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APPLICATION OF AN AUTOMATED FISH BIOMONITORING SYSTEM AT OLD O-FIELD FOR
CONTINUOUS ACUTE TOXICITY EFFLUENT MONITORING

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ABSTRACT

An Automated Fish Biomonitoring System was developed by the U.S. Army Biomedical Research and Development Laboratory (USABRDL) to identify developing toxic conditions in water by continuously monitoring the ventilation and movement patterns of the bluegill (*Lepomis macrochiris*). Physiological stress to the bluegills, characterized by changes in fish ventilation and movement patterns, is used as an early warning to identify developing acute toxicity of a treated groundwater (effluent) discharge at Old O-Field, Aberdeen Proving Ground, MD. An IBM compatible personal computer continuously monitors and records ventilatory rate, ventilation depth, cough rate, and whole body movement of up to 32 fish simultaneously.

Monitoring begins with 16 fish held in control water for a three-day acclimation period followed by four days of baseline data collection. The fish are then divided into two groups (8 fish in control water and 8 fish in effluent). During the subsequent continuous exposure to effluent, the computer provides immediate analysis of statistically significant departures from baseline conditions for fish in the control and effluent-exposed groups. After two weeks exposure to effluent, new fish are placed on-line to continue monitoring the effluent. The U.S. Environmental Protection Agency Region 3 included the automated fish biomonitoring system in a Record of Decision for the discharge of the effluent to the Gunpowder River.

The Automated Fish Biomonitoring System has been integrated with the Groundwater Treatment Facility at Old O-Field. When the monitoring system identifies a potentially toxic effluent (6 fish

responding to the effluent), an effluent sample is automatically collected for chemical analysis, a remote monitor in the treatment facility control room identifies the problem to the facility operators, and the discharge is diverted to storage tanks until the problem is resolved.

INTRODUCTION

The increasing use of biological approaches to evaluating water quality is illustrated by trends in regulating wastewater effluents over the last 25 years. Originally, chemical and physical measurements of effluent quality alone were considered sufficient for characterizing potential toxicity to aquatic organisms in receiving waters, but experience showed that some effluents that appeared nontoxic based on standard chemical measurements were nevertheless highly toxic to aquatic life. Consequently, laboratory tests with aquatic organisms are widely used in addition to physical and chemical measures to provide a direct measure of effluent toxicity¹. In addition, standard biological measurements have been developed for field evaluations of stream quality, including indices based on fish or invertebrate community structure². These measures offer a direct indication of toxicity and integrate the multitude of potential sources of toxicity that may be present. Even unsuspected and infrequent but highly toxic materials can be evaluated using biological approaches. One such biological approach is the monitoring of ventilatory response to environmental stressors. Ventilatory responses are often some of the first prelethal symptoms exhibited by animals to environmental stressors³.

The Automated Fish Biomonitoring System developed at USABRDL^{4,5} provides several additional biological measures that significantly increase the utility of the system as a biological monitor for developing acute toxicity of an effluent. The ventilatory system gives near real-time continuous toxicity evaluation which translates to near real time feedback to plant operations. The system gives an early warning to developing acutely toxic conditions leading to the prevention of acutely toxic releases to the environment. The ventilatory system has been automated to provide unattended and remote toxicity monitoring.

SITE BACKGROUND AND HISTORY

Old-O-Field is a 4.5 acre hazardous waste and ordnance disposal site located on the lower half of the Gunpowder Neck in the Edgewood Area of Aberdeen Proving Ground, Maryland. Surrounded by water on three sides (Watson Creek to the north and east, and the Gunpowder River to the west), Old O-Field is situated on a local topographic high with a 4 to 6 foot relief across the field. The site is mostly overgrown with small trees and scrub vegetation, with a few partially visible disposal pits.

Prior to its purchase by the U.S. Army in 1917 the site was mixed farmland and woodland, like much of the Gunpowder Neck Area. With the Army's purchase, the site became integrated into the Edgewood Arsenal, but disposal activities did not begin until the 1930s. During the 1940s and early 1950s, unlined pits and trenches were dug within Old O-Field and used for the disposal of military unique substances, munitions, contaminated equipment, and miscellaneous hazardous waste. Trenches were typically 12 feet deep, 20 feet wide, and covered with soil. No disposal appears to have been performed after 1953.⁶

The Army has performed several cleanup operations at Old O-Field. In 1949, a decontaminating agent was applied to the field to detoxify mustard agent that had been scattered over the area. However, recent analyses of Old O-Field groundwater have revealed chlorinated hydrocarbons that probably resulted from the decontamination effort. The early attempts at cleanup resulted in further contamination of Old O-Field groundwater. In 1953, another cleanup operation was conducted which involved soaking

the field with fuel oil and allowing it to burn for several days. Further decontamination and cleanup efforts were limited to removing and securing ordnance items recovered in surface sweeps across the field. Currently, the site contamination is being controlled by the installation of a permeable cap and a pump and treat system to remove the contaminants from the groundwater prior to entry into the Gunpowder River.

GROUNDWATER TREATMENT FACILITY

To address the problem of groundwater contamination, the U. S. Army Department of Safety Health and Environment at Aberdeen Proving Ground has built a treatment facility which extracts groundwater and treats it by 1) chemical precipitation to eliminate heavy metals 2) air stripping to remove volatile organic compounds 3) ultraviolet oxidation to chemically oxidize organic compounds, and 4) carbon adsorption to remove residual organic compounds that may not have been destroyed by the previous unit processes. The final effluent is monitored by an in-line continuous fish biomonitoring system to determine whether the effluent leaving the treatment facility is acutely toxic. Treated water is either discharged to the Gunpowder River, or passed through the treatment process again if the treatment has been found to be inadequate.

EFFLUENT DISCHARGE MONITORING REQUIREMENTS

The Record of Decision agreed upon by EPA Region 3 and Maryland Department of the Environment placed the following monitoring requirements on the Old O-Field groundwater treatment facility effluent for the first 2 years of operation. The effluent was to have routine sampling for chemical analysis (metals, volatile organic compounds, and mustard agent degradation products), quarterly biomonitoring using fathead minnows and ceriodaphnia, and the installation and operation of the Automated Fish Ventilatory Monitoring System.⁷

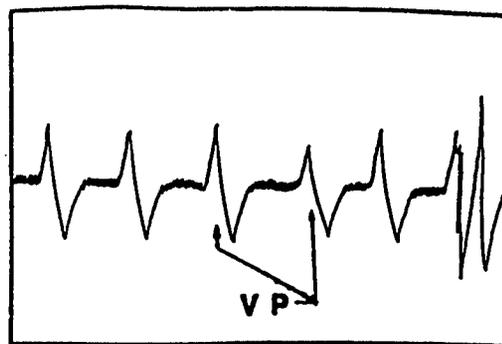
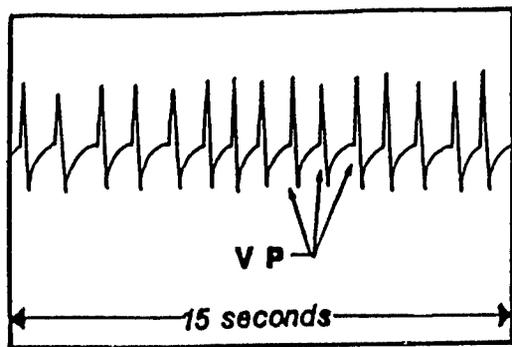
METHODS

TEST FISH

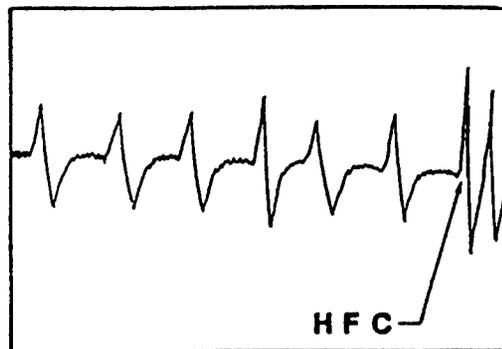
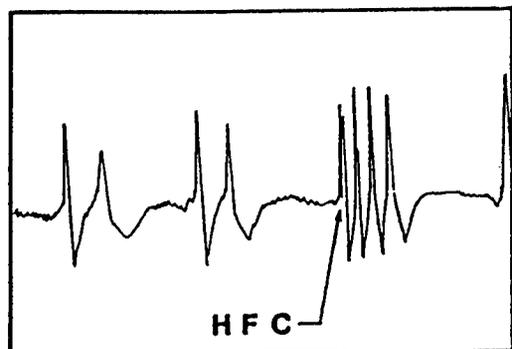
Bluegills, *Lepomis macrochirus* can be acquired from commercial hatcheries or field collected year round for use in the ventilatory monitoring system. The bluegills were placed on site, acclimated to continuous light and control water for a minimum of 2 weeks prior to testing. During acclimation and testing, wide spectrum fluorescent bulbs with a color rendering index of 91 were used. Testing was conducted under continuous light to eliminate diurnal changes in bluegill ventilatory patterns. During acclimation in the holding facilities, fish were fed Rangens #3 trout food (Zeigler Bros., Inc., Gardners, PA) plus frozen brine shrimp (Living World, Virginia Pet Supply, Fairfax VA). Fish were not fed during the 2-week effluent exposure period.

BLUEGILL VENTILATORY PARAMETERS

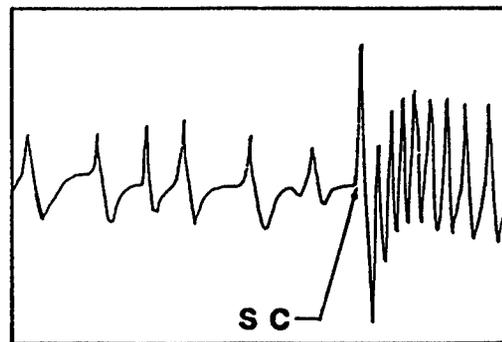
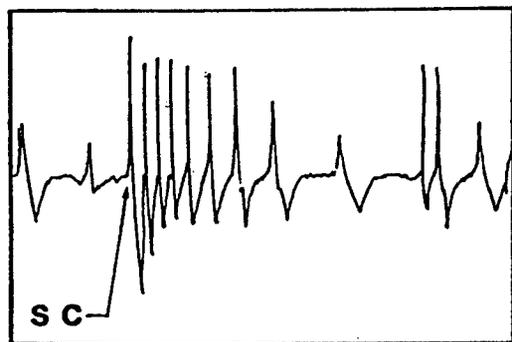
The monitoring system analyzes changes in the ventilatory activity of fish². The electrical impulses generated by muscular activity of the fish are amplified, filtered and analyzed to create a four parameter monitoring system. These parameters are: ventilatory rate, mean signal height (ventilatory depth), gill purge rate (coughs), and rapid irregular electrical impulses (whole body movement). Figure 1 shows typical ventilatory electrical signals from the bluegill.



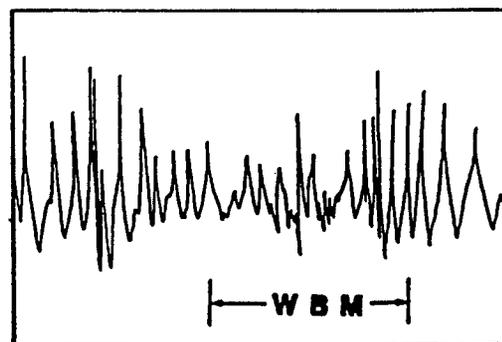
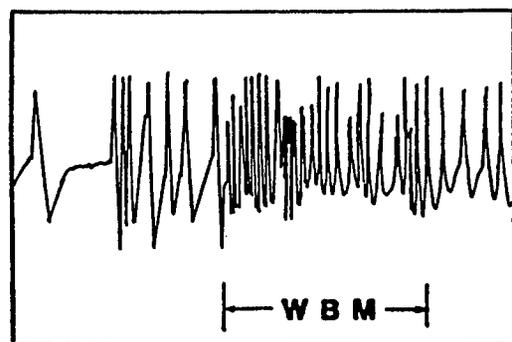
V P - VENTILATORY PEAK



H F C - HIGH FREQUENCY COUGH



S C - SPIKE COUGH



W B M - WHOLE BODY MOVEMENT

FIGURE 1. TYPICAL VENTILATORY SIGNAL RECORDINGS

EXPERIMENTAL DESIGN

The Automated Fish Ventilatory Monitoring System is a 21-day test that cycles new fish in for testing every 14 days; 3 days acclimation, 4 days baseline data collection, and 14 days monitoring (exposure) to the treated groundwater (see Figure 2). There were two effluent treatments and two control groups with 8 fish per treatment and control. Each of the 32 fish used in the test were placed into individual test chambers (See Figure 3). After one effluent treatment group and one control group have been monitored for 7-days, a second set of 16 fish are placed in effluent treatment and control groups. After the 7 day acclimation and baseline data collection for the second set of fish, they were placed on-line for effluent monitoring. The first set of fish were removed from the chambers, weighed, standard length measured, and placed in a holding tank for a 2-week observation period. Due to the exposed bluegills potential acclimation to the effluent, the fish were not re-used and were euthanized. The ventilatory electrical signal is picked up by the chamber electrodes that are connected to individual amplifier filter boards using shielded cable. Each fish ventilatory signal is then sent to a personal computer for analysis of ventilatory rate, depth, cough rate and whole body movement.

During exposure to the effluent, each fish ventilatory pattern was compared to its own baseline normal limits. If the exposure ventilatory pattern differed from the baseline pattern, the fish was considered "out of control". For a treatment to be "out of control", 6 fish in that group were required to be out of baseline limits⁸. The continuous ventilatory data collected was summarized and recorded on electronic media every 15 minutes during each monitoring cycle. The data was also sent to a host computer for remote data access. Data were then evaluated and archived at USABRD.. A solenoid dilution device was used to maintain a 50 ml/min. flow rate to all fish chambers.

WATER QUALITY MONITORING

Continuous monitoring of dissolved oxygen, pH, temperature, and conductivity were accomplished using a multi-probe transmitter (H-20, Hydrolab Inc.). The water quality data were directly imputed and recorded in the 15-minute biomonitoring records. When the fish respond to changes in effluent quality, a signal is sent to an automated sampler and an effluent sample is taken at the time of response. The sample was then preserved and held for future analysis.

RESULTS AND DISCUSSION

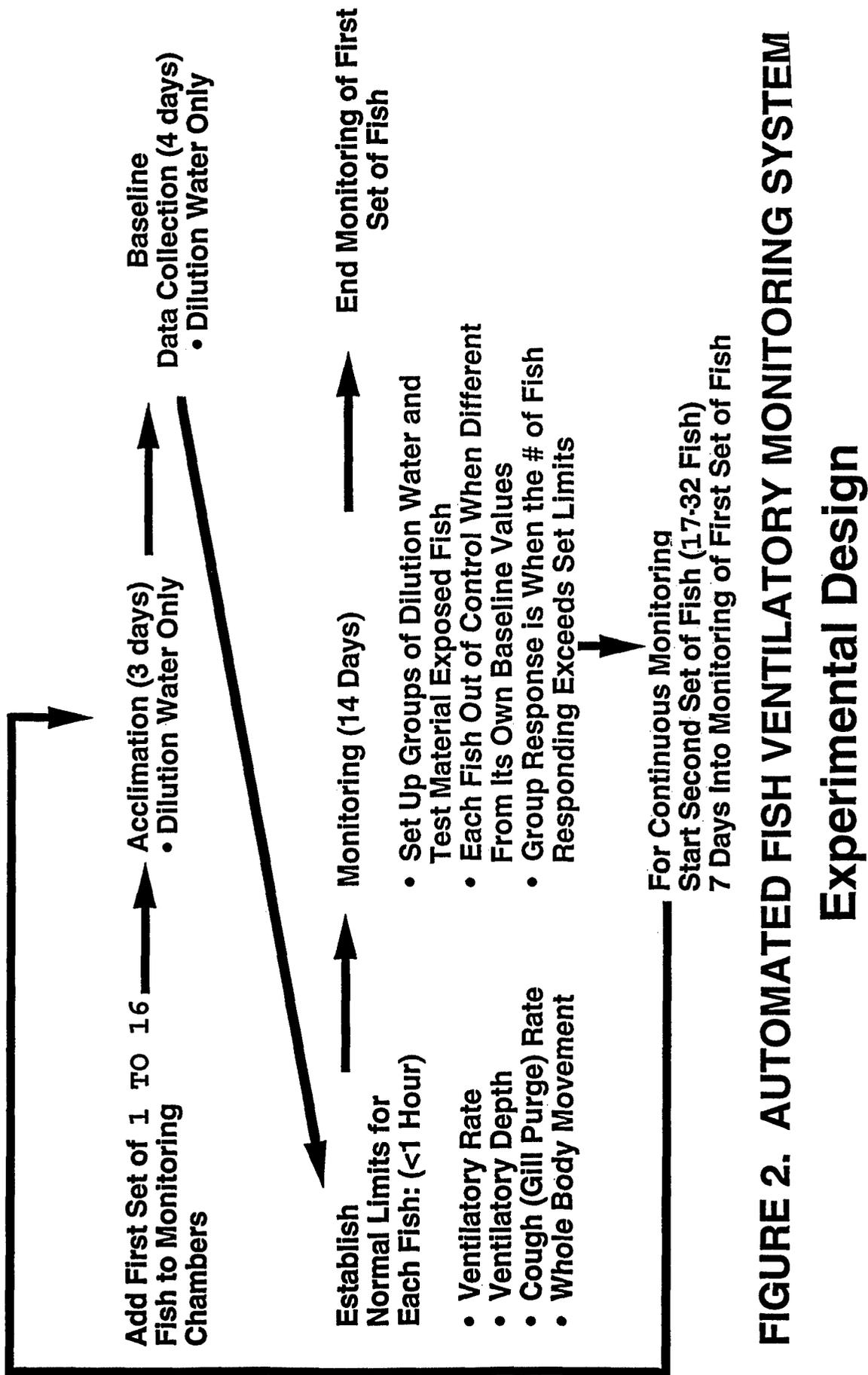
LABORATORY SYSTEM VALIDATION

The accuracy of the Automated Fish Ventilatory Biomonitoring System was established by comparing stripchart records (128, two and one half minute records from six tests) to the computer generated values for ventilation rate, average ventilation depth, and cough rate. Whole body movement accuracy was not established due to infrequent movement events. The accuracy of the ventilatory rate and average depth was 99% with an R^2 value of 0.997 (slope = 0.94) and a cough rate accuracy of 118% with an R^2 value of 0.781 (slope = 1.27).

Laboratory results of single compound testing have produced 0.5 hour response times for zinc and pentachlorophenol at concentrations near the 96 hour L for bluegills. Responses were similar to those reported by McKim et al.^{9,10}

AUTOMATED FISH VENTILATORY BIOMONITORING SYSTEM APPLICATION

This manuscript covers the operational period for the Automated Fish Ventilatory Biomonitoring System from June 23, 1996 to March 31, 1996 at Old O-Field. During this period, the data acquisition



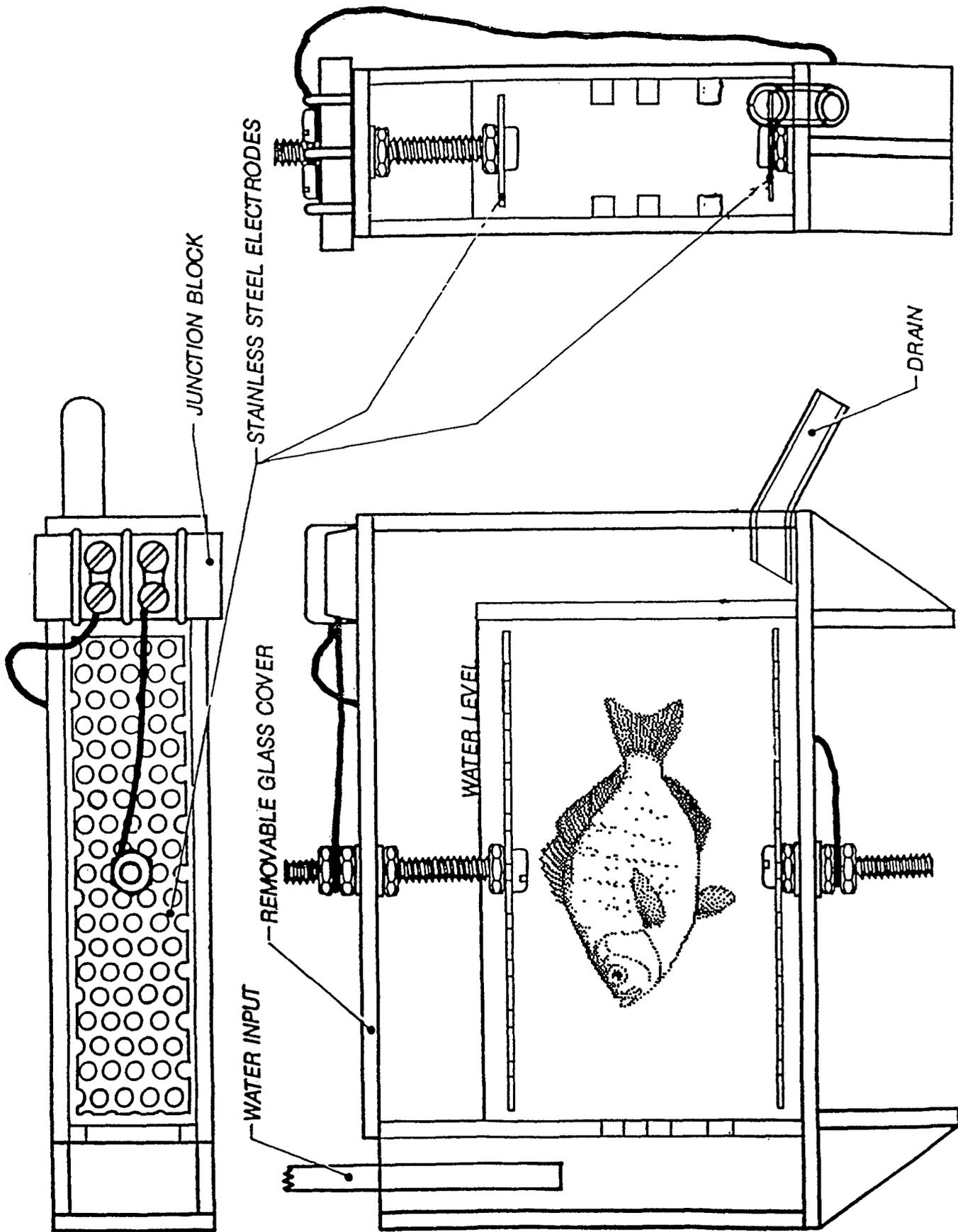


FIGURE 3. TEST CHAMBER, FULL SCALE

system was on-line for 273 days out of a total of 282 days. Data were not taken for approximately 9 days because the data acquisition system was off-line for various reasons (e.g., instrument calibration, data transfer, accidental program interruptions, power failures, etc.). No effluent discharge to the Gunpowder River occurred if the data acquisition system was off-line. No fish mortality was observed during discharge to the Gunpowder River as a direct result of groundwater process failure. This result was in part due to the early warning provided by the Automated Fish Ventilatory Biomonitoring System to the treatment plant operators. In most cases, corrections to effluent quality were made prior to the conditions becoming acutely toxic. However, acutely toxic conditions were identified during treatment plant maintenance and operational modifications were started.

A number of out of control responses occurred during the operational period. The total number of days the system obtained out of control responses was 21 days when the system was on-line. An explanation for the out of control responses was available for 20.6 days of the 21 days. No obvious explanation was apparent for 0.4 days. Since analytical samples were automatically taken when these responses occurred, analytical results of the preserved samples may provide additional explanations for fish ventilatory responses to the effluent.

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DISCLAIMER

The views, opinions, and/or findings contained in this manuscript are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by official documentation. Citations of commercial organizations or trade names in this manuscript do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 86-23, 1985 edition.

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