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Long-Term Operation of an Automated Fish Biomonitoring System for Continuous Effluent Acute Toxicity Surveillance

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Over the past 30 years, the use of aquatic organisms as biological early warning indicators for monitoring of water supplies and effluents has been extensive, and many applications of such biological early warning systems (BEWS) have been proposed. The fundamental components, design, and operating parameters of these BEWS have been reviewed elsewhere (Gruber and Diamond 1988; Kramer and Botterweg 1991). Fish were the sentinel organisms originally selected for BEWS, and they continue to be a popular choice. While examples of field testing of automated BEWS in the USA are relatively few, a number of systems have been evaluated in Europe (Koeman et al. 1978; van Hoof et al. 1994; Gerhardt 1999) and in South Africa (Morgan et al. 1982).

Automated BEWS are designed to record continuously certain established behavioral or physiological parameters for evaluation of changes that could be indicative of developing toxic conditions. Fish ventilatory parameters known to be sensitive to toxicity are ventilatory rate (opercular movement), depth or amplitude of ventilation, and coughing or gill purge rate. Whole body movement has also been used. A review summarizing the methodology for measurement and interpretation of fish ventilatory patterns as early warning signals of water quality deterioration and incipient toxicity was published recently (ASTM 1995).

This paper describes the long-term performance of an automated biomonitoring system utilizing the ventilatory and movement patterns of the bluegill, *Lepomis macrochirus,* which has operated continuously at Old O-Field, Aberdeen Proving Ground (APG), MD, since June 23, 1995.

Old O-Field is a 4.5 acre hazardous waste and ordinance disposal site located on the lower half of Gunpowder Neck in the Edgewood area of APG, and is surrounded by surface water on three sides (Watson Creek to the north and east and Gunpowder River to the west). During the 1940s and early 1950s, unlined pits and trenches were dug within Old O-Field and used for the disposal of chemical warfare agents, munitions, contaminated equipment, and miscellaneous hazardous waste. Several clean-up efforts by the Army resulted in further contamination of groundwater at the site. In 1949, a chlorinated decontaminating agent was applied

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to the field to detoxify mustard, and in 1953, the field was soaked with fuel oil and allowed to burn for several days. During the past five years, interim environmental remediation has been afforded by pumping water from the contaminated aquifer to a groundwater treatment facility (GWTF), where it is subjected to four stages of treatment to remove heavy metals and organic pollutants and discharged to the Gunpowder River. The site was selected for continuous automated biomonitoring of the effluent prior to discharge because of the chemical complexity of the untreated effluent, and the need of APG managers, state and federal regulatory agencies, and the public for assurance that discharge is within safe limits.

MATERIALS AND METHODS

Bluegills (length 4-8 cm, weight 1.2-13.3 g) are acquired from local sources and acclimated on-site in control water with continuous light for a minimum of two weeks. During acclimation they are fed commercial trout chow and frozen brine shrimp, but are not fed in the ventilatory chambers.

Water from the contaminated aquifer at Old O-Field is pumped to the GWTF for processing to remove heavy metals and organic pollutants. Treated effluent is then pumped to one of two holding tanks prior to discharge to the Gunpowder River, and a side stream is directed to the biomonitoring chamber. The flow-through chamber has an overall dimension of 23 x 15 x 12 cm and eight sub-chambers 2.5 x 9.5 x 6 cm, with individual water input and a common drain. The sub-chambers are designed to allow source water to enter the bottom, pass through the chamber, and exit the top over a spillway. If effluent processing is discontinued, effluent-exposed bluegills receive effluent recirculated from a holding tank. The holding tanks provide a mechanism to stop effluent discharge in the event of fish mortality. Control bluegills receive the same flow rate of dechlorinated tap water. Controls are used to monitor for alarms that may be caused by physical disturbances of the testing facility, cessations of water flow, or other factors that may produce system alarms unrelated to effluent effects.

Temperature, pH, dissolved oxygen and conductivity are measured in the effluent and control streams at 30 min intervals by alternating flows into a Hydrolab® monitor. Ranges of water quality parameters are shown in Table 1. While both control and effluent waters show similar temperature variations, effluent dissolved oxygen and pH tend to be lower and effluent conductivity higher than control water.

Ventilatory signals from individual fish are monitored by electrodes suspended above and below each fish in a chamber. The electrical signals are amplified, filtered, and passed onto a personal computer for analysis. Amplification is performed by PCI-20044T-1 and PCI-20045T-1 active analog signal conditioning termination panels (Intelligent Instrumentation, 6550 South Bay Colony Dr.,

Table 1. Ranges of water quality parameters, 1997 through 1999

Parameter	Field Studies	
	Control Water	Effluent
Temperature (°C)	16.4 - 26.2	19.2 - 26.2
pH	6.5 - 9.5	5.8 - 9.2
Dissolved oxygen (mg/L)	5.8 - 10.0	1.6 - 10.7
Conductivity (mho/cm)	0.20 - 0.66	0.41 - 3.65
Alkalinity (mg/L as $CaCO_3$)	36 - 75	36 - 55
Hardness (mg/L as $CaCO_3$)	42 - 103	120 - 325

Tucson, AZ 85706). Each input channel is independently amplified by a high gain true differential-input instrumentation amplifier; signal inputs 0.05-1 mV are amplified by a factor of 1000. Signal interference by frequencies above 10 Hz is attenuated by low-pass filters. The ventilatory parameters measured are ventilatory rate, ventilatory depth (mean signal height), gill purge (cough) frequency, and whole body movement (rapid irregular electrical signals) as shown in Figure 1. Each parameter is calculated at 15 s intervals, and any interval in which whole body movement was detected is excluded from calculation of the other three parameters. The computer ventilatory parameter accuracy was established by comparing the computer-generated values for ventilatory rate, average ventilatory depth, and cough frequency to those obtained from concurrent strip chart recorder tracings. Whole body movement was not addressed because of the infrequency of these events. For a total of 128 2.5 min records taken from six tests, the ventilatory rate accuracy (and, by inference, average depth) was 99% (R^2 0.997, slope 0.94), and the cough frequency accuracy was 118% (R^2 0.781, slope 1.27).

Figure 2 is a schematic display of the major components of the biomonitoring system and their functioning in relation to the GWTF at Old O-Field. Water flow through the system and fish ventilatory signal analysis are described above.

Continuous biomonitoring is achieved by alternating between groups of 16 fish, each of which is "on-line" for 14 days; within each group, eight fish receive effluent and eight receive control water. Each new group of 16 entering the system is monitored for seven days in control water before introduction of effluent; three days for acclimation followed by four days for collection of baseline data. If a ventilatory or body movement parameter of an individual fish becomes statistically different from its normal (pre-exposure) response, the response is said to be "out of control." If six of the eight fish receiving effluent

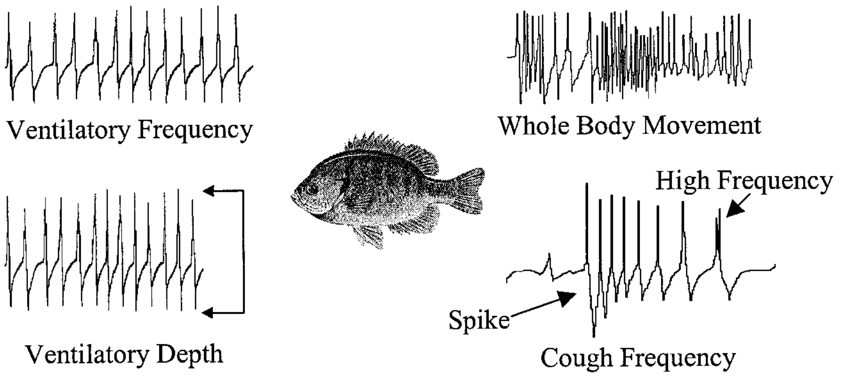

Figure 1. Bluegill Ventilatory Parameters

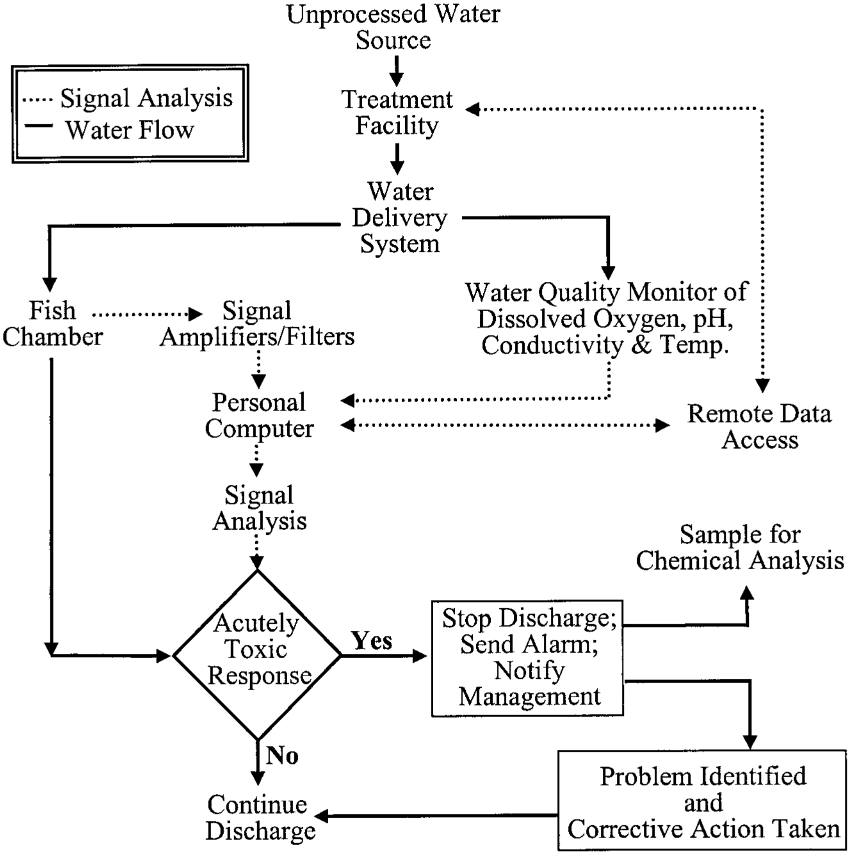

Figure 2. Effluent Monitoring Schematic

exhibit statistically different responses, the group response is said to be "out of control" and acutely toxic response is considered possible. The biomonitoring program then sounds an alarm at the GWTF and activates an ISCO® autosampler for an investigation of the effluent water quality parameters to help determine the probable cause(s) of the response. Water quality parameters are determined manually, and the sample is analyzed for heavy metals by inductively coupled argon plasma mass spectrometry and for volatile organic compounds by GC/MS to determine whether water quality excursion or inadequacy of treatment is a factor. If inadequate treatment is found, the effluent is recycled back through the GWTF. If four or more of the effluent test fish die, discharge of effluent to the Gunpowder River is stopped. Dead fish are replaced with fish having no pre-exposure base line data, so the system operates as a mortality monitor only until the next set of fish has passed through the acclimation and baseline periods. If only the control fish generate an alarm condition, biomonitoring system operation is investigated, but effluent discharge is not affected.

RESULTS AND DISCUSSION

The effluent biomonitoring system was installed at APG in early 1995, and was run in a start-up mode from March 27 through June 22. This period was needed for integration and adjustment of the detection parameters of the system with operational parameters of the GWTF at Old O-Field. Detailed accounts of the continuous operation of the system from June 23, 1995 through March 31, 1996 and from April 1,1996 through March 31, 1997 are available (Burton and Tieman 1996; *Shedd TR, Widder MW. Unpublished Report, U.S. Army Center for Environmental Health Research*). During the first period, the data system was operational for 272.9 days out of 282.4 days. (Effluent is not discharged to the Gunpowder River unless the system is on-line.) The majority of off-line time was due to scheduled events, e.g. instrument calibration and data transfer, and the remainder due to unplanned events, e.g. power outages and back-up generator failures. During the first four months of continuous operation in 1995, three events took place which allowed the system to function as a mortality monitor only, for a total time of 17.4 days. In the first two events, effluent and control fish were killed by a power outage and by a failure of the water delivery systems; consequently fish with no pre-exposure base line data were used as effluent acute toxicity monitors for a total time of 14 days. The third hiatus (October 1995) was due to a programming error which disrupted collection of baseline data for 3.4 days. No further operations of the system as a mortality monitor only were necessary through March 1999.

The system monitors control fish separately and in the same manner as effluent fish for evaluation of out of control responses (see Materials and Methods). During nearly four years of operation it was found that out of control events attributable to excursion of water quality parameters, activity at the GWTF, and health of fish were observed with both groups of fish; out of control events attributable to loss of water flow were observed only for the controls.

During the period June 23, 1995 through March 31, 1996, a total of 124 effluent group out of control events occurred while the system was on-line and not operating in mortality monitor mode. Summation of the duration of each out of control group event in hours and minutes and rounding off amounted to a total of 21.5 days out of 255.5 days. Explanations were readily available for all but 0.5 day of the 21.5 days of out of control group response time. The majority of explicable out of control responses (89%) were attributable to changes in the effluent water quality. A major problem in early operation of the biomonitoring system was a significant increase in conductivity of the effluent during the months of September and October, which triggered a number of out of control events in November 1995, but was subsequently eliminated by pre-exposure base line conditioning of the fish in water of equally high conductivity.

The water quality parameter implicated in the second largest number of out of control responses was dissolved oxygen (DO), and it continued to be a sporadic problem throughout the second reporting period. DO concentrations <5 mg/L trigger GWTF regulatory compliance actions. Low DO was alleviated during the treatment process by recirculating the effluent prior to delivery to the fish. Effluent temperature variations were also implicated in a number of out of control events during the June 1995-March 1996 reporting period, notably low temperature stress in November 1995 and February 1996, and a combination of DO shifts and rapid changes in temperature in March 1996. Out of control events not attributable to effluent water quality excursions were due to a previously mentioned power outage (6%) and malfunction of the water delivery system (5%).

During the April 1996-March 1997 reporting period, the automated data acquisition system was operational for 361 of the 365 days. Approximately 2.7 days of off-line time were due to scheduled events. During the operational period, the program recorded 118 out of control events lasting a total time of 21.7 days. Only 13 of these events, which lasted a total of 1.9 days, had no obvious explanation, and no mortalities resulted. Fluctuations in effluent water quality parameters were responsible for >99% of the explicable out of control time, with the remainder due to water delivery system malfunction and power failures. It is noteworthy that nearly all of the effluent out of control response events occurred in the first three months of the reporting period (51 in April, 46 in May, and 10 in June). None occurred in August, October, December or January. The majority of water quality excursions were due to low DO, but temperature variations were also frequent.

In June and July 1997, four out of control events not attributable to excursion of water quality parameters were observed. Control fish did not show a response, and metals analysis of the effluents revealed average concentrations of arsenic and antimony of 0.27 and 0.025 mg/L, respectively, well below the respective

USEPA water quality criteria levels (0.36 and 0.088 mg/L; USEPA 1991) but significantly higher than those averaged over the preceding three months (0.14 and 0.0007 mg/L, respectively), indicating a treatment process change. Thus, the system provided an early warning of sub-lethal toxicity, and the effluents were recycled for further treatment.

Continued operations of the biomonitoring system over the subsequent two years (April 1997 through March 1999) revealed similar on-line and response patterns to the previous reporting period. The automated data acquisition system was operational for 99% of the time (720 of 730 days) and the effluent group responses have stabilized at 2.5% of the operational time (18 days). Fluctuations in effluent water quality parameters continued to be responsible for >99% of the out of control time, with the remainder due to treatment facility maintenance and water delivery system malfunctions.

The earlier problem with increased conductivity of the effluent resurfaced between November 1997 and February 1998, and again was corrected by pre-exposure base line conditioning in water of equally high conductivity. In November 1998, however, the conductivity rise was so high as to affect the quality of the electrical signal. The problem has been solved by implementation of a variable gain amplifier that corrects for attenuation of the fish ventilatory signal under conditions of high conductivity.

A significant aspect of operation of the biomonitoring system is its positive effect on the management of the wastewater treatment operation. After an initial learning period when the biomonitoring system was adapted to the specific operational situation at the treatment facility, the engineers at the facility became more aware of effluent conditions, and biomonitoring alarms did not unduly disrupt effluent treatment operations. During the last two years of operation there have been only two biomonitoring alarms as a consequence of greater than 50% mortality due to mechanical failure, but none due to effluent toxicity. On-line operation of the biomonitoring facility has provided APG managers, state and Federal regulators, and the general public with increased confidence that effluent discharges will not be harmful to the receiving ecosystem.

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