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AUTOMATED MULTISPECIES BIOSENSING SYSTEM AND DEVELOPMENT: ADVANCES IN REAL-TIME WATER QUALITY MONITORING

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ABSTRACT

The objective of this study was to develop an automated computer-assisted multiple species biosensing unit for stream-side water quality monitoring. Groups of representative freshwater animals from several trophic levels were selected as test subjects for the automated biomonitor, including fish, immature insects, and mussels. Emphasis was placed on system design, hardware development, data management, and on initial observations of species-specific responses that could be used as criteria for judging water quality fitness. Specially designed differential amplifiers for detecting selected bioelectric responses from unrestrained subjects were interfaced to a 16-bit instrumentation minicomputer. Analogue response signals generated by fish gill ventilatory movements, mayfly nymph gill beats, and the mussel Quadrula, cardiac events were digitized and stored on disk files as events per unit time. Results from this study revealed that a multiple species biomonitor designed to detect selected biological responses generated by individuals from different freshwater tropic groups can be measured simultaneously and data bases managed for real-time acquisition. Changes in response rates may then be viewed in light of fluctuating environmental conditions and both biological and chemical/physical water quality data files may be accessible through various communication systems.

1. INTRODUCTION

Automated biological monitoring systems designed to detect toxicant-induced stress responses in aquatic animals have typically relied upon a single vertebrate animal, the fish, for sensing water quality changes [1]. From these systems, stress detection has been measured as a change in gill ventilatory frequency and cough activity, cardiac function, and selected behavorial and physiological responses. Though effective in many applications, it appears unlikely that fish alone will be responsive to the complex matrix of potential toxicants occurring in a multiple-use water resource. Therefore, in attempts to reduce the inherent bias of depending on a single-species or animal group for judging community toxicity and to better complement aquatic ecological quality control programs, we propose a computer-assisted biosensing unit designed to generate real-time biological response data from groups of different types of representative freshwater animals. By viewing biological response information from multiple species in light of simultaneously derived physical/chemical water quality data, and comparing this real-time information base to historical and projected trends, increased levels of confidence may be realized in a predictive/reactive program for maintaining aquatic ecological integrity [2].

2. RESEARCH OBJECTIVES

Our specific objective in this study was to design an automated computer-assisted multiple species biosensing unit for stream-side water quality monitoring employing freshwater vertebrates and invertebrates representing several trophic levels, including the bluegill sunfish (Lepomis macrochirus), the immature insect nymph (Hexagenia spp.), and the washboard mussel (Quadrula quadrula). In meeting this objective, emphasis was placed on system design, hardware development, and software managements programs. The selection of test organisms for this development study was based primarily upon availability since our initial research reveals that numerous members of these groups as well as selected types from various other groups work equally well as test subjects.

3. EXPERIMENTAL PROCEDURES

Initial research and development work was carried out in a mobile research laboratory operated by the U. S. Tennessee Valley Authority (TVA) near the Tennessee River, Muscle Shoals, AL. Sand filtered river water was used in these tests. During this phase of the study, techniques and procedures for monitoring bioelectric signals produced by various physiological functions from the mussel and mayfly nymph subjects were developed, incorporating modified methods previously tested in automated fish biomonitoring [3, 4, 5].

Once monitoring techniques had been refined, design requirements for response signal amplification, interface electronics, computer hardware, and data management systems were identified. Important to these requirements was the observation that each animal type induced rhythmic analog electromagnetic frequencies associated with specific physiological functions that could be selected for, i.e., heart rates, breathing patterns.

3.1. Biological Considerations

Fish test chambers were modifications of those used in previous studies [4, 5]. Employing a tube chamber design which housed a single free swimming fish, a pair of probe-type antennae were arranged on the tube in specific configurations for receiving the electromagnetic frequencies generated by each gill ventilatory response (breath).

Mayfly nymph chambers were designed along a similar plan, since the nymphs selected display a unique behavior of "burrowing" into acceptable substrate -- thus forming a tube-type tunnel within which they live. Once established this aquatic insect sets up a rhythmic peristolic oscillation of its many paired abdominal gills. Should an acceptable substrate for developing a burrow not be present, the nymph will typically seek an alternate tube chamber or similar shelter; in our case an artificial tube chamber. Our artificial tube chambers consisted of various sizes of tygon or plastic tubing which had been equipped with micro-probe antennae. Within these tube chambers, the apparent electromagnetic frequencies produced during gill oscillations could readily be measured by routine electrophysiology methods.

The washboard mussel cardiac activity was monitored by attacking micro-probe antennae to the valves and near the cardiac region of the animal. One technique required the placement of antennae through 1-2 mm diameter openings drilled in the umbo (raised area on each valve adjacent to the hinge) of the shell. These antennae were arranged so that they rested between the inner surface of the valve (shell) and the animal (mantle). They were not inserted into the tissue. This arrangement provided access to the bioelectric activities generated during peristolic contractions of the heart.

In the monitoring configuration, one of each species was placed in a modified 38 L rectangular glass aquaria with slate bottom. These aquaria were adapted with stand-pipes for the removal of water from the bottom and received continuous once-through flows of river water. Bluegills housed in tube chambers and mollusk were placed on the slate bottoms while an artificial tube chamber containing mayfly nymph was placed in a subcompartment and floated at the surface of the aquaria. Mayfly subcompartments also received continuous flows and were equipped with stand-pipes. For systems testing, four modified aquaria thus equipped were used.

3.2. Computer-Assisted Monitor

Specially designed differential d.c. powered amplifiers were constructed with variable gain and d.c. off-set. One amplifier was used for each animal and the gain and filter set to read the specific analog frequency desired. Undesirable high frequency noise was filtered at the initial stages of amplification. Amplifiers were interfaced to a 16-bit Texas Instrument instrumentation minicomputer with CRT terminals, printer, duel sided high density floppies (1.2 Mbite) and modem. Responses were amplified up to 10^6 times, digitized, and stored in registers until inputed to the computer for filing on disk as designated in the ROM program. The complete automated computer-assisted biomonitoring system (ACABS) was an updated version of the automated fish respiration monitoring system (AFIRMS) reported recently [5], which was fabricated by the Data Services Branch, TVA.

4. RESULTS AND DISCUSSION

An important task in meeting the objectives of this study was first to confirm that visually observed biological responses, such as heart rate and gill beats, were those represented by the analog frequencies seen after amplification. Ultimately, these responses then had to be verified with their digital equivalents seen in the computer out-put. Comparing visual counts of fish gill beats to electronically produced analog frequencies and digital equivalents was readily obtainable and is generally accepted [1]. Mayfly nymph gill oscillations were easily verified in a similar manner by directly observing the number of gill events produced per unit time and comparing this rate to the simultaneously recorded number counted on a strip-chart graph and computer out-put.

Verification of mollusk cardiac events actually observed to events simultaneously recorded electronically during the same time interval was not accomplished without modifying the mussel shell. This was done by cutting a "window" in one valve and sealing that opening with a glass plate. In doing so, the animal could maintain normal homeostasis and hydrodynamic pressures while observing cardiac responses. The test mussel was also equipped with a micro-probe antennae configuration via the umbo insertion method. Thus, direct observation of cardiac peristolic rates could be correlated to physiographic strip chart and computer printout data.

Employing several sets of observation/verification tests with mussels and mayfly nymphs, we provided confirmation that the physiological responses observed were indeed represented by the amplifier output. These responses were then seen as digital equivalents in the computer printout. Insight gained from these tests helped direct the final amplifier design, the specific computer hardware configuration and program management system for data files and retrieval.

The data management program was written and placed in ROM (read only memory) with a variable format for data input and display. This allowed continuous data acquisition from 1 to 59 min and was specified as "collecting" in the readout. A wait interval when no data would be taken had similar limits. Data filed on disk would be listed by year, month, day, hour, and minute as total events per individual test subject, collection interval in minutes, and the number of events per minute. The window displayed by the terminal included these listings for groups of eight individual test subjects at a time with an update each minute.

Strategies for early warning detections of water quality changes and increasing pollution have been discussed in detail [1, 6, 7, 8, 9]. Typically, by recording biological responses of test subjects while maintained under ambient or reference conditions, one develops a data base upon which to compare responses displayed during stressfull periods. This approach with groups of different types of aquatic animals could improve the detection limits and confidence level of a real-time early warning biomonitoring system. Combining the ACABS as a multiple species sensing device with simultaneously derived water quality data could provide positive reinforcement to decisions concerning developing toxicity. Arguments for incorporating more than a single species in an automated biomonitoring system, closely follow similar arguments against the use of a single species toxicity test for inforcing a toxics reduction plan intended for the protection of the receiving stream and its ecological quality [10].

5. CONCLUSIONS

The design and operation of the automated computer-assisted biomonitoring system for multiple species monitoring was shown to be feasible in this study. Though we were able to operate the system using groups of three different types of aquatic animals in a stream-side facility under ambient water quality conditions, additional testing needs to be done with toxic treatments and varying water quality regimes. In this study we selected to use a representative fish, immature aquatic insect and mollusk; however, preliminary work shows that a multitude of other invertebrate species within these and other groups may be employed as biosensors. Additionally, we have found that under stream-side laboratory conditions, bluegill can be maintained in the test chambers for up to 2 yrs while the burrowing mayfly nymph has been monitored continuously for several months in an artificial burrow under similar conditions. To date, mussels have been maintained in the automated system and their cardiac and other functions recorded intermittently for periods approaching 1 yr. We feel that future problems will not rest entirely with the electronics as it has in the past, but will primarily be encountered in the biological aspects of the monitoring system. Finally, we believe the use of multiple species in automated biomonitoring systems can provide a reasonable complement to ecological quality control programs and toxics reduction monitoring.

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