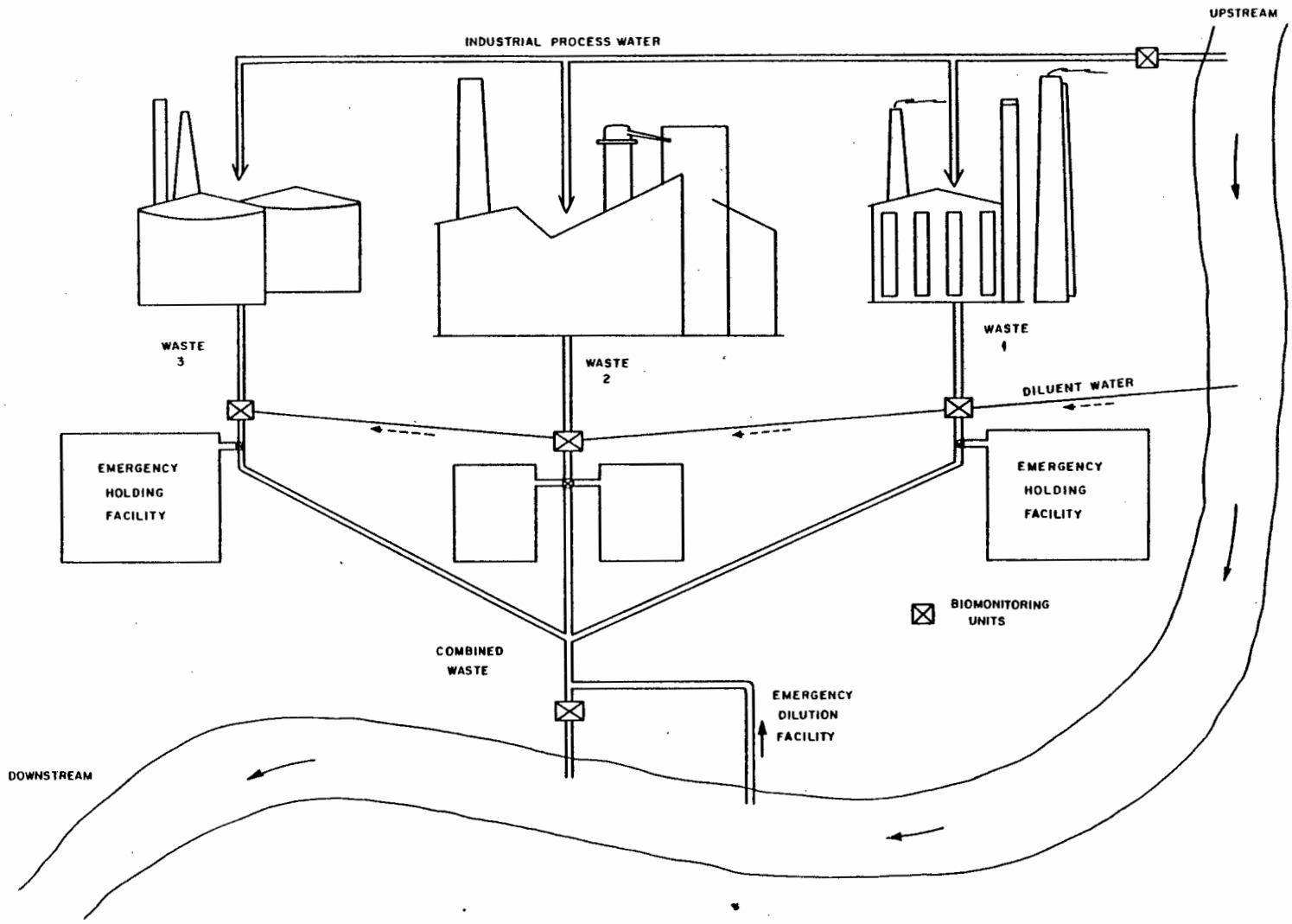

FIGURE 95. Use of several monitoring units at an industrial site

gical monitoring would lose credibility. On the other hand, it may be pointed out that besides real, continuous protection for the environment and the public respect that biomonitoring would bring, industries stand to benefit from discovering spills quickly, by cutting losses of valuable materials and by detecting costly malfunctions in their processes. One does not have to anticipate all the things that can go wrong. The biomonitor is the sensor for the unplanned event.

COST OF BIOMONITORING

There is, at present, no sizeable body of information regarding the cost of biomonitoring. However, installation and operating costs furnished by SAPPI KRAFT (Tugela Mill) Ltd for the location of a 15-channel continuous automatic biomonitoring system, based upon the ultrasonic detection of fish activity patterns, in the largest integrated pulp and paper mill on the African continent at Mandini, Zululand, have been calculated (Morgan *et al.*, 1982).

The total cost of installing the biomonitoring system at the mill was approximately R35 000 (R1 = U.S. $1,2). Most of this was accounted for by labour and building costs for the construction of the laboratory, and provision of ancillary services. At the time of installation, the cost of hardware for the electronic monitoring and alarm units was approximately R7 500, with each sensor chamber costing R200 to have made. It should be noted, however, that this relatively low expenditure resulted in a saving to the company of between R3 and R5 million, since it could be indicated that second-stage effluent treatment, as originally required by the regulatory authorities, has, to date, been unnecessary in this geographical situation. This will not always be the case. In many instances, more extensive treatment may be indicated.

The essential automatic and continuous nature of the biomonitoring system entails minimal operating and maintenance costs. Running expenses for the first twelve months of operation totalled R3 350, a sum which compares favourably with the cost of R3 500 incurred by the company in carrying out chemical-physical analyses of the effluent.

BIOMONITORING : WATERSHED QUALITY CONTROL AND MANAGEMENT

For centuries, fish have been recognized by scientists and lay-men as widespread and important indicators of river water quality. As such they play a role in the work of water authorities to maintain and, where necessary, to restore the vitality of rivers, to supply a wholesome water to the consumer and to maintin, improve and develop fisheries. A prerequisite to effective management of water quality for these purposes is to establish quality objectives which must take into account *inter alia* the quality of water required to support viable fish populations.

Fish kept in closely controlled conditions in the field can serve as valuable and continuous indicators of water quality. The use of automatic water quality monitors now appears to be a practical proposition and as such could play a most important role in water quality management in the future.

THE INCORPORATION OF BIOMONITORING SYSTEMS INTO A WATERSHED QUALITY CONTROL SCHEME

Potential uses of automatic systems utilizing fish include the monitoring of industrial effluents, river waters and raw waters abstracted for public supply. Monitoring of effluents from sewage-treatment works, particularly those treating highly industrialized sewages, is a further potential use.

Figure 96 illustrates how a system of such monitors might be organized on a watershed. There would need to be two different applications: in-plant and in-stream (river locations) monitors, the nature of which being dependent upon 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 measuring center, or centers, for analysis. The measuring centers throughout the watershed may be linked to a single co-ordination center at which the necessary decisions will be made regarding action to be taken in response to a pollution incident.

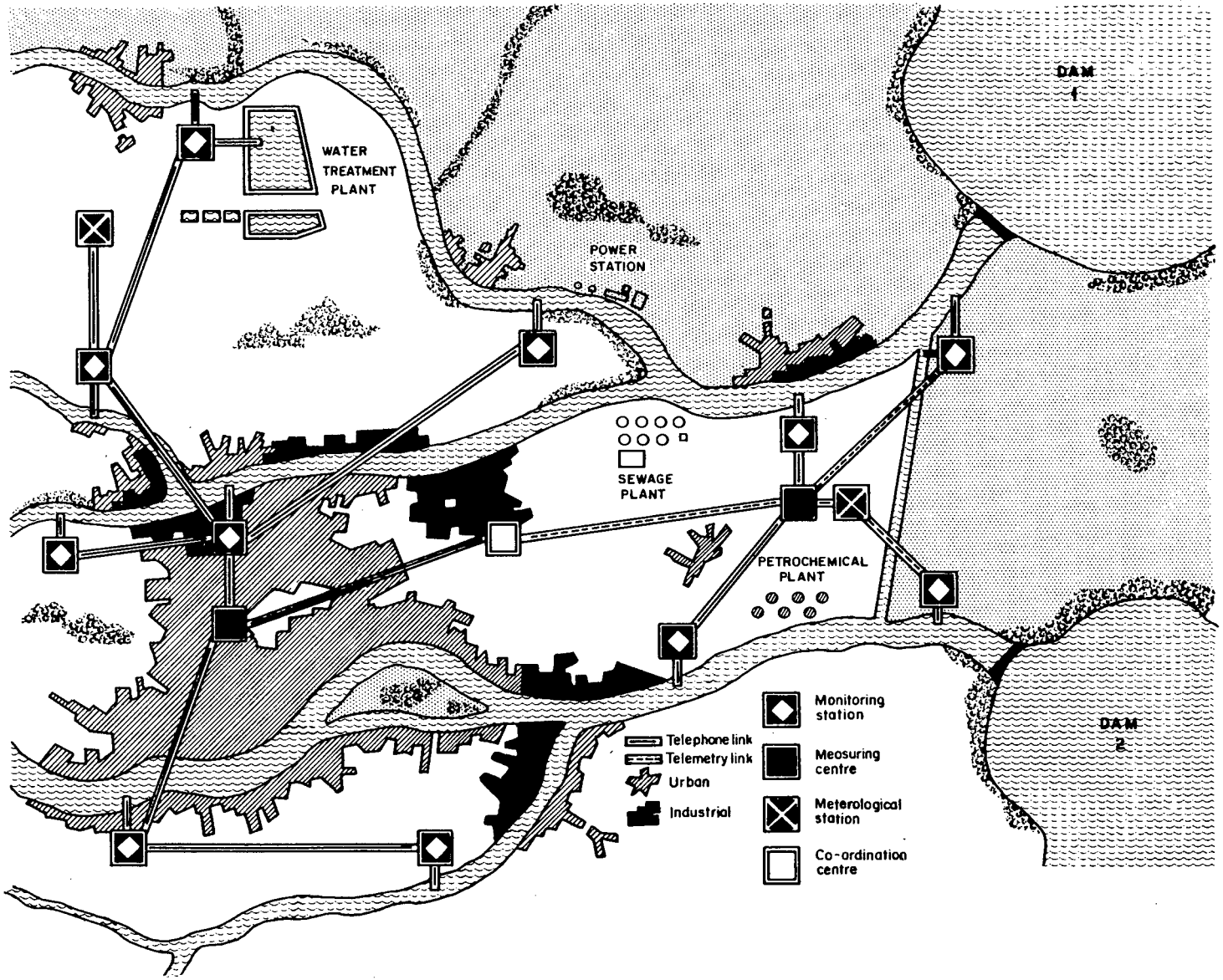

FIGURE 96. Monitoring network for a waterway system

At river locations it may be necessary to use telemetry links for data transfer, although in the majority of cases information will be transferred using leased telephone lines. In some areas such links from water quality monitoring stations have already been established. Many of these have been constructed to protect public water supply abstractions or to control inter-river transfer schemes. On the basis that it will become necessary to extend the number of determinands to include biomonitoring and that in many cases the necessary housing, pumping and information transfer facilities are already in existance it would seem appropriate that the first installations of automatic fish monitors should be made at existing water quality monitoring stations of this kind and integrated with established physical-chemical monitoring units.

The regional co-ordination centre would receive continuous information upon levels of both agricultural and industrial pollution and take the necessary steps to alleviate problems which may arise from deleterious increases in their levels. Other than the choices open to the managements of individual industries, as outlined previously, regional management would provide for two further options. To ameliorate pollution incidents, temporary river flow augmentation could be implemented from upstream dams; and industries, other than that causing the incident, could be instructed to temporarilly reduce their discharges so that the assimilative capacity of the watershed is not exceeded. The regional co-ordination center may also receive hydrological and meterological information so that the volumes of industrial effluent discharges are more closely related to actual river flow conditions, and accomodation be made for agricultural leachate during seasons when, for example, crop-spraying is taking place.

The use of fish in automatic water quality monitoring stations offers many advantages and would provide a far broader assessment of water quality than present monitoring stations can achieve. Many of the pollution incidents which occur result in toxicant concentrations in rivers well in excess of those to which a fish monitor would be sensitive. At present, an adverse water quality is indicated by the chemical analysis of a snap sample; the output from an automatic continuous water quality monitor measuring a very limited range of

determinands or mortality of the natural fish populations. An automatic water quality monitor using fish has the potential to assess water quality continuously for a wide range of determinands and to detect pollutants at a fraction of the concentrations lethal to fish. However, it is improbable that watershed users, especially industries, will employ biomonitoring systems without appropriate legal inducement. Accordingly, the responsible regulatory agencies will have to accept the conceptual soundness of the technique. Even then, it may be some time before such techniques play a major role, as discussed below, in regulating water quality.

LEGISLATIVE ADOPTION OF BIOMONITORING IN ENVIRONMENTAL WATER QUALITY CONTROL

The use of water for industrial purposes, and the control of effluents resulting from industrial processes, as well as the control of pollution of water arising from other sources are governed, in South Africa, by the Water Act No. 54 of 1956, as amended from time to time.

The sections most applicable to industrial effluent control and the prevention of water pollution are 21 (sub-sections 1, 2 and 5) and 23.

Section 21(1) contains three important provisions, namely:

1. Effluent purification forms an integral part of the normal manufacturing process.
2. Any person after using water for industrial purposes shall purify it to a predetermined standard which the Minister shall lay down after consultation with the (South African) Bureau of Standards.
3. The Minister may lay down a general standard or special standard in respect of any areas, area or stream or streams, or in relation to "any one or more industrial processes".

This section is made subject to the provisions of sub-section 5 of Section 21, and this means that unless the provisions of sub-section 5 apply, that is unless an industry has a permit, it must discharge only effluent which complies with the published standards. To date two standards have been laid down, being the general standard and a special standard for certain streams where the water is of a high quality, the so-called trout streams.

Section 21(2) lays down that water, including sea water, used for industrial purposes and purified to the required standard must be returned to the stream of origin, or to the sea.

The general standard, for effluent disposal, laid down by the Bureau of Standards, prescribes inflexible maximum concentrations of a number of determinands, and is applicable nationally. The application of a special standard is the exception rather than the rule. Legal requirements for industrial effluent discharges, therefore, do not take into account the variability in quality of receiving waters.

As far as control of pollution is concerned, sub-section 5 of Section 21, is probably the most important section in the water act. Stripped of its legal wording this section says that where it would be impracticable for an industry to purify its effluent to the prescribed standard the Minister may issue a permit exempting such industry from compliance. The degree of exemption must be specified as well as the point of discharge, which must be such that dilution will render the effluent harmless to fauna and flora, and that no other person will be prejudicially affected. It may be argued that, in such cases, the utilization of biomonitoring systems would ensure the protection of the receiving ecosystem, and a legal injunction, to this effect, could be employed whenever an industrialist seeks exemption from the general standard.

It is probably true to say that Section 23, because it requires technical proof of an offence in order that a prosecution in a court of law may succeed, is the most misunderstood section regarding pollution in the Water Act.

For the sake of clarity. Section 23(1) is quoted:

"23. *Prevention of pollution of water.* - (1)(a) Any person who wilfully or negligently, and where any provision of Section 21 applies, contrary to that provision, commits an act which could pollute any public or private water, including underground water or sea water, in such a way as to render it less fit -

(i) for the purpose for which it is or could be ordinarily used by other persons; or

(ii) for the propagation of fish or other aquatic life; or

(iii) for recreational or other legitimate purposes, shall be guilty of an offence.

(b) If in any prosecution under paragraph (a) it is proved that the accused committed any act which could pollute water referred to in that paragraph in any manner mentioned therein, it shall be presumed, until the contrary is proved, that the accused committed such act wilfully or negligently."

The purpose of this section is quite clear but procedural difficulties arise in administering the section.

"Rendering water less fit than" implies background knowledge regarding the quality of water before it was polluted and this is perhaps the biggest stumbling block in this section. Where background knowledge is lacking proof of pollution, from physical-chemical data alone, as required by the Act, is almost impossible. In some cases, in running water, samples at points above, at and below the source of pollution may provide proof, but the incident may have resulted from an interaction between an industrial effluent and natural variation in receiving water quality.

Where Section 21 applies, it is considered that it is very much simpler to act. Should a person discharge an effluent not complying with the relevant standard or with his permit, all that need to be proved is, in fact, that the effluent did not comply. One sample and a competent analyst is sufficient. That is if the sample is taken at the time, or just after, a pollution incident occurs. This is very rarely the case.

It would seem, therefore, that the Water Act, as promulgated and applied, mitigates against the environment in a number of important respects. How, then, can the introduction of biomonitoring systems play a role in alleviating what seems to be an unsatisfactory situation?

The most dramatic change would occur should biomonitoring requirements replace existing legislation in regulating specific toxicants at their point source. However, one cannot imagine this happening immediately, if ever, without a radical reappraisal by regulatory authorities. In any event, the application of biomonitoring systems to water quality control would need to evolve through a number of stages before its acceptance as a legislative element.

It is likely that, initially, standards for industrial effluent discharge will continue to be based upon definitive physical-chemical criteria formulated by governmental organizations, with biological monitoring systems being used solely as an extra safeguard against "fish kills". Such environmental catastrophies will continue and industries experiencing adverse public reaction may be induced to incorporate early warning systems into their effluent control programmes. Industries most likely to adopt biological control measures, without legal enforcement, would include those with effluents so complex as to be difficult to analyze chemically; those in extremely sensitive environmental situations and those wishing to enhance their public image. It is unlikely that, at this stage, a large number of industries will install biomonitoring systems unless required by law. The few that do will, however, provide a data base which will enable the concept to be fully evaluated.

A further stage, which may continue the evolution of the acceptance of biological monitoring, might involve the installation of biomonitoring systems by industries which apply for permit exemptions. This may be effected by a regulatory authority acting in an advisory capacity and, therefore, gaining access to information generated by biomonitoring systems. This would enable regulatory authorities to develop a capability for dealing with such data on a trial basis. Regulatory agencies may then be better equipped to enforce the introduction of biomonitoring technology on industries wishing to establish new sites in sensitive areas, as well as compelling them to adhere to the standard regulations.

The final, and most difficult, stage would involve a complete shift in the approach to environmental control by regulatory agencies, where biological parameters rather than physical-chemical ones are considered

the prime determinands for the regulation of discharges of industrial effluents. Each discharge would be considered on its own merit as to whether it adversely affects the biological integrity of the receiving ecosystem. It is very difficult to imagine this occurring, as it would require different attitudes and procedures than those prevalent today. It would entail the direct association of standards with the continually changing natural environment, and, although it may result in greater respect between watershed users and environmentalists, might be considered far too reformist by those reluctant to relinquish established principles.

It is to be hoped, however, that continuous automatic biological monitoring systems, of the type described in this study, will gain acceptance by the academic community, industrialists and regulatory agencies, so that they may assist in providing protection to our much used and greatly abused aquatic environment.

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APPENDIX

PUBLICATIONS

The following publications on work included in this thesis have been published in the open literature.

1. MORGAN, W.S.G. and KÜHN, P.C. 1974 A method to monitor the effects of toxicants upon breathing rate of largemouth bass (*Micropterus salmoides* Lacépède). *Water Research.* 8, 67-77.

2. MORGAN, W.S.G. 1975 Monitoring pesticides by means of changes in electric potential caused by fish opercular rhythms. *Prog. Wat. Tech.* 7, 33-40.

3. MORGAN, W.S.G. 1976 Fishing for toxicity : Biological Automonitor for continuous water quality control. *Eff. Wat. Treat. J.* 16, 471-475.

4. MORGAN, W.S.G. 1977 Biomonitoring with fish : An aid to industrial effluent and surface water quality control. *Prog. Wat. Tech.* 9, 703-711.

5. MORGAN, W.S.G. 1977 An electronic system to monitor the effects of changes in water quality on fish opercular rhythms. *Biological Monitoring of Water and Effluent Quality ASTM STP 607.* John Cairns Jr., K.L. Dickson and G.F. Westlake, Eds. American Society of Testing and Materials, pp 38-55.

6. MORGAN, W.S.G. 1979 Fish locomotor behaviour patterns as a monitoring tool. *J. Wat. Pollut. Control Fed.* 51, 580-589.

7. MORGAN, W.S.G., KÜHN, P.C., ALLAIS, B. and WALLIS, G. 1982 An appraisal of the performance of a continuous automatic fish biomonitoring system at an industrial site. *To be published in Progress in Water Technology.*