# THE IMPORTANCE OF BLUEGILL FEEDING BEHAVIOR WITHIN AN AUTOMATED BIOLOGICAL MONITORING SYSTEM FOR INDUSTRIAL WASTES


by

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# INTRODUCTION

The objectives of this investigation were to:

1). design an aquarium with sufficient self-cleansing properties to permit prolonged, automated feeding with no attendance by personnel;

2). determine a ration level that would:

   a). minimally tax the self-cleaning capability of the tanks, and

   b). be of sufficient quantity to sustain the fish in good health;

3). determine whether or not feeding had any effect on the gradual decline in the mean daily activity level of bluegill sunfish (*Lepomis macrochirus*, Rafinesque) maintained in individual aquaria;

4). identify and define any specific activity patterns associated with feeding; and

5). evaluate the response to feeding as it relates to the toxicant detection capability of the biological monitor discussed herein.

Cairns et al. (1973) suggested the management and control of our watershed could be much improved if current systems for monitoring chemical and physical parameters were augmented by biological monitoring techniques. The principle involved in the type suggested by Cairns is the continuous measurement of some physiological parameters of an aquatic organism exposed to the same changing sublethal concentration of waste loads as those in the watershed. Should measurements of these parameter start to exceed predetermined critical limits, industrial effluents may be diverted from the watershed until the cause is ascertained and corrected. In this way the need for difficult, costly, and time consuming analyses of complex waste mixtures is eliminated.

The monitoring system used in this study continuously measured swimming activity levels of bluegill. The basic principle involved is as follows. The activity of a fish is continuously recorded for 4 days. From this data a model of "normal" activity is computed for that individual for any given time interval throughout a 24 h period. During the test period which follows, if at any time that fish's activity is sufficiently above or below the computed "normal", a response is tallied. A warning is constituted by 3 fish responding simultaneously for a 1 h period. The development has been reported by Cairns et al. (1970a, 1970b, 1970c), and Cairns et al. (1973) and will be explained in greater detail in the body of this paper. Waller and Cairns (1971) and Cairns and Sparks (1971) used the monitor to detect sublethal concentrations of zinc. Their results show that responses appeared in both respiration and activity, and bluegills exposed to lethal concentrations of zinc responded abnormally well in advance of irreversible damage.

Prior to the initiation of this study, none of the fish used in the monitor developed by Cairns were ever fed during experimentation. The suspicion developed that during some of the longer experiments fish activity showed a gradual decline. The daily pattern of movement with its changing activity levels remained, but was superimposed on a gradual downward slope. It was suspected that a lack of food might be related to this problem.

Coupled with this reduction in activity levels were the obvious problems associated with lack of feeding. Anticipating that the monitor would be used in industrial situations, it would be advantageous to minimize the amount of attention required of plant personnel. Thus, if the fish were not fed, extended operation periods would certainly result in a deteriation in their condition and a corresponding loss in the efficiency and reliability of the monitor.

However, feeding posed certain problems. It was not desireable to feed the fish by hand for three reasons. First, it would require additional attention by plant personnel. Second, the entrance of any personnel into the chamber in which the fish were housed would cause abnormal readings in respiration and/or activity. It seemed unlikely to suspect that hand fed fish would be fed at exactly the same time every day, thus making it impossible to compensate for the effect of the feeding. Third, hand feeding would decrease the flexibility of the monitor. In instances in which some fully automated production facility were isolated from the main plant, daily trips to feed the fish would seem unrealistic. Thus it appeared that automated feeding was mandatory, and that this automation needed to have a high degree of reliability over extended periods of time.

Biological considerations also placed constraints on feeding and the manner in which feeding was carried out. The possibility that excrement or uneaten food might contaminate the tank had to be considered. Such contaminants might, in some manner, affect the action of a given toxicant, and also provide a medium for the concentration of harmful bacteria or other pathogens. Thus limitations needed to be imposed on the design of the monitor and on the amount of food. Tanks containing fish would need a self cleansing capability while ration size would need to be small enough to insure complete consumption to result in a minimum of residue so as not to tax any cleansing capability;

and large enough to meet the maintenance requirements of fish in the monitor situation.

Provided that feeding could be accomplished under these criteria, other questions presented themselves. Would the behavioral response to food, as recorded through the sensors of the monitor, be of a sufficiently consistant nature so as to be predictable? That is, would fish feed when not in the presence of any toxicant in such a manner that subsequent feedings would not be reguarded by the monitor as abnormal and trigger a response or false warning?

Furthermore, if feeding was feasible both from a mechanical and a behavioral standpoint, could it in any way affect the sensitivity of the monitor? Would fed fish, presumeably of better health, not respond to as low levels of toxicant as unfed fish thus diminishing the value of the monitor; or would the presence of certain toxicants be initially reflected in an alteration of feeding behavior, thus increasing the sensitivity of the monitor and its overall value? This study was an attempt to answer those questions.

## LITERATURE REVIEW

### Ration Levels

Fish ration levels and food utilization have been extensively studied (Gross et al. 1965; Seaburg and Moyle, 1964; Doudoroff and Shumway, 1967). For a comprehensive list of such references and others on related material, see Raney, Menzel and Weller (1973), and Dean Bibliography of Fishes (1968). Most investigators were concerned with finding ration levels and feeding regimes that would produce the maximum growth. From them it is apparent that the amount of food eaten is dependent on a large number of endogenous and exogenous conditions.

Gerking (1952; 1971) and Seaburg and Moyle (1964) report that as the size of a fish increases, both metabolic rate and daily ration size decrease. The sex and age of sunfish (Centrarchidae) apparently influence ration levels. Male sunfish grow significantly faster than females (Allee et al. 1948), and food is less efficiently utilized as age increases (Gerking, 1955). Paloheimo and Dickie (1966) said, concerning growth efficiency, that relatively large variations are seen within individuals at different times, as well as differences between individuals.

Environmental conditions can effect ration levels through various pathways. Metabolic rate determines the caloric requirement for energy and growth. Seasonal changes in the environment may alter this rate, modifying energy requirements (Hoar and Randal, 1969). Savitz (1968) indicates that for protein, the maintenance level changes with the season.

Doudoroff and Shumway (1967) report that when fish are on an unrestricted diet, any lowering of dissolved oxygen below air saturation results in reduced growth. The activity of digestive enzymes of fish may vary with the pH and temperature of the water (Morishita et al. 1966). Brown (1957) reports that the relationship between food requirement and temperature is not simple: for fish of equal size, the maintenance requirement in creases gradually from 5 C through 9 C, then rapidly to 11 C, and less rapidly above 11 C becoming nearly constant about 20 C. Pentelow (1939) and Gerking (1971) support this by saying that feeding and maintenance are greater at high temperatures than low.

Studies by Gross et al. (1965) reveal photoperiod influences growth in green sunfish (Lepomis cyanellus). The fish generally grow at the highest rate in increasing light, the lowest during periods of decreasing light. Within the same species, Allee et al. (1948) said a positive correlation exists between position of the individual in the social hierarchy and the amount of growth achieved.

Under natural conditions, the size of a fish's daily food intake is influenced by the average degree of concentration of the food species and their aggregation (Ivlev, 1961). The laboratory situation poses some additional factors affecting food intake and utilization. The rate of feeding itself is directly related to growth (Gerking, 1955; Palmer et al. 1951). Comfort (1956) demonstrated that female guppies (Lebistes), living alone and on similar feeding schedules, attained specific sizes directly related to the size of their containers. Allee et al. (1948) reported a similar condition; fish isolated in a larger space grew better than those isolated in a smaller one. However, Brown (1957) says that fish having much living space have considerable fluctuations in appetite. In an earlier publication Brown (1946) said that for maximum productivity among trout, an optimum degree of crowding was necessary. And Seaburg and Moyle (1964) concluded that population density is related to growth among Centrarchid panfish.

Brown (1957) reported that fish can gain weight only if they eat more food than is necessary to maintain their basal metabolism and to provide energy for their activity. The sum of these two quantities she called routine metabolism while the amount of food necessary for a fish to neither gain nor lose weight is the maintenance ration. The level of this maintenance ration varies with the size, age, and physiological state of the fish. Also, the efficiency of conversion of food to fish flesh is highest at near maintenance levels and decreases with an increase in the amount fed (Brown, 1957).

In an attempt to determine the maintenance requirement of groups of 2-year-old brown trout, Brown (1957) found that as the amount of food was reduced, the fish first lost weight and then became adapted to the new ration level and gained weight. Pentelow (1939), also using brown trout, reported that with careful adjustment of the rations, it was possible to keep the body weight approximately constant from week to week.

Two deductive approaches toward approximating the maintenance ration for bluegill suggest themselves; a) begin feeding the average daily ration of bluegill during winter, or b) find a daily ration reported in the literature and take half of that amount. Winberg (1956) suggested that the metabolic rate of fish in confinement should be doubled to correct for activity in nature. If that is so, any estimate of the daily ration in nature could be placed over two to yield a maintenance ration level worthy of testing.

Seaburg and Moyle (1964) calculated a daily ration for bluegill based on the average stomach volume of fish caught during the summer, the percentage of food found experimentally to be digested in four hours and the number of such four hour digestion periods in a day. They support this approach by referring to field data indicating that bluegill feed fairly constantly throughout a 24 h period. Using this approach they found the average daily ration for bluegill usually declined as the size of the fish increased, but was most often in the range of 1-2% of a fish's body wt.

Anderson (1959) reported that for Third Sister Lake, Washtenaw County, Michigan, the average weekly ration of bluegill throughout the winter period (Nov. - Apr.) was 0.5% body wt. Fish lost a small amount of weight and did not increase in length.

Gerking (1955) made measurements of energy expenditure of bluegill in the lab. He found that 30 g fish require about 0.7 kg cal/day. Bomb calorimetric measurements of the mealworm diet of those fish indicate that the maintenance ration of 30 g fish is about 0.32 g/day, or approximately 1% body wt.

In this study the search is for a ration level that will maintain the fish in good health and result in the least amount of debris to tax the cleaning capacity of the aquaria. Brown (1957) gives cause for some optimism in this endeavor. She said that fish given more food than their maintenance requirement (although allowed unlimited food) have appetites which fluctuate, but generally eat amounts which vary directly with the amount provided.

Feeding Behavior

Bluegill feeding behavior is far from a stereotyped behavior. Flemer and Woolcott (1966), working in Tuchahoe Creek, Va., report the bluegill is a very opportunistic feeder. Moffett and Hunt (1945) report that bluegill feeding behavior varies with different waters such that no definite dietary list can be compiled.

Seaburg and Moyle (1964) said that plant materials were a regular dietary item of bluegills in mid-summer. Many investigators report that bluegill stomach contents contain aquatic plants (Flemer and Woolcot, 1966; Seaburg and Moyle, 1964; Keast, 1968). However, the nutritional value received from plants is not thought to be significant (Gerking, 1955), and may simply be ingested in the process of feeding on aquatic insect larvae (Etnier, 1971).

Bluegill, in the natural state, are primarily visually oriented feeders normally attracted to food by its movements (Miller, 1963). Insects and insect larvae seem to be the most important dietary inclusion (Etnier, 1971; Miller, 1963; Keast, 1968) and may be taken from the waters surface, drifting underwater, or from the bottom (Keast, 1968; Etnier, 1971).

Volumes of food found in bluegill stomachs during 24 h sampling periods indicate that they feed dairly regularly throughout the day and night (Seaburg and Moyle, 1964). Boyer and Vogel (1971) report that Longear sunfish (Lepomis megalotis, Rafinesque) have been observed surface feeding on late summer nights when there was a bright moon.

Changes in stomach volumes also indicate that bluegill exhibit a pattern of changing feeding levels through the summer; high in early summer, low in midsummer and possibly a rise toward autumn (Seaburg and Moyle, 1964).

Bluegill feeding behavior within a laboratory situation is poorly documented. The fish accept artificial food readily and soon learn to expect it at regular times (Brown, 1957). Miller (1963) reported that this learning is facilitated if an experienced fish is present.

## Exploratory Behavior

Fish exhibit a changing pattern of activity which is repeated daily. Kapoor (1971) noted that *Lepomis gibbosus* were more active during early morning and late afternoon, but also reported that following three to four days of captivity the fish showed a trend toward a decline in movement.

This kind of activity reduction, even among fish which are adequately fed, is reminiscent of the course of events seen in exploratory behavior. Barnett (1958) said exploratory behavior may be interpreted as stimulus hunger; and, although best known in the laboratory rat, other animals from cockroaches to monkeys behave in a similar manner. Glanzer (1953) concurred with this when he stated that each moment an organism perceives a stimulus-object, a quantity of stimulus satiation toward that object is developed. The effect of this satiation is to reduce the organism's tendency to respond to that stimulus-object. The longer the organism perceives that object, the greater the amount of satiation developes. This might be described as a boredom-like effect.

Working with goldfish (*Carassius auratus*) Kleerekoper et al. (1974)

have associated locomotor activity with exploratory behavior. He postulated that the highly organized pattern of locomotion shown by goldfish might be the expression of appetitive habitat exploration, and the rate of activity is affected by the time elapsed since the first exposure of the naive animal to the novel environment. Hinde (1970) cautioned that we not limit our thinking of exploratory behavior to activity alone, that even within one species the types of behavior which come within the broad category of exploration or investigation are diverse. He said it is difficult to give a more precise definition than to say exploratory behavior is constituted by those behaviors which function to familiarize the animal with its environment or with a source of stimulation.

Many investigators agree that exploratory behavior results in a decrease in activity over time. This is particularly evident in the case where an animal is continuously exposed to the same constellation of external stimuli (Glanzer, 1953). Glickman (1958) reported that rats demonstrate extensive locomotory activity when placed in a new stimulus situation, and the amount of activity declines as a function of the length of time the animal remains in the situation. Montgomery (1953) supported this finding in rats and DeLorge and Bolles (1961) and Carr et al. (1959) reported that significant activity decreases occurred within 10 min tests.

Some work has been done on exploratory behavior in fish. Welker and Welker (1958), using the silver jenny (Eucinostomus gula) described an initial "freeze" reaction followed by an increase in activity when a strange object was placed in the tank. Russel (1967) looked at this reaction further and noted that guppies (Lebistes reticulatus) showed a decrease in exploration of an unfamiliar object after the initial activity increase. Such decreases in activity may in some cases be habituation, a product of exploration, which Hinde (1970) defined as the relatively persistent waning of a response as a result of repeated stimulation which is not followed by any dind of reinforcement.

There is some disagreement over the effect of feeding or food deprivation on exploratory behavior. Finger (1951) recounted that rats deprived of food responded with an increase of activity on the activity wheel. Adlerstein and Fehrer (1955) and Stackhouse et al. (1960) reported that deprived rats explore more, and at a more consistent rate than do sated rats. Seeming to substantiate this, it has been demonstrated that deprived rats would investigate the outside of their cages significantly more quickly than sated subjects (Bolles and DeLorge, 1962); and Fehrer (1956) said that hungry animals would leave a familiar box to explore an unfamiliar box sooner, more often, and for longer total time than sated animals.

Other studies appear in opposition to the above findings. Carr et al. (1959) said he found a consistently higher level of exploratory behavior for white rats which had not been previously deprived of food than those which had been deprived. Zimbargo and Montgomery (1957) noted that with rats food and water deprivation results in decrements in exploratory behavior.

Some explanation for this dichotomy was put forth by Hinde (1970). He said hunger usually decreases exploratory behavior, but hungry rats are more likely to leave a familiar environment and explore an adjacent strange one than are satiated animals; it is probably best described as appetitive to feeding.

Exploratory behavior is, then, a complex set of interrelated behavioral patterns. Certainly the precise effect of hunger varies with the degree of hunger and the conditions of testing.

Feeding and Toxicant Detection

That behavioral patterns can be modified when organisms come in contact with some toxicant is not a recent revelation. Aquarium hobbyists have long known that a cessation of feeding is often indicative of ill health among their fish. However, the fact that such modifications, or deviations from the accepted behavior, can be monitored and used to indicate the presence of sublethal concentrations of toxicant is only recently being tested. Response to food is one such behavior which more readily lends itself to monitoring.

Edwards and Brown (1966) reported that trout left part of their pelleted food uneaten when subjected to 0.6 toxic units of zinc. Even temporary and sublethal conditions of turbidity caused trout to seek cover and cease feeding (European Inland Fisheries Advisory Commission, 1965). And Sprague (1971) Gives the general report that feeding behavior of fish is badly affected by some pollutants.

Feeding behavior seems particularly well adapted to scrutiny in pollution detection. It can become a characteristic response in the lab. Unlike some other more striking behaviors (e.g. mate selection, territoriality, courtship, etc.) feeding behavior is not restricted to certain times of the year, and the releasing stimulus is easily controlled by the experimenter.

The physiological pathways through which response to food may be altered are various. In tests of the effects of sublethal concentrations of detergents on the flagfish, Jordanella floridae, one manifestation was a change in feeding behavior (Foster et al. 1969). The gross effect seemed to be that the fish's appetite had become markedly lessened, but examination of the details of the changes in feeding behavior indicated that the specific effect of ABS was to inhibit the input of sensory information by which the flagfish distinguishes between palatable-edible material and unpalatable-inedible material. And Badach et al. (1965) demonstrated that detergent eroded the taste buds of catfish so they were unable to find food.

Thus it would appear that by also monitoring feeding behavior, an investigator is provided a means to detect abnormalities in an additional array of physiological pathways. That is, in the monitor used in this study, some hypothetical toxic condition could result in reduced visual acuity of bluegill. This would not necessarily result in any abnormality reguarding activity per se, but may be detected through reduced feeding.

## METHODS and PROCEDURES

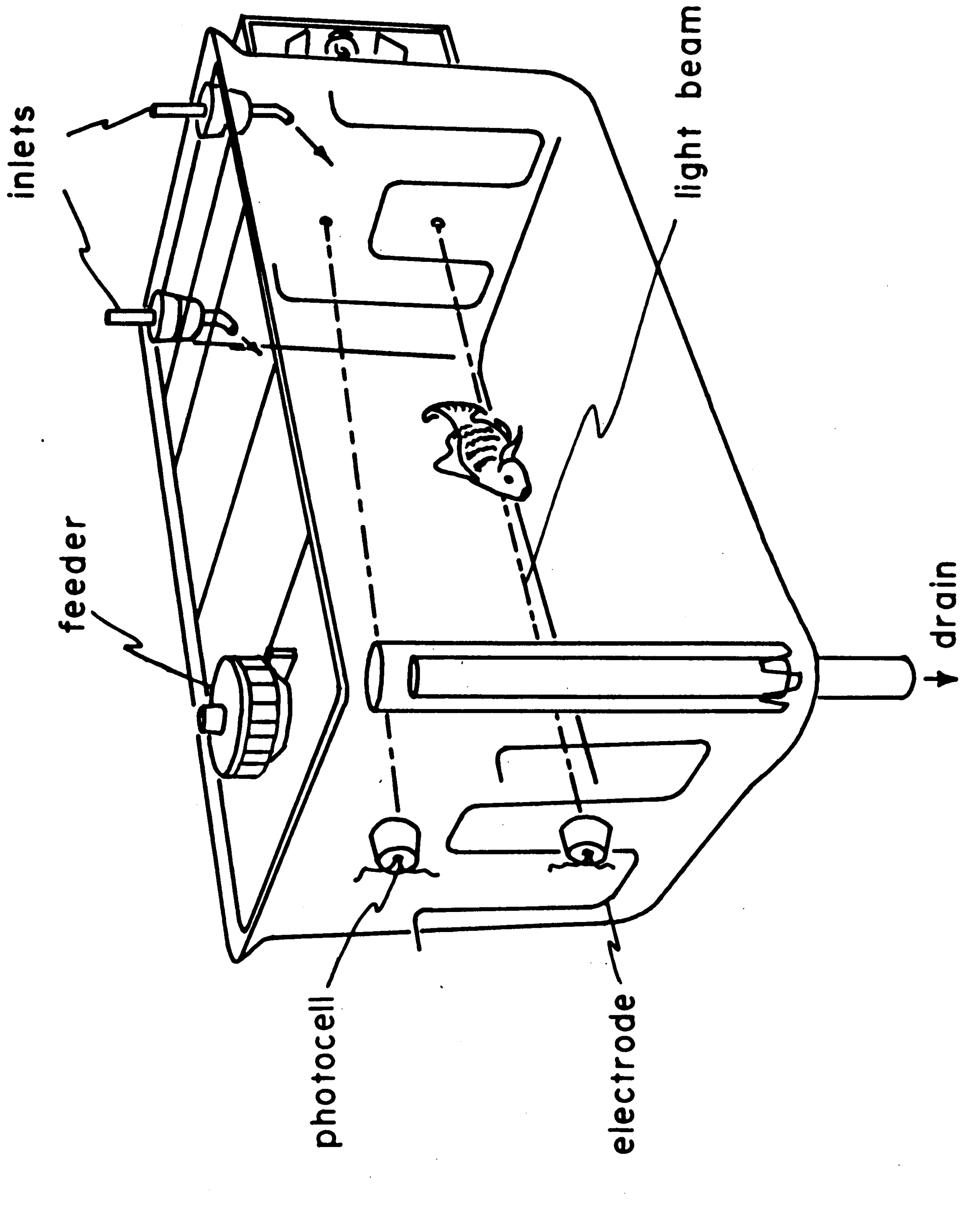
### The Biological Monitor

The biological monitoring system used in this study consisted of 10 tanks equipped to continually record respiration and locomotor activity of the fish. The design decided upon was custom molded fiberglass tubs basically rectangular in shape (32x60x29 cm deep) and having a 45 l capacity (Fig. 1). To aid in removal of debris the bottom sloped toward one corner where a standpipe similar to that described by Alderdice et al. (1966), fit into a drain connector by means of a ground glass joint. This arrangement allowed easy removal of the standpipe in order to drain the tank.

Water and the toxicant involved (a waste product from the manufacture of T.N.T. referred to as "pink" water) were supplied to the tanks on a continuous flow basis through Mount and Brungs type diluters (mount and Brungs, 1967; Brungs and Mount, 1970). These had a mean cycle time throughout all experimentation of 3 min 2 sec and a delivery of 3800 ml (760 ml per tank). The influents were introduced above the surface of the water through two glass nozzels, the direction of which could be altered to adjust flow patterns. Thus a current could be produced which efficiently swept wastes toward the standpipe where they were removed by the outflow.

Each tank was supplied with a cover divided into three sections. Either end of the cover was formed of clear plexiglass to permit easier attachment of influent conduits at one end, and automatic feeders at the other. The center section was formed of glass with a black band

Figure 1. The aquarium design used in this study.

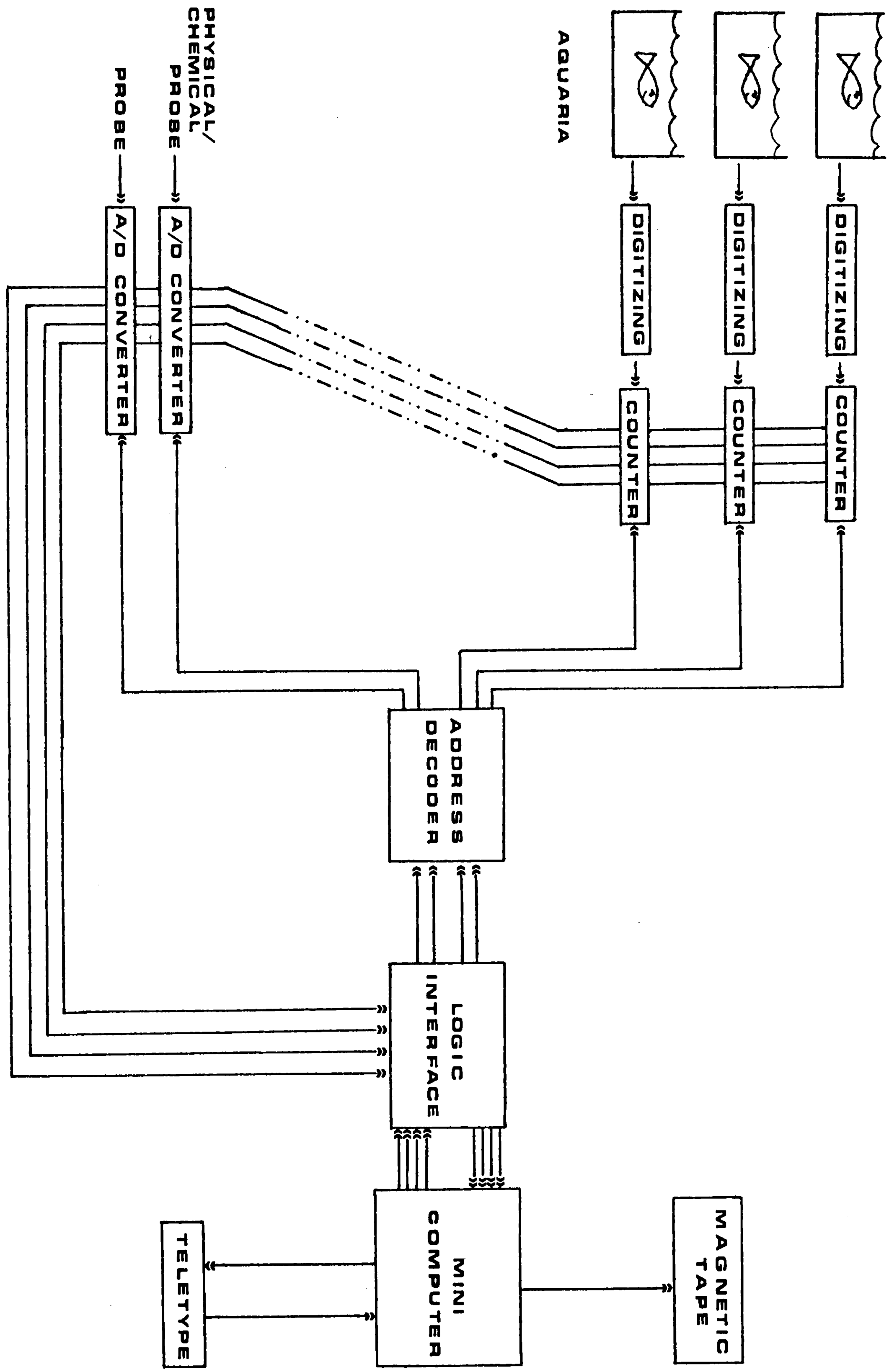

inlets
feeder
light beam
drain
photocell
electrode

approximately 17 cm in width. This produced a shadow in the tank and reduced the tendency of fish to remain near the sides. The tanks were enclosed in a 3x3x3 m chamber insulated so as to reduce extraneous auditory stimuli and control photoperiod (maintained at 12 h light and 12 h dark). Since bluegill are known to be sensitive to seismic stimuli (Sparks et al. 1972; Cairns et al. 1973), efforts were made to reduce foot traffic in the area so as to keep transient vibrations to a minimum.

Feeding was accomplished through the use of commercially available automatic feeders ("Lazy Susan", double A brand, model No. AA-100) driven by electric motors. These were mounted on the tank covers and the food allowed to drop through a hole into the tank. When feeding once per day, they had sufficient capacity to feed for 14 days.

Fish locomotor activity was monitored through the use of photoresistors in a manner similar to that described by Waller and Cairns (1972). Affixed to the rear of the tank was a light source which created two beams by means of adjustable mirrors and small windows in the side of the tank. These illuminated two photoresistors mounted on the outside of the opposite end. The signal from the photoresistors of the tank passed through a comparator circuit and the number of pulses within a given unit of time, after passing through a digitizing circuit, was recorded by an electronic counter (Fig. 2). At 30 min intervals a PDP8 minicomputer censused each of the 10 counters, recorded the number of pulses from that interval, and reset the counters to zero. A teletype was used for a continuing display of the data and external control of the minicomputer. Permanent data recordings were simultaneously

Figure 2. A schematic representation of
the biological monitor.


AQUARIA
DIGITIZING
DIGITIZING
DIGITIZING
COUNTER
COUNTER
COUNTER
PHYSICAL/
CHEMICAL
PROBE
PROBE
A/D CONVERTER
A/D CONVERTER
ADDRESS
DECODER
LOGIC
INTERFACE
MINI
COMPUTER
TELETYPE
MAGNETIC
TAPE

recorded on magnetic tape.

The technique specifically designed for handling data from this system was developed by Hall (1972). It was found that both individual and diurnal variation in activity must be accommodated. This was accomplished by recording data for an initial standardization period of at least four days during which only dilution water was supplied to the fish. This four day period commenced only after newly introduced fish were permitted a 24 h acclimation interval to adjust to the tanks. The standardization period was used to establish the "normal" pattern of activity for each fish for each 30 min interval of the day. Statistically this was done by estimating the parameters for negative binomial distributions from the observations of each 30 min period and each fish during the standardization period. A fortran program modified from Hall (1972) was used to calculate upper and lower "critical limits". For each normal reading two critical levels of activity were calculated, one above normal and one below. If during any 30 min interval the activity of a given fish deviated sufficiently from the normal to exceed these critical values, the monitor indicated the activity for that fish during that recording period as abnormal. The direction (high or low) of the response was also shown and if three or more fish respond for two or more consecutive periods, a warning was given (Fig. 3). Thus the behavior of the fish indicated when toxicant levels became potentially hazardous.

Figure four is a sample plot of the type of activity patterns an unfed control fish showed when the data from the monitor are graphed.

Figure 3. A representative page of printed output from the monitor; the left most column is time recorded in minutes of the year.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 209700 | 72 | 35 | 121 | 22 | 184 | 17 | 379 | 28 | 36 | 19 | 192 | 42 |
| | | | | | | | | | CODE 1L | | | |
| 209760 | 174 | 36 | 124 | 14 | 45 | 17 | 527 | 22 | 38 | 20 | 178 | 38 |
| | | | | | | | CODE 1H | | | | | |
| 209820 | 74 | 38 | 157 | 18 | 100 | 17 | 338 | 26 | 41 | 26 | 167 | 40 |
| 209880 | 59 | 36 | 169 | 18 | 46 | 19 | 339 | 22 | 46 | 16 | 187 | 40 |
| | CODE LH | | | | | | | | | | | |
| 209940 | 33 | 26 | 170 | 17 | 72 | 18 | 316 | 32 | 122 | 27 | 160 | 48 |
| | CODE 1L | | | | CODE 1L | | | | | | | |
| 210000 | 10 | 43 | 224 | 16 | 70 | 26 | 409 | 39 | 221 | 18 | 362 | 38 |
| | CODE 1L | | | | | | | | | | CODE 2H | |
| 210060 | 190 | 40 | 192 | 17 | 219 | 19 | 375 | 45 | 164 | 19 | 243 | 40 |
| | CODE 2H | | | | | | CODE 2H | | | | CODE 2H | |

**WARNING**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 210120 | 7 | 39 | 76 | 16 | 103 | 20 | 407 | 64 | 63 | 17 | 162 | 58 |
| | CODE 2H | | | | | | | | | | | |
| 210180 | 23 | 22 | 57 | 13 | 70 | 21 | 565 | 47 | 70 | 15 | 204 | 40 |

**WARNING**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 210240 | 35 | 25 | 15 | 12 | 43 | 17 | 361 | 26 | 79 | 17 | 180 | 40 |
| | | | | | | | CODE 3H | | CODE 1H | | CODE 3H | |

**WARNING**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 210300 | 11 | 28 | 44 | 16 | 2 | 15 | 388 | 28 | 75 | 12 | 153 | 58 |
| | | | CODE 1L | | | | | | CODE 1H | | CODE 3H | |
| 210360 | 102 | 25 | 0 | 14 | 53 | 16 | 524 | 23 | 39 | 13 | 122 | 40 |
| | | | CODE 1L | | | | CODE 1H | | | | CODE 3H | |

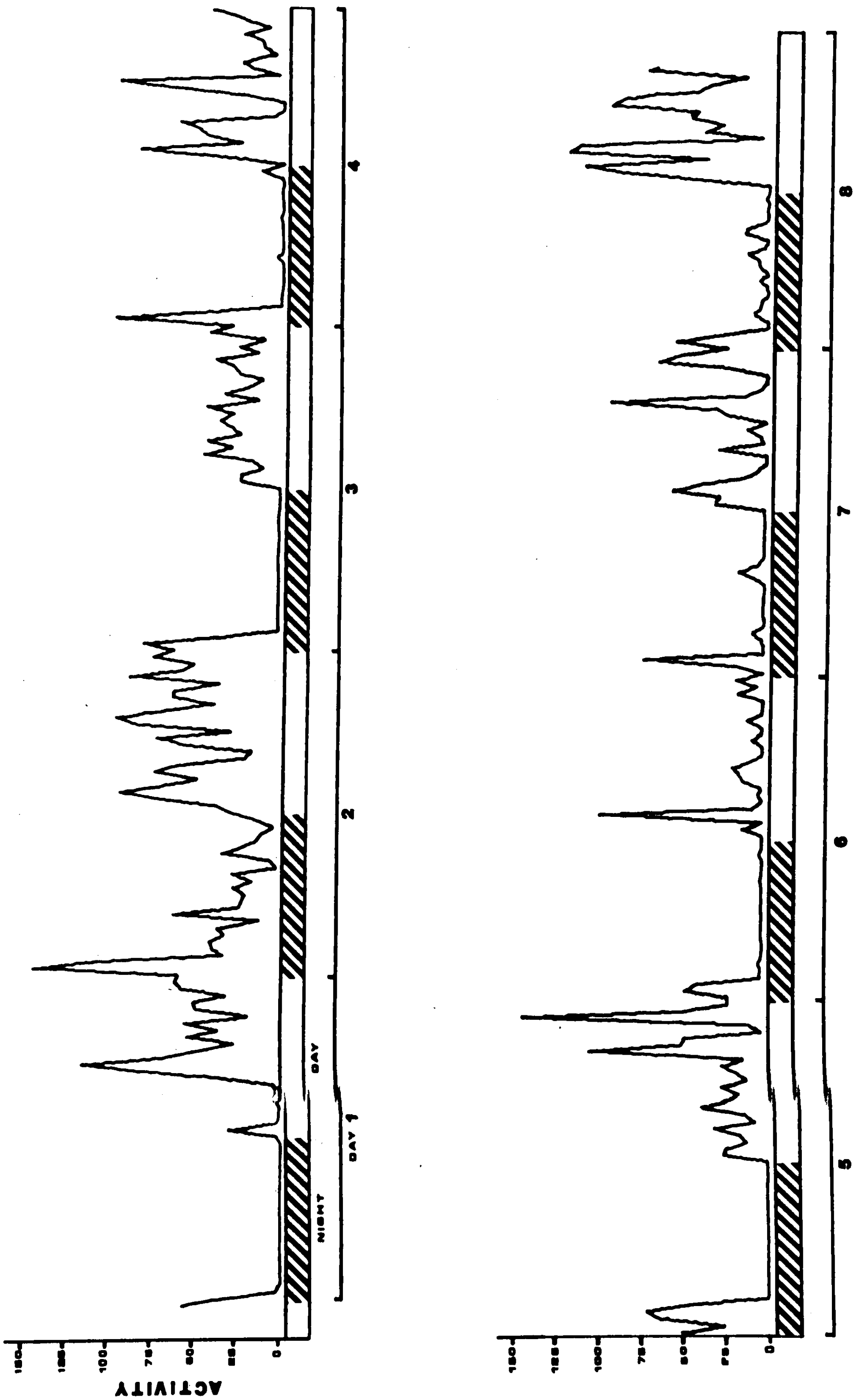
**WARNING**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 210420 | 45 | 21 | 52 | 11 | 27 | 15 | 259 | 24 | 24 | 15 | 89 | 51 |
| | | | | | | | CODE 1H | | | | CODE 3H | |

**WARNING**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 210480 | 56 | 34 | 37 | 11 | 1 | 15 | 418 | 37 | 20 | 11 | 54 | 41 |
| | | | | | CODE 1L | | | | | | CODE 3H | |
| 210540 | 72 | 21 | 40 | 10 | 27 | 15 | 351 | 22 | 38 | 14 | 38 | 47 |
| | | | | | CODE 1L | | | | | | | |
| 210600 | 39 | 21 | 13 | 14 | 16 | 19 | 165 | 33 | 29 | 15 | 29 | 35 |
| | | | | | | | | | CODE 2L | | CODE 2H | |
| 210660 | 89 | 17 | 15 | 16 | 17 | 16 | 168 | 29 | 21 | 18 | 23 | 35 |
| | CODE 2L | | CODE 1L | | | | | | | | CODE 2H | |

Figure 4. A plot of the activity of a control fish; note the diurnal rhythm.


ACTIVITY
NIGHT
DAY
DAY 1
2
3
4
5
6
7
8
0
25
50
75
100
125
150

Determination of Ration Size and Feasibility of Feeding

To determine the amount of food required per day, fish weights were checked before and after a nine day experiment. Prior to experimentation all fish were maintained in 50 gal aquaria on a 24 h photoperiod of 12 L - 12 D. Throughout the holding period the fish were fed Purina trout chow (number 3 grade) at random intervals. No fish were ever held less than two weeks prior to experimentation and all readily accepted the artificial food. A total of 30 fish were involved having a mean body wt of 22.37 g (SD 5.3).

For each ration level trial, 10 fish, visually estimated to weigh 20 g, were netted by hand. This proved to be an optimum size for use within the monitor due to the spacing of the light beams through the tank and the size of the tank. Each fish was allowed to drip in the net for 15 s and then transferred to a preweighed beaker of water. In an effort to reduce the stress from handling, the fish were not anaesthetized or blotted dry as some investigators have done (Gerking, 1971).

The fish were then placed in the monitor, one per tank. They were provided one 24 h period within which to recouperate from handling and to acclimate. Miller (1963) stated that Lepomis gibbosus can fully recover from the shock of being transferred from one aquarium to another within 30 min. O'Hara (1971a, 1971b) reported that for bluegill a 24 h period of adjustment is required.

Five fish were randomly selected and fed from automatic feeders once each day for the remaining eight days of the experiment. The daily feed dose was loaded into the feeders through the use of a small scoop constructed so as to deliver a set percentage of the mean body wt of the fish involved.

At the end of the ninth day the fish were removed and again weighed in the same manner as described above. The weight changes in all fed fish verses unfed fish were tallied. Three trials, using different fish and decreasing ration levels, were run.

The problem this experiment intended to solve was one of a very applied anture. Throughout the development of the biological monitor, design efforts had been made to eliminate the need for any specially trained personnel. It is for this reason that experimental fish were selected on a visual estimate of their size and wt; and why a small feed scoop was designed rather than critically weigh out the daily feed dose of each individual fish.

Following each trial a visual comparison was made of the condition of the water from tanks of fed and unfed fish. All tanks were drained, the water filtered and any residue in the tanks scraped out. The amount of filtrate and residue from tanks of fed and unfed fish was visually compared. Also the oven dry weight of all food introduced into the tanks of fed fish was compared to the oven dry weight of filtrate and residue recovered from those tanks.

Determination of Response to Food

In order to test the response to food, 10 fish, naive to the monitor, were selected in the manner described above except they were not weighed. Four trials were run involving a total of 40 fish. All fish were afforded a 24 h acclimation period. This was followed by a four day standardization period during which no fish were fed and then a four day test period in which five randomly selected individuals were fed at the estimated maintenance ration level. Automatic feeders fed once daily at a prescribed time. Brown (1957) reported that fish soon learn to expect food at regular times. With the exception of the final trial (accomplished using Tetra Min brand food in the form of flakes), the food used was Purina trout chow grade number 3. This was a food to which the fish had become accustomed and responded readily.

The hypothesis was as follows: using the diagnostic and analytic capabilities of the monitor, fish were unfed for a four day period from which their "normal" activity levels were computed for each 30 min period of the day. Then five fish were fed at a prescribed time each day for four days. It could be anticipated that increased activity would be associated with feeding. If the suspected increase in activity rose to a level significantly above the calculated "normal" for that period of the day, a response would be reported by the monitor; if not, the increases would show up in activity plots of experimental (fed) vs control (unfed) fish.

Determination of the Effect of a Toxicant upon Feeding Behavior

Once established that feeding was a physical possibility within the structure of the monitor and the response to feeding had been documented, the question presented itself as to whether or not the established feeding regime and associated behavior would affect the sensitivity of the monitor. Would fed fish be hardier and more resistant to toxicants than unfed fish? Conversely, would fed fish be a more sensitive indicator of very low concentrations of toxicant due to a disruption of some physiological pathway associated with feeding (vision, olfaction, gustatorial senses), and manifest that disruption through altered activity patterns during feeding time?

In order to test this the monitor was again employed, much in the fashion described above. The fish were alloted a 24 h acclimation period. During the 4 day standardization period 5 fish were fed while 5 were unfed. During the 4 day test period which followed, all fish were subjected to "pink" water introduced through the diluters into each of the 10 tanks to produce an in-tank concentration of 0.59%. Previous static bioassay work indicated 0.50% to be a low sublethal concentration; physical properties of the dilution apparatus yielded a concentration of 0.59%.

Two trials were run, each involving 10 fish, 5 fed and 5 unfed. The resultant data from the two classes were then analysed and compared to determine: 1) whether fed fish showed a significantly different number of responses than did unfed fish, 2) whether responses occurred with different frequency between fed and unfed fish, and 3) whether responses occurred at different times between the two classes (i.e. whether fed fish responded sooner or later than did unfed fish).

## RESULTS and DISCUSSION

### Ration Size and Feasibility of Feeding

The ration level tested in the third trial (0.48% mean body wt) was considered acceptable for use within the monitor (Table 1). This agrees with previous work. Gerking (1955) reported a maintenance ration for 30 g bluegill in the lab as approximately 1% body wt. Seaburg and Moyle (1964) reported a natural summer ration for bluegill in the wild was most often in the range of 1 - 2% body wt, and Winberg (1956) suggested that figures obtained for wild bluegill should be reduced by 1/2 for a laboratory situation.

As mentioned earlier in the paper, fish ration size (as a percentage of body wt) decreases as fish increase in size and nutritional requirements vary with changing seasons, temperature, and dissolved oxygen. Brown (1957), in an effort to measure the maintenance requirement of brown trout, discovered that as the amount of food was decreased the fish at first lost wt, then adapted to the new level of feeding and gained wt.

In the experiments conducted in this study, fish were not maintained for a sufficient length of time to allow aging to interfere; furthermore, photoperiod was constant (12L - 12D) to preclude seasonal complications, and dissolved oxygen content was always at saturation. Temperature was not controlled, but was monitored to detect any large fluctuations. Through the course of all three trials the water temperature rose gradually from 18.5 C to 23.0 C with no rapid changes. During the third trial, which resulted in the accepted ration level of

Table 1. Weight changes of bluegill within the biological monitor associated with different ration levels.

| Trial | Number of fish fed | Number of fish unfed | Ration level- (% mean body wt) | Mean wt change (g) - fed | Mean wt change (g) - unfed |
|---|---|---|---|---|---|
| 1 | 5 | 5 | 1.60 | +1.96 | -1.20 |
| 2 | 5 | 5 | 0.74 | +0.52 | -1.92 |
| 3 | 5 | 5 | 0.48 | -0.06 | -0.50 |

0.5% body wt, the temperature varied only between 22.2 C and 23.0 C.

If Brown's work with brown trout is applicable to bluegill, there is some possibility that the maintenance ration level for bluegill within the monitor is somewhat lower than these results indicate since all fish were maintained on an ad libitum diet before experimentation.

Filtrates from the water and tank residues from fed fish from all trials at all ration levels tested amounted to 5.7% oven dry wt of all food introduced. Accumulations of debris and bacterial build-up on the sides and bottom ranged from slight to undetectable upon a visual inspection. Therefore, even if an actual maintenance ration level was not determined, the level found was sufficient to maintain the fish in good health and not too large to tax the self cleansing properties of the monitor tanks.

Response to Food

In order to test whether feeding affected the gradual decline in activity over time, a mean activity level was computed for each day of an experiment (day 1 through day 8) from data of all unfed and then all fed fish. The means were then plotted and the slope of the line calculated. Both lines had a negative slope; the line for unfed fish had a slope of -0.285 while that for fed fish was -0.177. This would seem to indicate a decrease in activity with time. It was interesting that the decrease was twice as great for unfed fish as for fed fish, however, neither line deviated significantly from zero ($P < .09$).

If this decline in activity was real, it is possibly associated with increased familiarization with the environment. Glanzer (1953) suggested that continuous exposure to an environment, that is, to the same constellation of external stimuli, an animal becomes less active. He continued to say that stimulus satiation might be described as a "boredom-like" effect. Hinde (1970) refers to this phenomenon as habituation and defines it as "the relatively persistent waning of a response as a result of repeated stimulation which is not followed by any kind of reinforcement"; and Russel (1967) demonstrated the phenomenon using guppies.

Habituation appeared to take place in these experiments and was not significantly altered by feeding. The decline in activity was considered to be at such a rate as to have no effect on the operation of the monitor. Long term confinement of fish under these conditions would be desireable to ascertain the length of time the decline persists and whether or not it levels off.

Concerning activity specifically associated with feeding, the original supposition was that activity levels during time of feeding would be detectably different between the standardization (unfed) period and the test (fed) period. This proved to be false. The monitor reported no abnormal activity at feeding time, and plots of activity (Fig. 4) did not clearly show any well defined peak of activity associated with feeding.

In a further effort to determine whether or not the monitor detected feeding, mean activity counts were calculated for each fish for the three hour period of the day which encompassed feeding time summed over all days of an experiment (Table 2). Any one feeder required 40 min to empty a day's ration into a tank. To take into account the variation

Table 2. Mean activity during feeding time of those fish which were given food and those not given food; also of those fish treated with "pink" water and those not treated.

| | Untreated | | Treated | |
|---|---|---|---|---|
| Day | Fed | Unfed | Fed | Unfed |
| 1 | 155.90 | 97.17 | - | - # |
| 2 | 151.67 | 99.43 | - | - |
| 3 | 164.40 | 54.83 | - | - |
| 4 | 156.14 | 48.87 | - | - |
| 5 | 152.00 | 75.80 | 109.97 | 21.83 |
| 6 | 119.30 | 60.13 | 85.05 | 27.72 |
| 7 | 133.10 | 38.87 | 114.02 | 14.57 |
| 8 | 136.45 | 57.27 | 134.14 | 23.65 |

# In this series of experiments feeding constituted the test period and did not begin until day 5.

between feeders, activity counts for the hour preceeding and following feeding time were used in computing a mean hourly activity rate for each fish during feeding time. This figure was contrasted with the mean hourly activity rate of the remainder of the daylight period. An analysis of variance of the data shows that fish activity during feeding time is significantly higher from fish activity at other periods of the day ($P < .02$, $df = 1,30$).

To test whether this difference was due to feeding or was an artifact associated with the time of day at which feeding occurred (although feeding time was varied between experiments), the same analysis was applied to unfed fish. There was no significant difference between feeding time and other hours of the daylight period for unfed fish.

The Effect of a Toxicant on Feeding Behavior

If there were some broad interaction between bluegill activity and "pink" water, this would have become apparent by contrasting the 4 categories: fed-treated, unfed-treated, fed-untreated, and unfed-untreated. A factoral analysis of variance showed no significant difference between the overall activity of any category and no significant interaction between feeding and "pink" water.

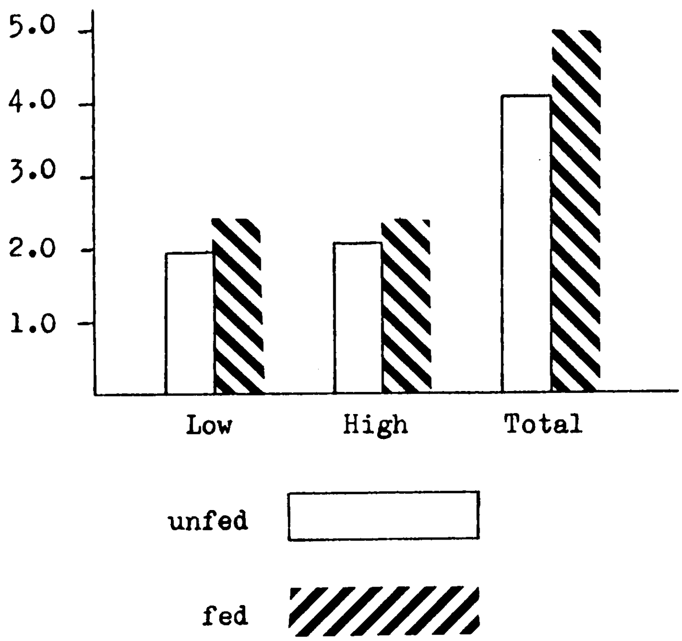
In the previous series of experiments a response to feeding was not detected until the activity of fed fish at feeding time was contrasted to the remainder of the daylight period. It was demonstrated that fed fish showed significantly greater activity at feeding time than at other times, while unfed fish showed no such response. The same approach was taken in this series of experiments, that is, the activity at feeding time was contrasted to the remainder of the daylight period for groups of fish fed and treated, and those fed and untreated. Fed-untreated fish showed significantly greater activity during feeding time ($P < .02$) while fed-treated fish showed no significant difference ($P < .56$).

When considering actual responses (as opposed to overall activity) to "pink" water (0.59%) as recorded by the monitor (Fig. 5), fed fish responded more than unfed fish although not significantly so ($P < .08$).

Although any individual test did not show results of the most lucid nature, when viewed collectively they lead to the conclusion that feeding augments the ability of the monitor to detect "pink" water. The fact that there was not a significant difference (significant at the .05 level) in the number of responses between fed and unfed fish may seem a bit worrisome. However, the effectiveness of the monitor is measured on a continuum from total nondetection through instantaneous detection, its effectiveness cannot be measured in discrete units. Attempts to detect a difference between the responses of fed and unfed fish did not begin with an apriori test level of .05 for the F statistic. The test was conducted through the use of the Statistical Analysis System on the V.P.I. & S.U. IBM370 computing facility. The resultant significance level was .08. This is deemed insufficient to reject the hypothesis that feeding effects the ability of the monitor to detect "pink" water, and that the effect is in a positive direction. I conclude that feeding does augment the ability of the monitor to detect "pink" water.

Although this has not been demonstrated before in bluegill, other

Figure 5. Total mean daily responses to "pink" water of fed vs unfed fish. High indicates a response was signaled due to excessive activity, low indicates a response due to insufficient activity.


5.0
4.0
3.0
2.0
1.0
Low
High
Total
unfed
fed

investigators have found toxicants to affect the feeding behavior of trout (Edwards and Brown, 1966), flagfish (Foster et al. 1969) and catfish (Bardach et al. 1965). Sprague (1971) reported that feeding behavior of fish is badly affected by some pollutants although he did not name them specifically.

The overall results of this work show that an average daily ration of 0.5% body wt of Purina trout chow, number 3 grade, is sufficient to maintain bluegill sunfish of an approximate body wt of 22 g in the environment of the biological monitor described in this paper. Some problems were encountered with the commercial feeders used (e.g. pellets jamming the feeder) and their maximum untended operation time was only 14 days. I have demonstrated the feasibility and value of feeding, however, this is partially dependent upon the food delivery technique which could be optimized through additional research. It is conceivable that a feeding unit that delivered the total ration instantly, as opposed to the 40 min span required by the feeders used in this study, would elicit a much more pronounced feeding response. This would make easier the detection of any abnormality of that response and perhaps further increase the sensitivity of the monitor.

## CONCLUSIONS

The conclusions drawn from this study apply specifically to the biological monitor described herein and are:

1). prolonged automated feeding without attendance from personnel is feasible;

2). a ration level of 0.5% body weight is large enough to sustain bluegill sunfish in good health under the conditions present in the monitor and small enough to maintain an acceptable level of cleanliness;

3). feeding has no significant effect on the gradual decline in mean daily activity;

4). feeding is associated with a distinct and detectable level of activity compatible with the design of the monitor; and

5). feeding increases the capability of the monitor to detect "pink" water.

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THE IMPORTANCE OF BLUEGILL FEEDING BEHAVIOR WITHIN
AN AUTOMATED BIOLOGICAL MONITORING SYSTEM
FOR INDUSTRIAL WASTES

by

Irvine D. Prather

(ABSTRACT)

Cairns et al. (1973) described a biological monitoring system designed to detect sublethal levels of industrial effluents. Measurements of fish (Lepomis macrochirus, Rafinesque) activity, as well as respiration, were employed as indicators of toxicity. Prior to this study, fish used within the system were unfed throughout the duration of an experiment, and casual observations indicate the mean level of activity decreased over time.

A complete redesign of the aquaria was accomplished (Westlake et al. unpublished) to provide a means of long term automated feeding. An approximate maintenance ration level was determined (0.5% body wt). The response to food was a flurry of activity at the time of feeding, but this did not significantly alter the overall decline in activity. Feeding did increase the capability of the monitor to detect "pink" water (a toxicant resulting from the production of TNT) as shown by a significant increase in the number of responses from fed fish over unfed fish.