INVESTIGATION OF A VIDEO-BASED
MOTION ANALYSIS SYSTEM
FOR EARLY WARNING OF TOXIC SUBSTANCES
IN DRINKING WATER SUPPLIES

[Prepared in partial fulfillment of the workplan,
"Toxic Substance Detection and Early Warning
for the Russian River," May 14, 1985.]

by

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November 15, 1988

ACKNOWLEDGEMENTS

We thank Robert Klamt of the California Regional Water Quality Control Board, Bob Morrison of the Sonoma County Water Agency, and John Greaves of Motion Analysis Corp. for all their help on this project. Special thanks to Bridgette Hejtmanek, Tracy Carone, Peter Otis, and Joe Williams for their assistance in conducting these experiments, and to Don Winkle for his work on software. Howard Bailey helped in many ways with sample design, equipment improvement ideas and statistical discussions.

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INTRODUCTION

Background

The project, "Toxic Substance Detection and Early Warning for the Russian River", (NCRWQCB, 1985)* was a cooperative effort among the Regional Board, the Sonoma County Water Agency (SCWA), Motion Analysis Corporation (MAC), and NET Pacific (formerly ANATEC Laboratories, Inc.). Seventy-five percent of the projects funds came from a Section 205(j) grant provided by the Environmental Protection Agency (EPA), while the remaining twenty-five percent was provided in materials and services by the contributors. The Regional Board administrated the project, using a contract with Sonoma State University to provide student help for most of the experimentation. The SCWA, MAC, and NET provided laboratory space, a field site, equipment, expertise, and additional manpower as needed.

This report fulfills tasks 4 and 5 to that Section 205(j) project. The primary objective of task 4 was: "... to test the prototype early warning (EW) system under laboratory and field conditions and evaluate its performance ...". The intent of this task was to evaluate the feasibility of using motion analysis equipment for detection of toxic substances in water. Although the study did not determine minimum concentrations at which an organism will respond, it did determine four behavioral responses to toxic chemicals that can be measured by a computerized motion analysis system. Further, a prototype field design was evaluated and modified through field testing at a site on the Russian River.

The primary objective of task 5 was: "... to determine the criteria for issuing an alarm based on detection by the EW system." (Klamt, 1985). Determining the actual criteria that would be applied to a system to judge whether or not to issue an alarm was beyond the scope of the project. More realistically, the data were evaluated with respect to various statistical approaches and the applicability of various tests in evaluating the data prior to issuing an alarm. The study results shed much light on the situation of evaluating behavioral data with respect to alarm issuance. The concepts of deriving such criteria and designing an evaluation procedure have been investigated and are discussed, but must be tailored to each individual situation. Factors such as sensitivity of the organisms, policy of the entity responsible for issuing an alarm, and the desired sensitivity for each early warning station may affect the design of alarm criteria.

Early Warning System Theory

An early warning (EW) system as conceptualized in this project would provide advance notice to a water purveyor of a hazardous substance in

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* Primary funding for this study has been provided by the State Water Resources Control Board using Section 205(j) grant funds made available by the U.S. Environmental Protection Agency. This does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency or the State Water Resources Control Board, nor does mention of trade names of commercial products consititute endorsement or recommendation for use.

the water supply. Obviously, an EW site would be located downstream of most potential chemical agents to a domestic supply, and far enough upstream to provide advance notification.

The system investigated for use as an EW system during this project was a computer-linked video system. The motion analysis system utilizes changes in motion patterns of aquatic organisms in response to toxicants in the water supplied to the organisms.

Using aquatic organisms for continuous toxicity monitoring is not a new concept. Cairns and van der Shalie (1980) presented a detailed review of early warning systems employing behavioral response. In the 1960's, Jackson and Brungs (1966) monitored fish survival in flowing wastewater. Later Hasselrot (1975) employed long-term monitoring of fish activity in Sweden. Besh et al. (1977) used rheotaxis as a measurement of response by observing whether fish could maintain position in a moderate current during and after exposure to a toxicant.

The advent of computers and closed-circuit video systems opened new possibilities for development of continuous biomonitoring systems (Lubinski et al. 1977, Gruber et al. 1981, Smith and Bailey, 1988). The Grieder system produced by Endeas and Hauser Instrument Co. uses a change in electrical pulse frequency from a Nile River fish, _Gnathonemus petersi,_ as an alert of chemical exposure. This fish normally emits pulses at about 18 Hz, but exposure to some toxic substances decreases both activity and pulse rate (Ewen 1987). Aquatest, manufactured by Quantum Science, Ltd. (QSL 1986), continuously monitors the ability of fish to swim against a current (positive rheotaxis). The system is designed to respond to acutely toxic levels of contaminants. The WRC MK III Fish Monitor uses ventilation frequencies of several trout. The response criterion is the difference between current ventilation rates and those measured 1 and 2 hours earlier (Evans et al. 1986).

Basic Program

The approach used in this study was to modify a commercially available (Motion Analysis Corp.) computer controlled, video-based automated system described by Greaves and Wilson (1980) for research on behavioral patterns of test animals. The modified system was tested for its ability to remotely monitor behavior of animals continuously exposed to a flow of river water.

Task 4 involved three phases:

1 - Laboratory testing in which three species of fish and _Daphnia magna_ (water fleas) were monitored for motion pattern changes in response to toxicants injected into the aquaria at known concentrations,

2 -Field testing of the basic laboratory system at a site on the Russian River provided by the SCWA, and

3 - Field operation of the system once the initial field testing and subsequent modifications were completed.

Task 5 investigated the concept of issuing an alarm based on information from the EW system, from both technical and management standpoints. An alarm necessarily incorporates knowledge of the type and nature of a substance in the water and the management implications of whether or not to shut down a domestic water supply. The amount of information on the type and nature of a substance hinges on the sensitivity of the EW system, i.e., will it detect specific classes of chemicals, and at what levels? The management concerns regarding an alarm issuance include liability from failing to issue an alarm when the public is at risk and issuing too many "false-alarms", thus incurring inconvenience and excessive cost.

This report presents the investigative approach to the EW system, the findings, and the conceptualization of alarm criteria. The additional needs for development of such a system are discussed with respect to modifications to the actual hardware and additional research on the sensitivity of the system.

MATERIALS AND METHODS

Experimental Design

The basic purpose of this study was to test the feasibility of using computerized motion analysis techniques to detect toxic substances in water, providing an advance warning to water users. The investigations were divided into laboratory testing and field testing. The conceptualization of alarm criteria followed the laboratory and field investigations and was not an experiment.

The laboratory tests were designed to observe the responses of aquatic organisms to toxicants injected into flow-through aquaria. Toxicant injections began at one-half the LC_{50} with three discrete periods for each test: pre-injection (control), injection (test), and post-injection (recovery). Since toxicant concentrations were monitored for each test, some idea of the sensitivity of the organisms was apparent. That is, if a response was detected prior to the toxicant reaching full concentration for a test, the next test was performed at or near that "first response" concentration. The investigations did not determine a lower threshold or minimum concentration at which a response was seen, rather were concerned with evaluating the ability to detect a response using the motion analysis equipment.

The field testing and operation phase was designed to ground-truth the laboratory system under field conditions. The initial design was installed at the field site, and modifications were made as problems arose. The final prototype system that was operated at the site incorporated those changes to the physical setup and the computer software. The field operation consisted of data collection from the river water and a recirculating control. Those data were then compared to the laboratory control data to determine if the field situation produced significantly different responses than seen in the laboratory tests.

Behavioral Response Measurements

A standard infrared-sensitive CCD video camera was used to capture the images of the test organisms. The test aquaria were placed between an infrared light source and the camera. This arrangement caused strong contrast between the organisms and background, a requirement for digitizing the video images. The analog video images were digitized by a video processor and then processed by a microcomputer-based graphics workstation (Sun Microsystems or IBM). The raw and processed images can be displayed on a computer monitor, giving the operator immediate visual information. The information can also be stored on a standard VHS video cassette tape.

Once the digitized images were stored on the workstation's hard disk, graphic images were displayed depicting the paths of motion. After the paths have been recorded (automatically by the computer program), attributes of the motion were calculated. Motion patterns which can be measured include linear and angular velocity, position in two dimensions (termed the x and y coordinates), change in direction, and others. The motion parameters can be displayed in tabulated summaries and/or in graphic form (line graphs, bar charts, three dimensional representations). Results can be merged into a previously existing database in order to accumulate information on behavior. All information displayed on the screen was available to the operator through "hard copy" provided by the system's printer/plotter.

While the system has been successfully used to determine avoidance/preference responses in both Daphnia and fish (Smith and Bailey, 1988), this approach was not used in the early warning behavior evaluations. The major objective of this study was to detect behavioral responses which were statistically different from the control conditions, not to establish the type of response pattern. If characterization of the types of behavior patterns are important for understanding the toxin or chemical that the organism was responding to or to assess receiving water for discharge violations, the behavior patterns could be easily included in the final analysis of the data.

The parameters selected for analysis of behavior follow the ones suggested by Giattini et. al. (1982), as well as some additional measurements made possible with the new computer and software:

> the speed of the organism through time (speed or linear velocity)
>
> the horizontal position of the organism through time (X-coordinate)
>
> the vertical position of the organism through time (Y-coordinate)
>
> rate of change of direction, regardless of the direction of the turns (angular velocity) It is defined for every point or instant in an object's path and is expressed in units of degrees per second.

The motion pattern data are time-dependent and therefore represent behavioral response through time. This same approach was used by Ishio (1964) to determine toxicant concentration by plotting the organisms' position against test chemical level.

Selection of test species.

The following criteria were used for preliminary selection of species:
(1) easy to acquire and maintain,
(2) known to be sensitive to a wide range of toxicants,
(3) in constant motion under normal or control conditions,
(4) suitable for testing in flow-through systems,
(5) relatively small in order to minimize the size of test chambers and, perhaps, to increase sensitivity,
(6) sufficient toxicological data (on behavior or LC50) should exist for the species that possible response thresholds can be estimated, and
(7) the species should be tolerant to the ambient water quality of the river (e.g. diel and seasonal temperature levels and variations of temperature, dissolved oxygen and turbidity).

The first four species selected for preliminary testing were rainbow and steelhead trout (Salmo gairdneri), Chinook salmon (Oncorhynchus tshawytscha), fathead minnow (Pimephales promelas), and a daphnid or water flea (Daphnia magna). Both an invertebrate and fish were included in order to widen the range of possible behavioral responses to toxicants and to increase the number of possible contaminants eliciting response.

Steelhead were obtained from the Warm Springs Hatchery located on a tributary of the Russian River. Rainbow trout and fathead minnows were purchased from the Thomas Fish Company in Novato, California. Chinook salmon were obtained from the Nimbus Fish Hatchery on the American River. The range in size of steelhead and rainbow trout was 35 to 65 mm and the range for salmon 30 to 40 mm. Fish were transferred from suppliers to recirculating systems (Living Stream by Fridged, Inc.) at NET Pacific and were maintained for a month before testing started. Holding temperature was held at 12 C. Fathead minnows were hatched from eggs at NET Pacific and raised to 25 - 35 mm before testing. The minnows were raised and tested at 22 C. Daphnia magna were obtained from Stanford Research Institute and cultured at NET Pacific at 22 C.

Laboratory Testing

As previously mentioned, the laboratory testing involved exposing organisms to known levels of toxicants and monitoring the changes in motion patterns. Two basic types of aquaria were used for these tests - avoidance/preference chambers in which organisms had a choice of exposure to the toxicant or control water and test chambers that allowed no escape from the toxicant. Tests were conducted in a standardized manner and the data analyzed by the computer system automatically. All tests were conducted in constant temperature rooms which were adjusted depending upon the test organism used. Temperature remained within ± 1 C of the selected temperature throughout the test period. pH was also held constant during any one test and ranged between 7.5 and 8.1 depending upon the test chemical used. Dissolved oxygen was held above 7.5 mg/L (maximum of 8.5) with hardness and alkalinity adjusted to match Russian River water conditions and held constant during each test. The following section describes the actual test systems, the sequence of the typical test, and the data collection and subsequent analysis of responses.

Flow-through Test System - Fish

The first fish testing system was an avoidance/preference chamber modified from a system developed by Westlake and Lubinski (1976). Later testing was done with a modified flow-through tank that contained only one fish chamber (same size as original chamber). The overall inside dimensions of the chamber were 91.4 cm long, 48.3 cm wide and 7.6 cm deep. The chamber was made of glass and divided into three compartments: control, test and avoidance/preference (Figure 1). Test fish were placed in control, test, and avoidance/preference chambers. The flow was channeled to the three chambers and the toxicant discharged into the appropriate channel. After the water had flowed through the two

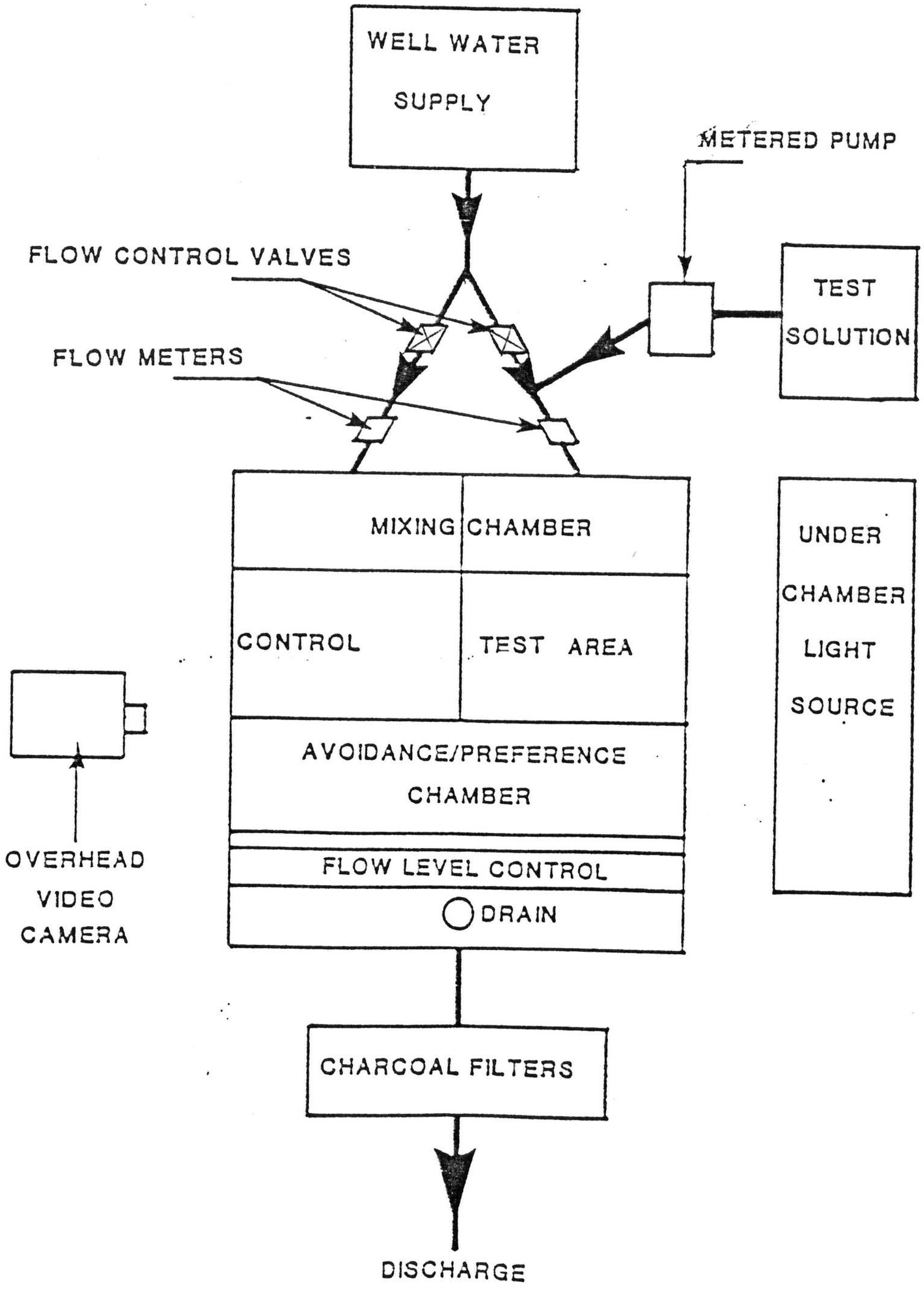

Figure 1. Diagram of the avoidance/preference fish behavioral testing system used in the laboratory. The later tests employed a similar system without the control and test areas, i.e., only one fish chamber.

divisions it entered the avoidance/preference area where the fish had a choice to either avoid or be attracted to the test material, or not to change their response pattern from the control conditions. The glass partitions between the different areas were sandblasted so that the fish in each area could not see the behavior of neighboring fish nor their mirror image. During the early stages of testing, control water was dechlorinated city water, filtered through activated carbon; later, well water was used. Control and toxicant could be supplied down either channel of the test chamber depending upon behavioral response and to verify avoidance/preference behavior.

Since a single test concentration was used for each test, a large difference in concentrations between test and control solutions was selected. This steep gradient provided the necessary cues for successful directed movements and the best opportunity for the sense organs of the fish to discriminate between opposing bodies of water (Larrick et al., 1978).

The system was the same with that one exception.

Flow-throuqh Test System - Daphnia

The Daphnia test system also employed two types of flow delivery and observation tank systems during the project. The first system consisted of a control water source (as described earlier), supplied to a gravity tank (Figure 2) in order to provide constant head pressure. This dilution water was allowed to flow freely at a rate of 60 mL/min. into a funnel which served to break any siphoning effects. This flow rate was selected because it was not strong enough to affect the swimming behavior of Daphnia and still allowed relatively rapid turnover of the chamber (approximately one volume change every seven minutes). To the bottom of the funnel was attached a line feeding into the Daphnia chamber. A continuously stirred stock toxicant solution was dripped into the funnel at a predetermined rate from either a metering pump or Marriotte bottle. An algal food suspension (Selenastrum capricornutum) at a concentration of 33,000 cells/mL was delivered to the funnel at a rate of 1 mL/min to achieve a final concentration of approximately 500 cells/mL in the chamber. Barera and Adams (1983) report that daphnids should be fed during testing, and algae seems to supply the best nutrition (Buikema, 1960).

In the early prototype design (Figure 3) the top of the chamber was sealed and the entry plug was placed near the bottom of the chamber. The units were difficult to clean and to add organisms, therefore the removable top unit was designed.

The second prototype chambers were plastic aquaria 12.7 cm long by 17.8 cm wide and 1.9 cm deep. Supply and drain holes cut through aquaria walls, and rows of horizontal holes along the sides of the aquaria provided a path for water to flow evenly into and out of the test chamber. The tops of the aquaria were removable, and secured by four stainless steel wing nuts and a silicon rubber gasket. The four sides of the viewing chamber were milled at an angle to eliminate parallax problems with the video system.

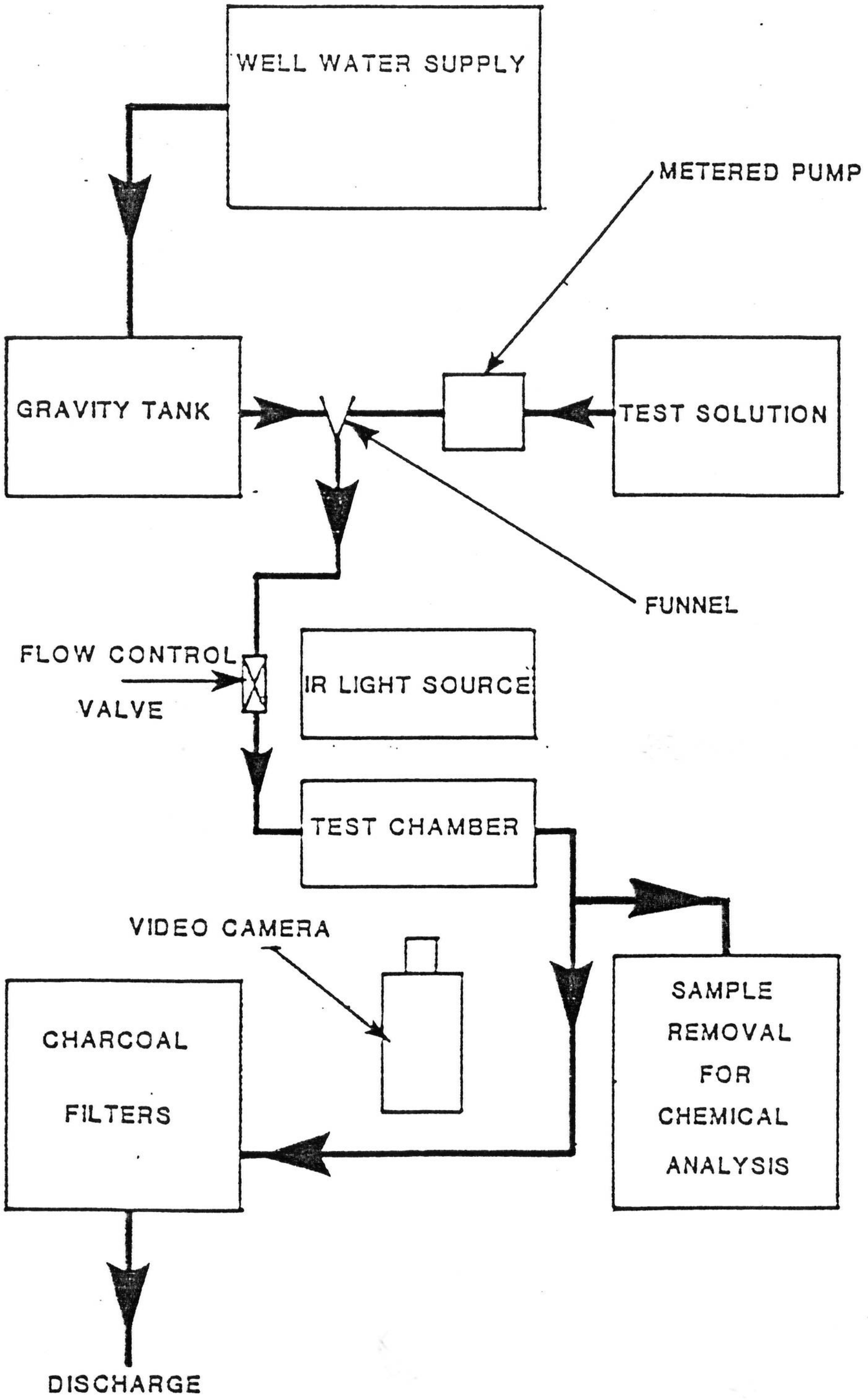

Figure 2. Diagram of the original *Daphnia* behavioral testing system used in the laboratory. The final system employed a peristaltic pump and valve manifold in place of the metered pump and funnel.

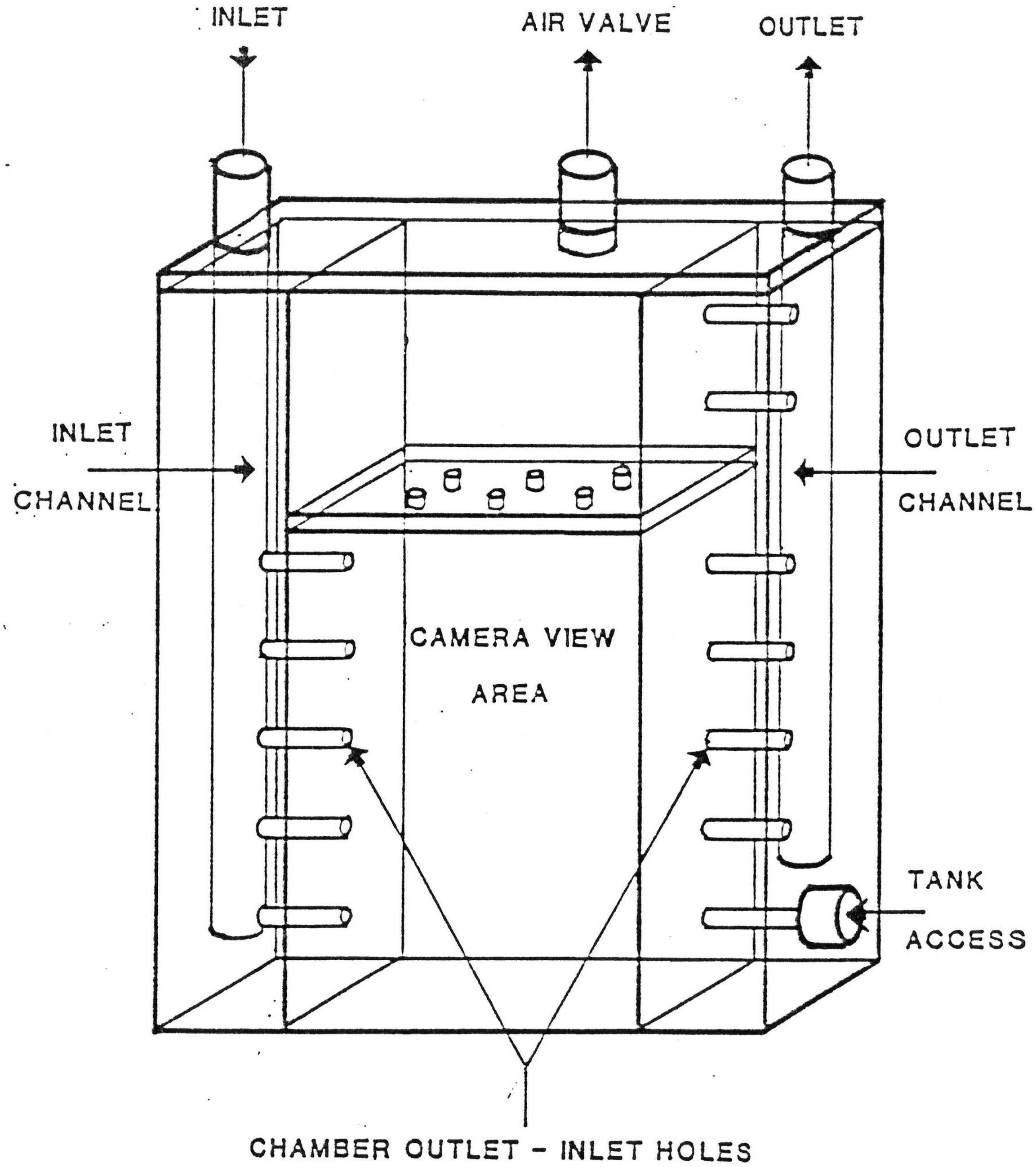

Figure 3. Diagram of the early prototype Daphnia test chamber. The later chamber had a removeable top for ease in cleaning and replacement of organisms.

Control water, described earlier, was supplied to a gravity-feed tank, thence to a distribution manifold, and to the test chamber via a variable speed peristaltic pump. A flow rate of 24 mL/min was used for these laboratory tests, providing a turnover rate of about one volume change every 12 minutes. Toxicant solutions were pumped into the distribution manifold, where valves switched the flow from control water to toxicant solution. The flow of algal suspension was metered by a variable flow intravenous injection valve and continuously entered the test chamber through the manifold at the rates and cell counts described for the early test design.

Test Sequence and Data Collection

Fish were acclimated in the recirculating system for 4 weeks, then individuals were transferred to the test chambers for a three day acclimation period. Salmonids were held at 12C, fathead minnows and Daphnia at 22C. Hardness and alkalinity was about 120 mg/L and 130 mg/L (as CaC03), respectively. Full acclimation was determined using the video system by measuring linear velocity data . The procedure was similar for daphnids, although acclimation periods were shorter (3-24 hrs).

Generally, one hour of control behavioral data was recorded on videotape before the toxicant was added to the chamber. Another hour of data was recorded during exposure. Test water flow was then switched to control water, and another 1/2 to one hour of data was collected. Some variation in lengths of exposure occurred among the tests.

During the laboratory test period, the behavioral response was captured on video tape rather than recording digitized image information on the computer's hard disk. This was done because the actual video images are really the original data, video tapes can be replayed and examined visually, and video tapes can serve as input to the system's image analysis. Additionally, video tapes could be reanalyzed using different time sequences and/or different measurements of motion.

Toxicant samples were taken from the system to characterize the build-up of toxicant and determine the actual toxicant levels. Basic physiochemical measurements like pH, disolved oxygen, temperature, and turbidity were also recorded.

Data Analysis

Following the test and data collection the data were examined statistically to determine which differences in behavior resulted from exposure of organisms to the test material. Variables selected for the analysis of behavior follow suggestions by Giattini et al. (1982). Additional measurements were added due to the increased scope of measurements made possible by a video-computer system. Linear velocity and location (x and y coordinates) are standard measures of locomotory behavior. Rate of change of direction was added as an index of turning (for an organism moving in a straight line, rate of change of direction is equal to zero, regardless of speed).

For this project, several types of statistical analysis were used. Comparisons of the means of the control and test data sets were made using the Kolmogorov-Smirnov two sample test and Mann-Whitney U test, both nonparametric methods (Sokal and Rohlf, 1980). Unpaired t-test, a parametric test, was also used. Several other tests were later used in an investigation of the statistical distribution of data and a comparison of the effectiveness of non-parametric and parametric tests for developing alarm criteria. Distribution of data were examined visually using histograms and tested for normality using the procedure of Martinez and Iglewicz (in Hintze 1987). Equality of variance between control and test conditions was tested using Bartlett's test (Sokal and Rohlf, 1969).

The data also were examined from the viewpoint of first response. That is, the first few means after toxicant injection were compared to the control data set (grand mean of the control). Two approaches were used: 1) comparison of the first 10 minutes' means with the control data set, and 2) comparison of each mean for the first 10-15 minutes with the control data set. The reasoning for these analyses is keyed to alarm criteria and timeliness of an alarm issuance, discussed in more detail in the alarm criteria section of this report.

Field Testing

A site on the Russian River was provided by the SCWA with easy access, protection from vandals, and protection from winter floods. A small building was constructed on top of the Wohler diversion caisson that housed exposure chambers and related equipment, a computer system, and resin column sampling equipment.

Figure 4 illustrates the early warning system design for field testing. Water was supplied from the river by a submerged pump encased in a 15.2 cm diameter PVC pipe, perforated by a series of small holes and anchored to a concrete fish ladder in the river. A 1.3 cm diameter stainless steel tube carried the water to the platform 24.6 m above the river bed. Water flow delivered by the system to the platform was 17.1 L/min. During the project, only the daphnid system was operated at the test site, although plans were made to add fish tests.

Two types of systems were used to provide both test and reference or control measurements. The control was a closed system, in which water circulated from the Daphnia chamber to a biofilter andsterilizing UV light and then back to the Daphnia chamber. Flow rates were set at 40-60 mL/min. The water supply was reconstituted deionized water adjusted to match hardness, pH, and alkalinity of river water or well water from the laboratory.

River water was supplied to the test chambers through a 38.1 cm x 122 cm PVC venturi sediment trap and pressure relief tank, the water was filtered through 30-u and 5-u filters to avoid accumulation of particulates and increased turbidity in test the chambers. This system mimics the SCWA supply system which was not particularly concerned with particle-borne toxicants, which would be removed by natural filter beds and would not reach the distribution system. (Also, toxicants tested for this project are not closely bound to the fine silts held in suspension

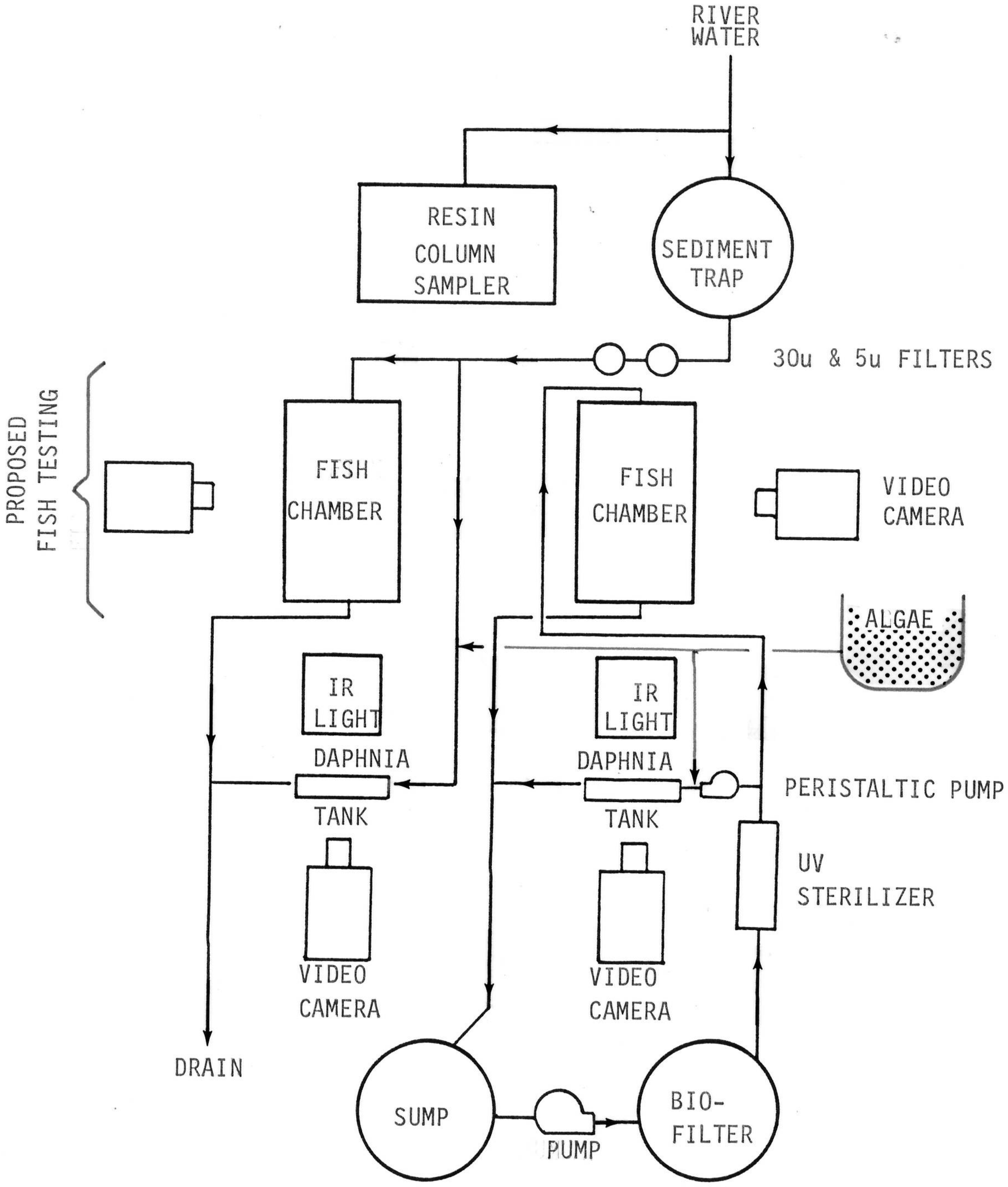

Figure 4. Diagram of the Daphnia field test system and proposed fish testing portion of an early warning system.

during periods of high flow.) A heat exchanger made of stainless steel tubing immersed in a water bath held the water at 22 C. The flow rate was 60 mL/min, the same as the control chamber. A reserve container and peristaltic pump provided a continuous supply of the alga Selenastrum capricornutum to feed the Daphnia.

The conceptualized computer interface to the test system is illustrated on Figure 5. Each video camera sequentially would capture 15 seconds of video images, and send analog image information to the video processor where the image could be viewed on a monitor and/or recorded on a video cassette recorder (VCR). The processed video information then would be sent to the host computer (IBM-AT with a 72 mb hard disk) to summarize the behavioral information from each camera in separate files. Data sets would be compared statistically to detect differences from the control. The analyzed data can be distributed to disk, to cartridge tape, or sent by modem to a host computer at a different location. Conceptually, statistical analyses being performed as data are gathered could detect significant changes from normal behavior. If conditions satisfied a predetermined set of warning criteria, a speech synthesizer and dialer could activate a voice alert or interact directly with the water purveyor's computer system (say to turn off valves or pumps).

The Early Warning System (EWS) operation and control shell tested during the project and as it currently exists is an interactive, menu driven program. It's purpose is to facilitate an automated method of timing control and system setup. The system automatically captured behavioral information from the video cameras and physical/chemical data from sampling probes placed in the water and connected to a Hewlett-Packard data logger. The behavioral data was analyzed and summary files created.

These files were then taken to a central computer at NET. for further analysis. The behavioral data were analyzed the same as for the laboratory tests. Control data were compared to the river (test) data, as well as the control data for Daphnia testing in the laboratory. In addition, the field data were analyzed from the viewpoint of first response. That is, each new mean was compared to all previous means as a group ("control" or normal behavior database).

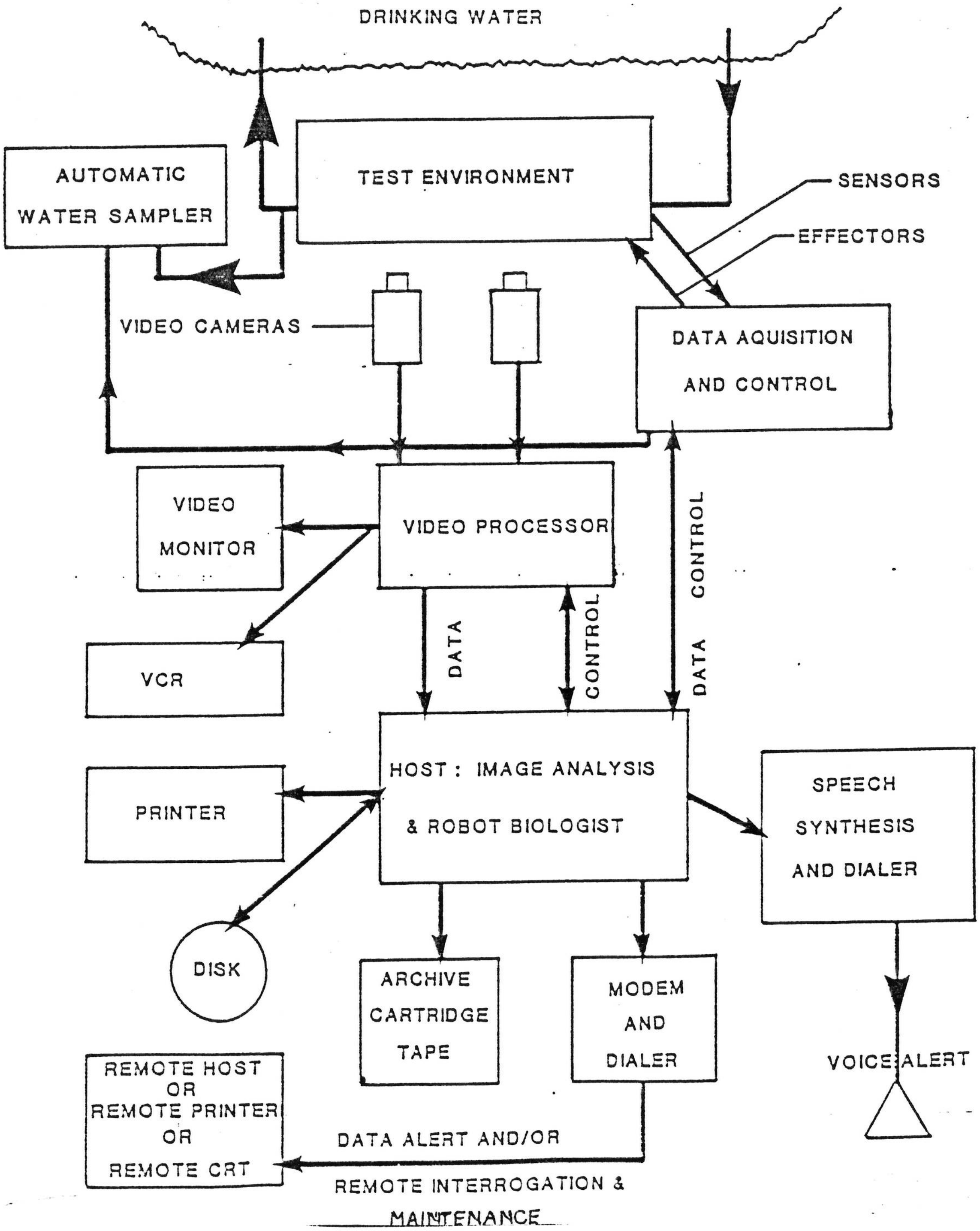

Figure 5. Conceptualized early warning system interface with the computer, video processors, and alarm equipment.

RESULTS

Laboratory Testing

Laboratory testing was directed toward two goals: testing behavioral response of organisms to known toxicants and testing a prototype computer system that could operate at the remote site.

From a list of toxicants that have been found or are potential contaminants in the Russian River (e.g. formaldehyde, DDT, pentachlorophenol, phenol), phenol was selected for the initial tests on salmonids, fathead minnows and daphnids. Phenol has been used as a reference toxicant (Lewes 1981, Klaverdamp et al. 1985), and good data base on phenolic compounds exists.

The effect of chlorine (as sodium hypochlorite) on fathead minnow behavior was also tested. Chlorine is an oxidant and represents a different class of toxicants. The effect of both copper sulfate and xylene on Daphnia were also tested. Copper sulfate was chosen because it is frequently used as a reference toxicant and as an easy to use representive metal, another class of toxicants. Xylene was tested because it is a carrier for many pesticides and is in itself toxic.

Salmonids

Preliminary tests on salmonids were conducted with a phenol concentration of 12.0 mg/L, which is higher than the reported LC50 value of 8.9 mg/L for rainbow trout (DeGreave et al. 1980). Figure 6 is a plot of phenol concentration in a test tank through the test period for a maximum concentration of 12.6 mg/L phenol. It illustrated the realtively short time for the tank to become filled with toxicant. The first detected resonse of the fish to the phenol occurred eight minutes after toxicant injection, six minutes after phenol actually entered the tank.

Statistical comparisons of control data sets vs. test data sets using unpaired t-tests are summarized for all salmonid tests in Table 1. Observed changes in behavior were statistically significant ($P = 0.05$) in all cases. Response times based on significant change in speed varied from 7.5 minutes for salmon to 13 minutes for rainbow trout. Tests on rainbow trout were generally similar. Rainbow trout show a marked difference in swimming speed when exposed to phenol. In all tests, fish responded to phenol by moving away from the source of introduction in the tank. Swimming speed decreased in rainbow trout (Salmo gairdneri) in response to phenol, but increased in chinook salmon (Onchrhynchus tshawytscha).

The response of four elements of steelhead behavior to phenol at 8 mg/L are shown on Figure 7. On each graph, every dot is the mean response of 10 fish and the vertical bars are standard deviation of the data set. Speed increased rapidly when phenol first entered the test chamber and then decreased below the level of the control situation (Figure 7a). The "vertical position" (along the direction of flow) of fish also changed significantly (Figure 7b) as the fish moved away from the incoming phenol. Changes in the horizontal position (across the flow) show the fish moving away from the side of the chamber where the phenol was

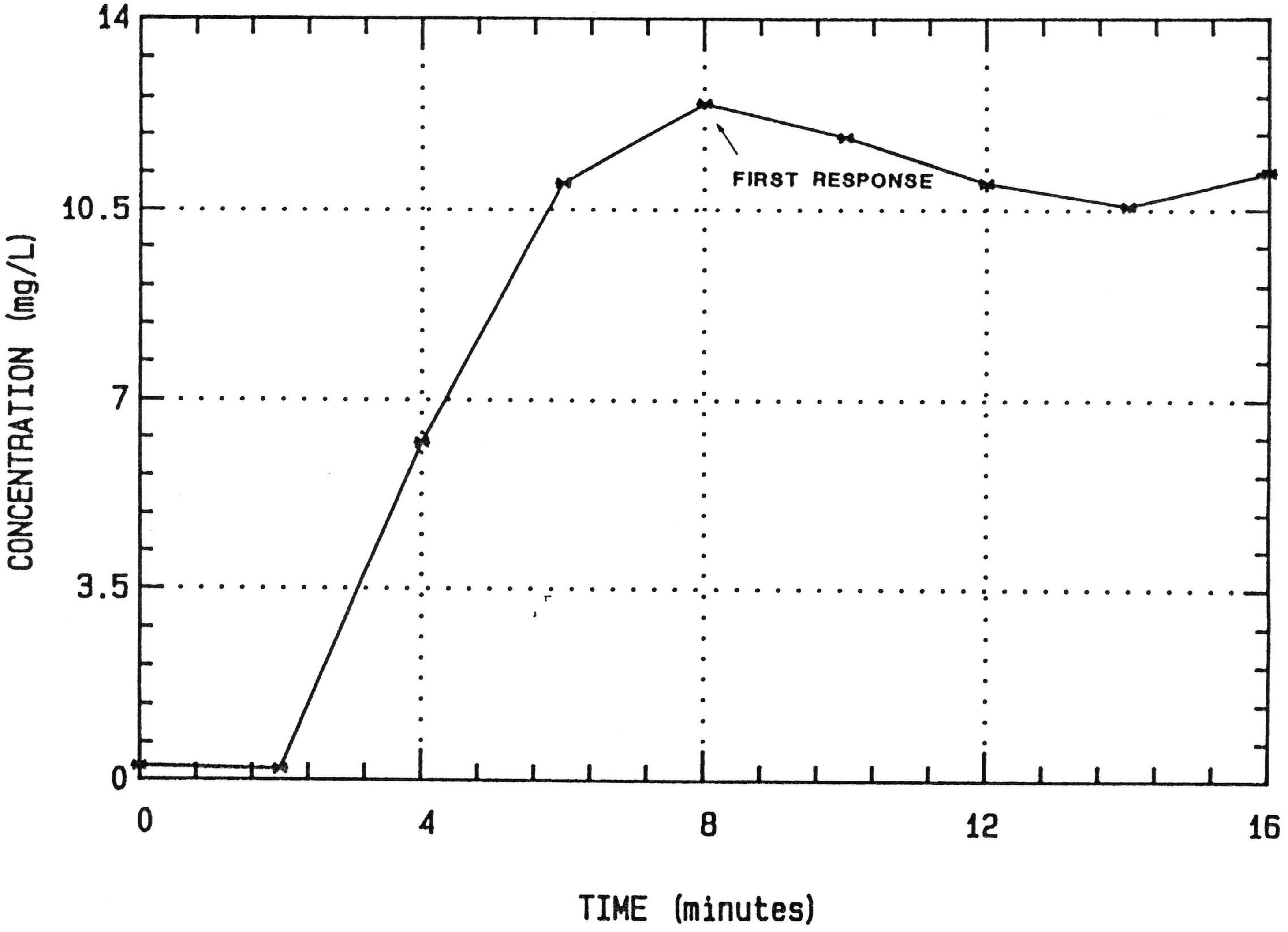

Figure 6. Concentrations of phenol during a test with chinook salmon at 12.6 mg/L final phenol concentration. (first response = first detected response to phenol seen as a change in speed)

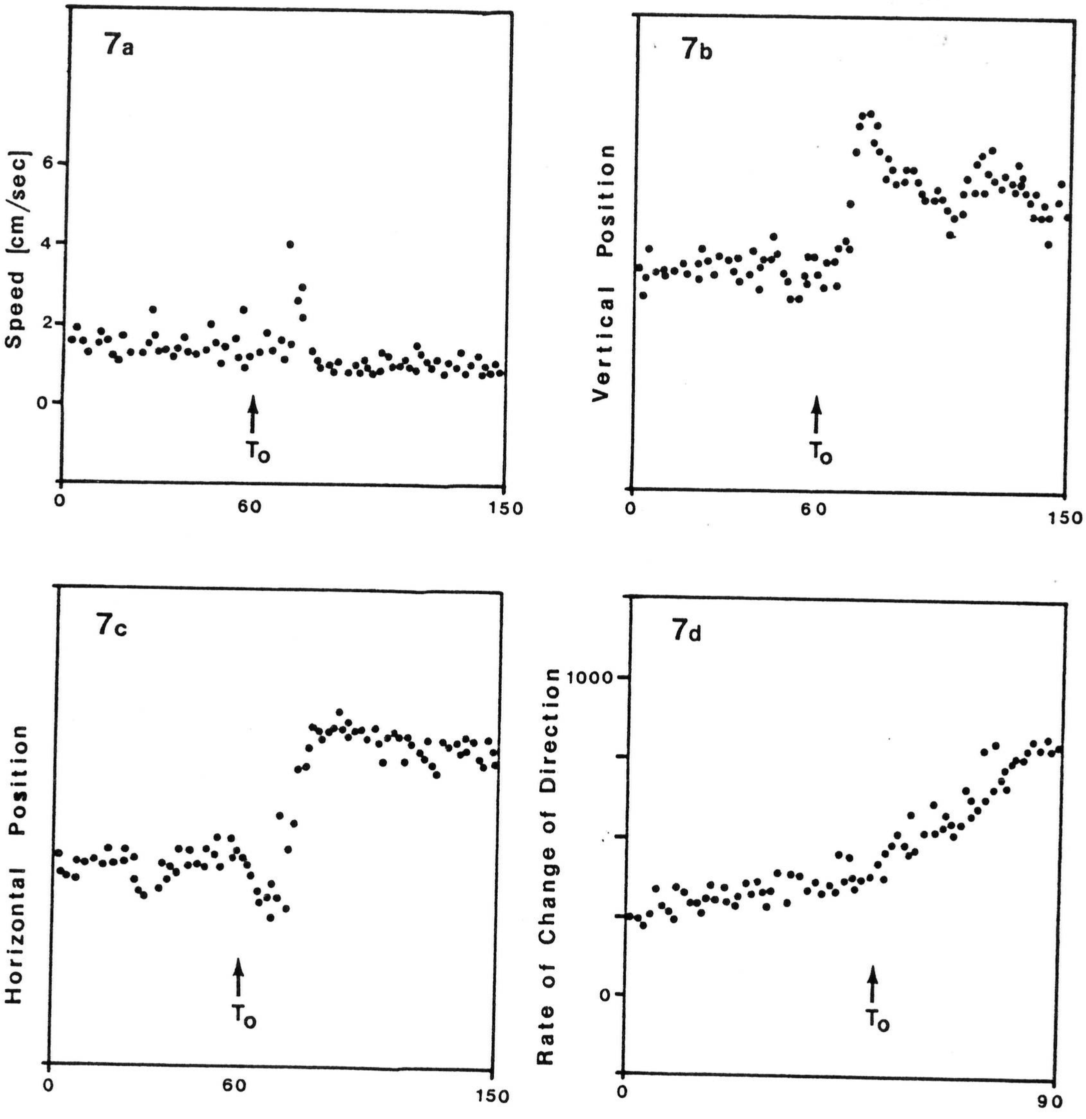

Figure 7. Plots of the change in the means for various behavioral parameters measured during steelhead exposure to phenol. T_0 = toxicant injection. a) linear velocity (speed), b) vertical position, c) horizontal position, d) rate of change of direction

Table 1. Summary of the behavioral responses of salmonids to phenol. Significance tested with Student's t-test.

Species and Concentration	Observed variable			
	Vert(x)	Horz(y)	Spd	Detection[1] Time (min)
Salmo gairdneri, rainbows				
10.8 mg/L	*	*	*	11
10.6 mg/L	*	*	*	13
Salmo gairdneri, steelheads				
8.0 mg.L	*	*	*	7
Chinook salmon				
12.6 mg/L	*	*	*	7.5

[1] = detection time based on significant change in speed
* = Significant difference in speed or position between control and test (P=0.05)

injected (Fig. 7c). Rate of change in direction also indicated a behavioral response to phenol seen as an increase in angular movement and change in fish direction (Figure 7d).

Another way of looking at the response data is to plot difference between any data point and the mean value of the control data. Such a mean difference plot (Figure 8) for steelhead illustrates the response pattern for horizontal position.

Daphnia magna

Long-term (90 hr) behavior of _Daphnia_ in control conditions is illustrated on Figure 9. Each dot represents the mean speed of 20 daphnids and the vertical bars are standard deviations. Tests were run for up to 164 hr with only two statistical outlying data points. Only one outlier (P = 0.05) occurs on Figure 9. Investigation on the frequency of unusual data points (outliers) is important for developing reliable alarm criteria.

Tests were conducted to determine both response time and level of acclimation to multiple exposures of phenol (with the same group of organisms). As was done for salmonids, response time vs concentration curves were established for each exposure to phenol. To determine a threshold of response, test concentrations started with the LC50 value of 17 mg/L (Figure 10) and sequentially decreased at about half of each previous value.

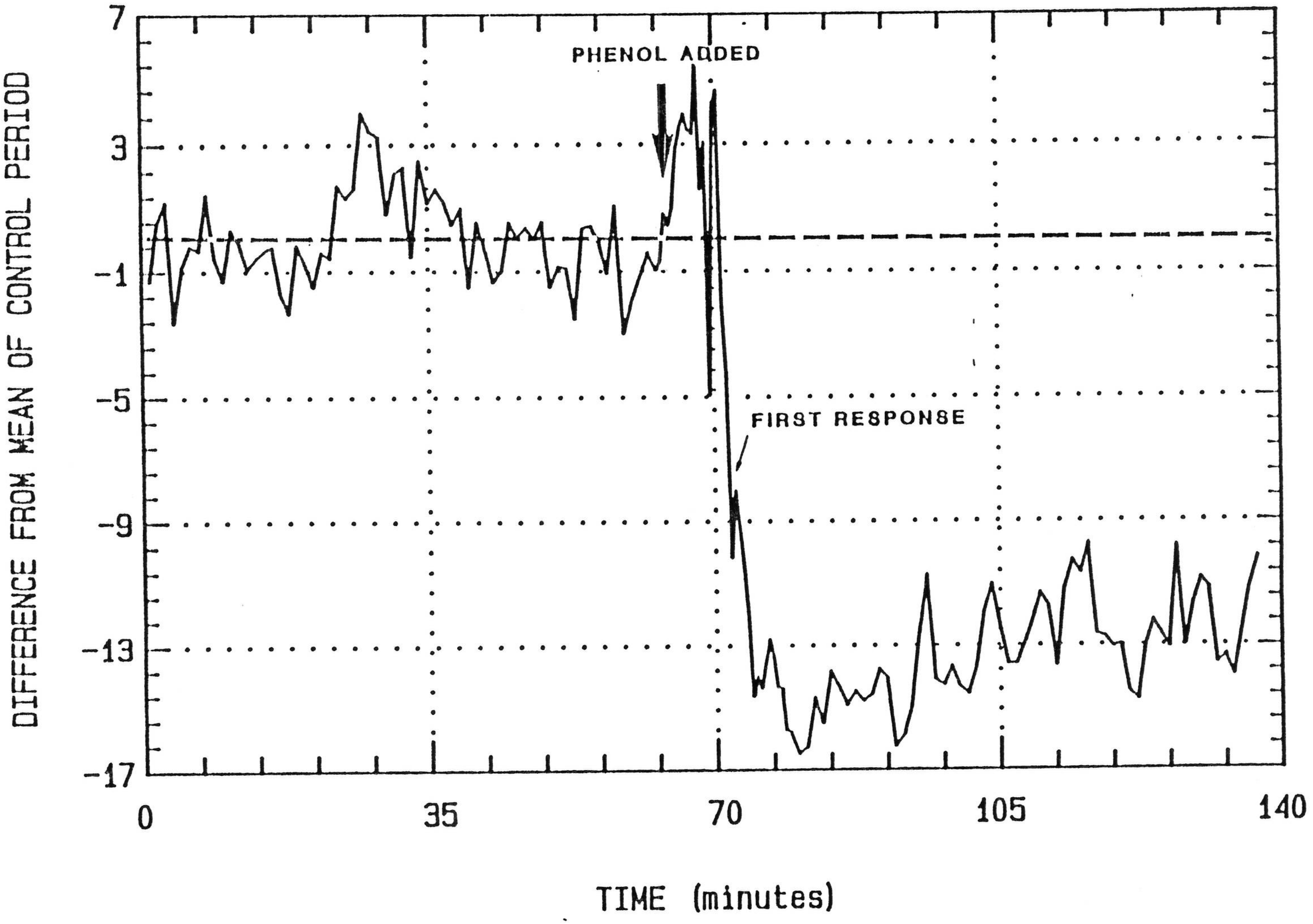

Figure 8. Plot of the mean difference from the control for horizontal position of steelhead exposed to phenol.

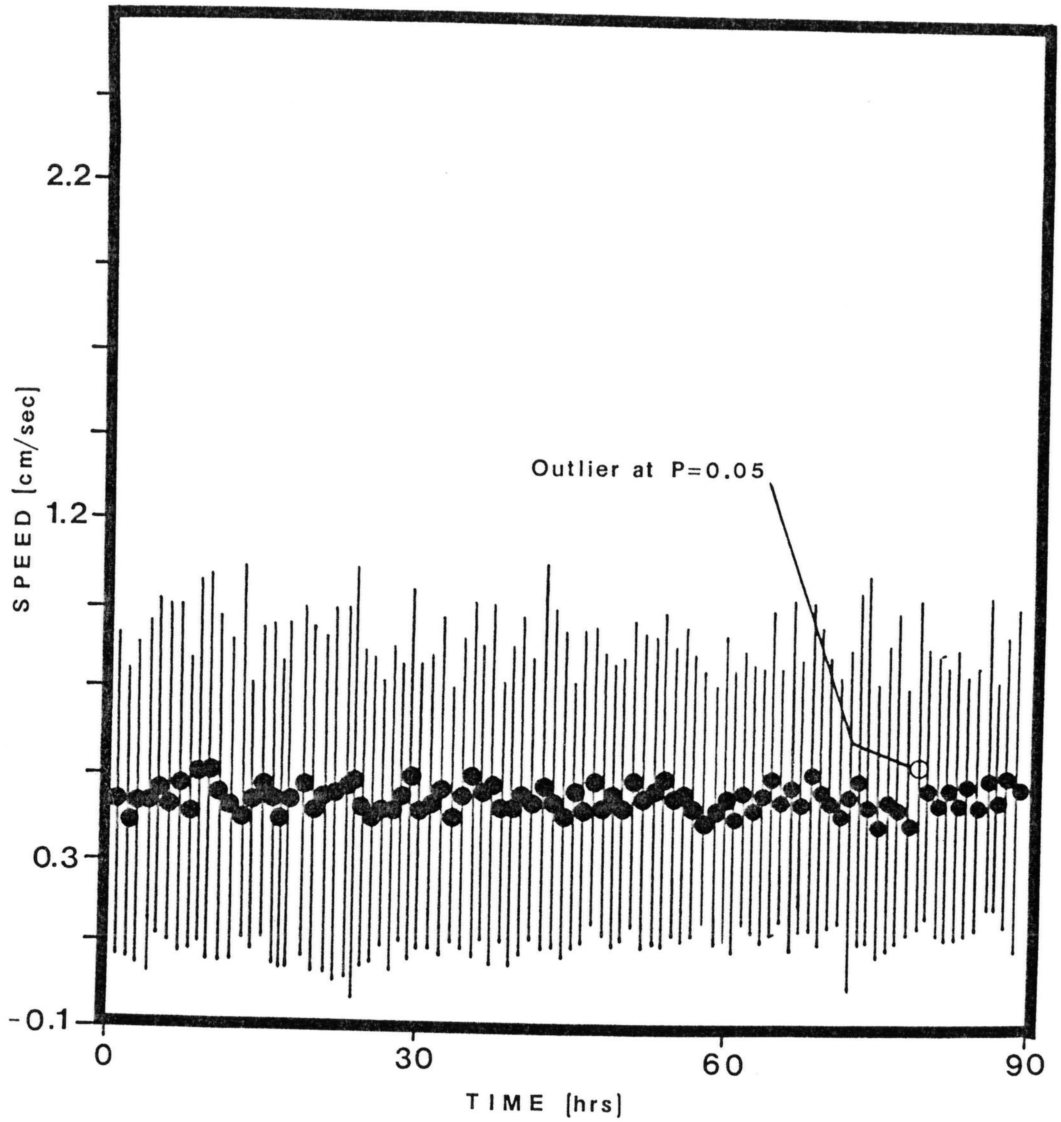

Figure 9. Means and standard deviations of speed for a longterm *Daphnia* behavior test under control conditions.

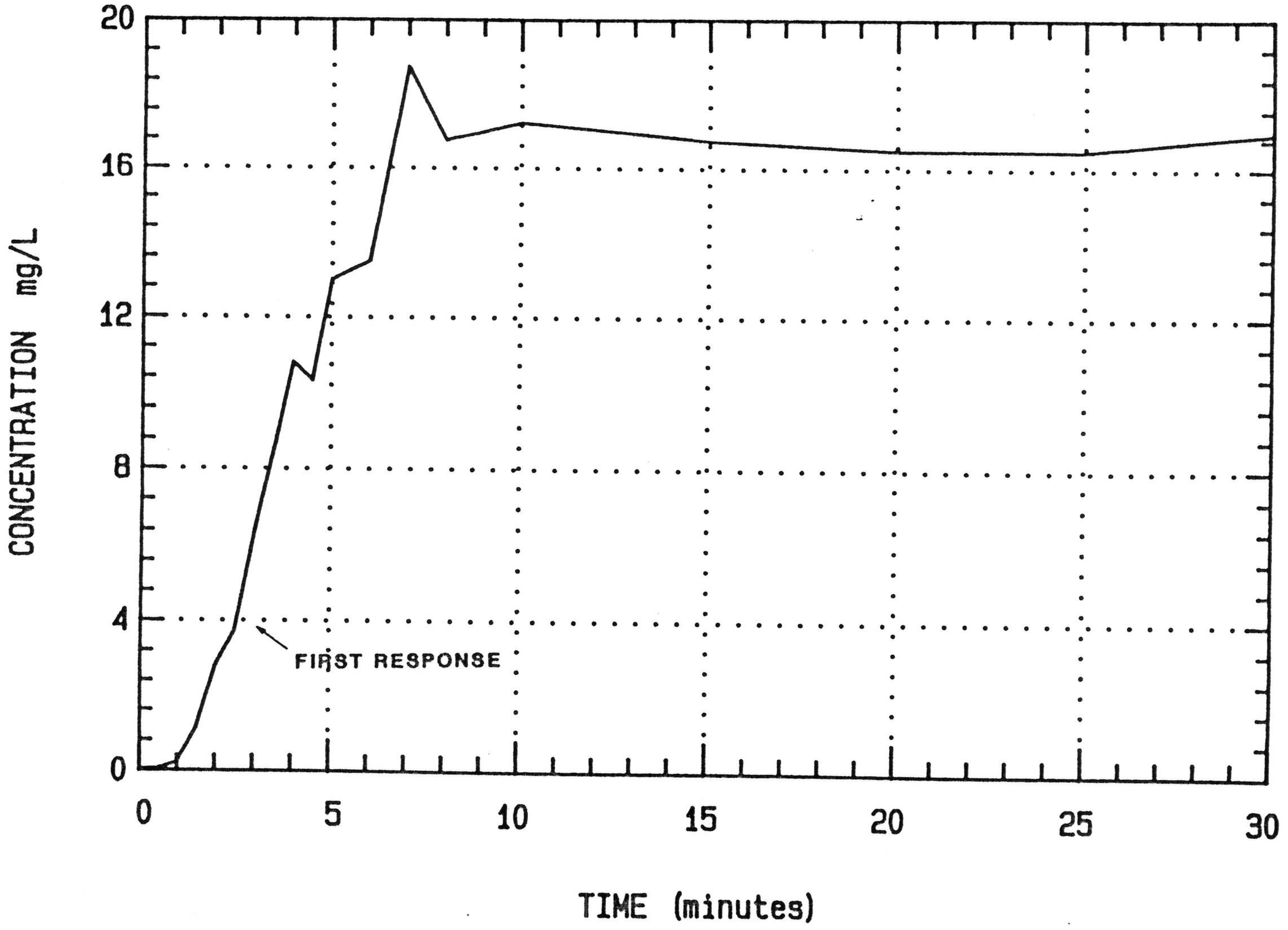

Figure 10. Phenol concentrations during a test with *Daphnia* at 16 mg/L final phenol concentration. (first response = first detected response to phenol seen as a change in speed)

Results of statistical comparisons of three elements of daphnid behavior are summarized on Table 2. Comparisons were made using unpaired t-tests. Response times were faster for daphnids than salmonids and averaged 1.8 minutes. Like salmonids, D. magna avoided the phenol at all concentrations tested.

Table 2. Behavioral responses of Daphnia magna to phenol. Significance tested with Student's t-test.

Concentration	Observed variable Vertical	Horiz	Spd	Detection[1] Time (min)
2.0 mg/L	*	*	*	1.6
1.6 mg/L[2]	*	*	*	2.1
1.2 mg/L[2]	*	*	*	2.0
0.8 mg/L	*	*	*	1.9
0.43 mg/L	*	*	*	1.5

[1] = detection time based on significant change in speed

[2] = Second exposure of Daphnia to phenol after 24 hours of purging with control water

* = Significant difference in speed or position between control and test (P=0.05)

Figure 11 shows that swimming speed increased in response to exposure to phenol and returned to the pre-exposure level after phenol was purged from the test chamber. Movement in the vertical direction (Figure 11) changed from a non-directed pattern in the pre-exposure period to a movement away from the side of the chamber where the toxicant entered. In both measures of position (Figure 11), behavior returned to a more nondirected pattern as toxicant was purged from the chamber.

In the case of multi-exposures to toxicants (using the same organisms) with a 24 hour purge period between exposures, the response level was detectable above the control level even after three separate exposures to the same concentration of phenol. The level of behavioral activity did not return to the same level as the first control period. The purged control levels after toxicant exposure were statistically significant from the orginal control period. However, when the organisms were exposed to a new introduction of toxicant the response level was elevated so that it was statistically significant from the control after purging. Pre- and post-exposure data were significantly different, even after 24 hours of exposure to control water. However, this fact did not result in a no-detection response.

A second set of experiments was performed without multiple exposure. These tests were not done in the first prototype chamber, but were done in the newer chamber. Test concentrations were 10, 5, 2, and 1 mg/L (Table 3).

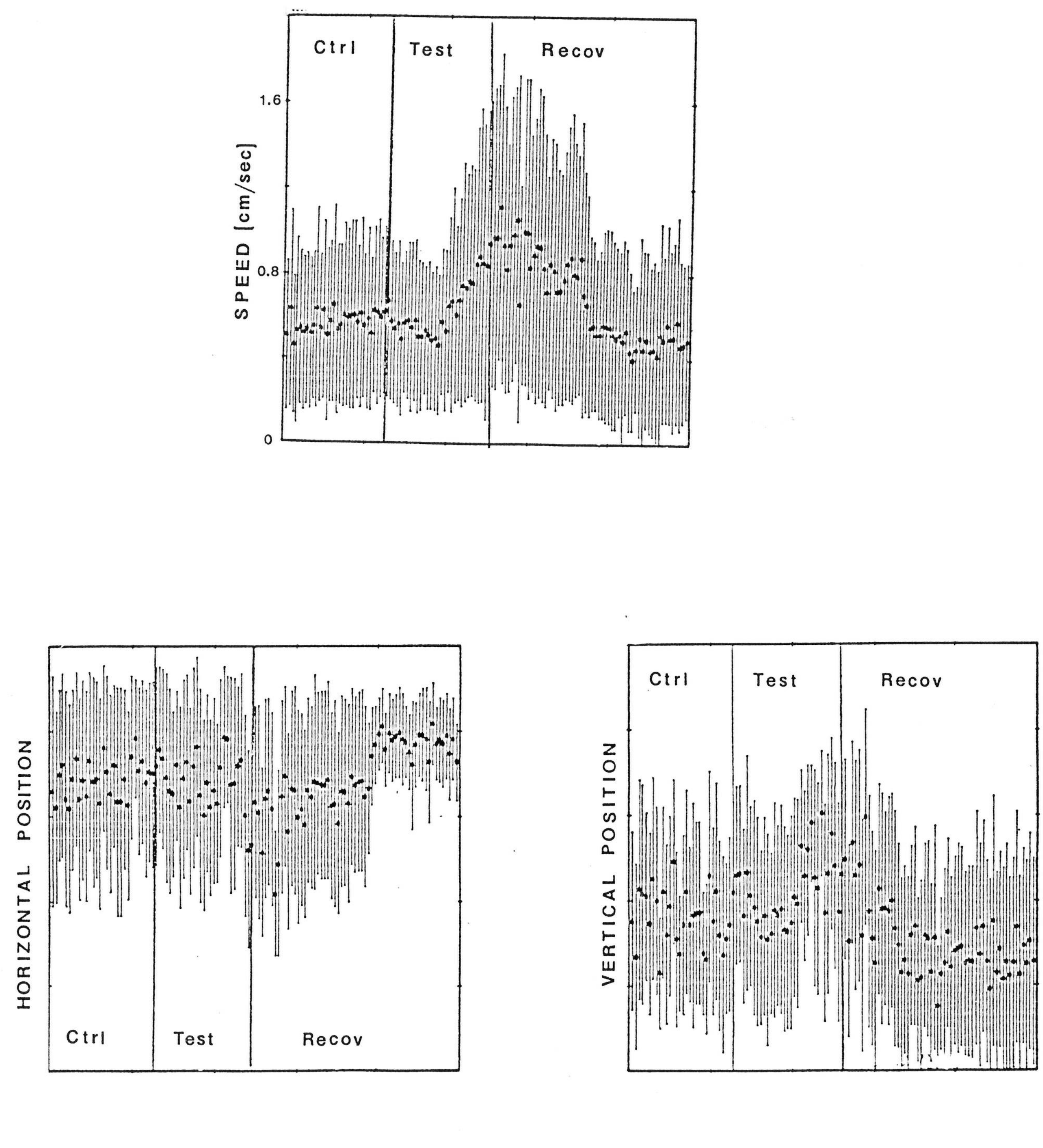

Figure 11. Behavioral responses of *Daphnia magna* to phenol at 10 mg/L. Ctrl = control, Test = toxicant exposure, Recov = post-test (recovery period)

Position in the x-direction (along the flow) changed significantly at all tested concentrations. Change in the y-direction did not. This result might be expected because in these experiments phenol was entering the chamber at all locations (because the tube with holes extended from top to bottom of the chamber) in the y direction at the same time. Change in speed was significantly different than the control conditions at the higher concentrations of phenol, and rate of change in direction was significantly different at all concentrations. The threshold of response in these tests was at least 1 mg/L and in the previous tests at least 0.8 mg/L.

Table 3. Behavioral responses of *Daphnia magna* populations to three different toxicants.

Toxicant and Concentration, replicate	Observed Variable			
	Vert(x)	Horz(v)	Spd	Chng Dir
CuSO4				
7 ug/L, rep. 1	NS	*	*	NS
rep. 2	NS	**	NS	**
rep. 3	*	NS	#	NS
Phenol				
10 mg/L	**	NS	*	**
5 mg/L	*	NS	**	**
2 mg/L	**	NS		**
1 mg/L	**	**	NS	**
Xylene				
3 mg/L	**	**	NS	NS
1.5 mg/L	**	NS	**	*

NS = test and control not significantly different at $P = 0.10$
# = test and control different at $P = 0.10$
* = test and control different at $P = 0.05$
** = test and control different at $P = 0.01$

Daphnia magna response to copper sulfate and xylene were both tested (Table 3). Three replicate experiments of the response of the daphnids to 7 ug/L were conducted to test replicablility of results and response to low concentrations of copper (pH=8.02, dissolved oxygen=7.8 mg/L, temperature=22 C). Response patterns at this low concentration were not consistent. Analysis indicated both statistically significant and nonsignificant results in different replicates of four elements of daphnid behavior. Information on replicability can be used in development of alarm criteria. The response of daphnids to two concentrations of xylene were also tested. At both 3 and 1.5 mg/L, the change in position along the flow (x-direction) changed significantly. Statistically significant responses of both speed and rate of change in direction to xylene were both found at 1.5 mg/L.

Fathead minnows

Fathead minnow tests were not conducted in the avoidance/preference chamber used for salmonids, but in the early warning system chamber without a partition. Unlike the avoidance/preference chamber, fish had no region of the aquarium in which they could avoid the phenol. Two sets of tests were conducted using the same concentrations of phenol.

The first set of experiments is summarized on Table 4. The fish responded to the presence of phenol at 0.5 mg/L. In terms of position along the flow, they moved away from the phenol in all cases. For data on position, the equality of variance between control and test data were tested using an F test (Statgraphics Ver. 1.2). Differences in variance were significant in all cases, even when the unpaired t test indicated no significant difference between test and control situations. This indicates an increase in activity in response to the toxicant, even though mean position did not change. It also suggests the importance of examining the assumption of equality of variances when testing behavioral data or developing alarm criteria for this project.

The second set of experiments is also summarized on Table 4. These data support conclusions reached in the first set of experiments. Once again, response to phenol was detected at 0.5 mg/L; a lower response threshold was not found. The two sets of experiments suggest that the most reliable elements of response behavior for fathead minnows may be speed and rate of change in direction. In addition, the response of fathead minnows to chlorine was tested at concentrations of 2 mg/L and 0.5 mg/L (Table 4). The response of speed was significant at both concentrations. Response in position was inconsistent. In this chamber there is no area of refuge from the chlorine, and so a consistent response would not be expected.

Field Testing and Operation

The field testing phase of the study resulted in plumbing and computer design changes too numerous to detail here. The final physical design is that presented in Figure 4. Problems were encountered with the river intake clogging with silt and could be solved with a back-flushing device and/or by using a sub-gravel intake. The computer interface problems that were encountered were dealt with on an as-needed basis. The final result was the computer software briefly described in the field testing section of this report's "Material and Methods."

Data were collected from the field system during the operation phase and analyzed for differences between the field control (recirculating system) and river test (water from the river). No significant difference was observed between the two. The river control and test were also compared to the longterm Daphnia control data. No significant differences were observed for those comparisons either.

No substantial changes in temperature, pH, or dissolved oxygen were observed beyond the expected diel fluctuations.

Table 4. Behavioral responses of fathead minnow populations to two different toxicants.

Toxicant and Concentration, replicate	Observed Variable			
	Vert(x)	Horz(y)	Spd	Chng Dir
Phenol				
first set				
4.45 mg/L, rep. 1	*	*	*	*
rep. 2	NS	NS	*	*
2.25 mg/L, rep. 1	*	*	NS	*
rep. 2	*	*	*	*
0.05 mg/L, rep. 1	NS	*	*	NS
rep. 2	*	NS	*	NS
second set				
4.5 mg/L	NS	NS	**	*
2.25 mg/L, rep. 1	NS	*	**	**
rep. 2	**	**	**	**
0.5 mg/L	*	NS	**	#
Chlorine				
2 mg/L	NS	**	#	NS
0.5 mg/L	**	NS	**	**

NS = test and control not significantly different at P = 0.10 (P=0.05 used for first set of phenol tests)
# = test and control different at P = 0.10
* = test and control different at P = 0.05
** = test and control different at P = 0.01

DISCUSSION

Most of the early warning systems reviewed use fish gill ventilatory response based on bioelectric signals that are most likely generated by the muscles that move the buccal apparatus and opercular plates (Cairns and Gruber 1980). Such systems use a single species and a single behavior response characteristic. The system described in this report is based on a multiple species approach that monitors several elements of actual behavior of the organisms. While more recent commercially available systems use various approaches to early warning response (e.g. Aquatest or Grider System), they use only fish as test organisms. We have tested a system using both fish and invertebrates in hope that a more sensitive response to a wider range of potential toxicants can be developed. Compared to other systems, the present one can use a larger number of organisms (up to 50 individuals) that can be smaller in size (like early developmental stages or small invertebrates). The possible combination of continuous biomonitoring coupled with an invertebrate such as Daphnia can offer a potentially more sensitive system both in level and speed of detection and in sensitivity to a wider array of chemicals. Any number of test species could be used in the system including both fresh water and marine. Our selection of test species was based upon availability, size of the data base, previous use as a bioassay organism and the behavioral characteristics of the organisms. Brown (1976) noted the importance of the multi-species approach to ensure a detectable response to a wide selection of toxic compounds.

There are some operational problems with all early warning systems reviewed. Observed amplitudes in ventilatory response can be effected by changes in the dissolved salts and the direction of the test fish in relationship to the detection electrodes (Cairns et al., 1981). The use of video images can be affected by seasonal variations in turbidity and contrast between the organism and the background. The turbidity problems can be greatly reduced by the use of filtration, although some regulatory agencies are concerned about filtration removing some toxicants. However, with early warning systems the quick response will most likely be to toxicants in solution. Materials sorbed to particulate matter may be unavailable for uptake by organisms and thus not detected (van der Shalie, 1981).

The importance of selecting organisms which have good contrast between the organism and the background is paramount with this system. Some invertebrates are almost transparent in water and thus are extremely difficult to digitize. Large, adult Daphnia fed with dark food can be digitized while juvenile fish illuminated from below are very easily followed by the system.

The organism must be active and in constant movement as was noted by Scherer and Nowak (1973) in their work on measuring avoidance movements in fish. Early work done by the authors on juvenile channel catfish Ictalurus punctatus demonstrated that while catfish are sensitive to phenol the control produced a difficult long-term pattern. The background noise caused by very infrequent activity followed by sharp, short locational changes and hyperactivity was extremely difficult to use in an early warning system.

While many toxicological testing procedures require diel lighting regimens, the test procedures selected for these experiments were based upon continuous lighting. This reduces the sharp transitions between light and dark which produces increased locomotor activity and opercular rate (Drummon and Carlson, 1977).

Cairns and van der Schalie (1980) pointed out that a basic factor in the successful operation of continuous behavioral biomonitoring was the ability to quickly and reliably detect deviations from normal conditions. The MK III Fish Monitor adopted four statistical methods; Cusum, Mann-Whitney U, nonparametric regression and percentiles. Based upon the tests conducted for that system (Evans et al., 1986) the percentile test performed most satisfactorily on the data series. Cairns et al., 1981 used time series analysis in his gill ventilatory system. The behavioral data generated by our video system can be analyzed in many different ways. We are still exploring the best methods for inclusion in an early warning system context. We have used the Kolmogarov-Smirnov two-sample test, Mann-Whitney U, Mann-Whitney Wilcoxon test, percentiles and numerous parametric tests in our analysis of the data. While we have not made a final selection, the concept developed for the MK III system, or some modification, seems to be a good starting point. Confidence limits or acceptable percentiles can be set with the computer system checking control data by determining if each new data point falls within these preset boundaries. The sensitivity could be set based upon the number of consecutive data points falling outside the allowed confidence limits. The control data developed for Daphnia indicated that the number of single outliers are not frequent and that for tests lasting up to 164 hours we had no two outliers in succession. Preliminary data review of information from the remote site indicated the same trend. Fish behavioral data show more variability in the controls for salmonids while fathead minnows exhibit less.

Laboratory Testing

Comparing this video system to existing early warning systems, this system now seems more sensitive than those which are based on ventilatory response. For example, the Grieder system using the Nile River fish Gnathonemus petersi detected the presence of 0.2 mg/L cyanide after 2 hours of exposure and 0.5 mg/L after 5.5 hours (Ewen 1987). The MK III Fish Monitor detected phenol at 2 mg/L with an alarm raised in 28 minutes (Evans et al. 1987). In our tests, Daphnia magna responded to phenol concentrations down to 1 mg/L (Table 3) and 0.43 mg/L (Table 2), and fathead minnows responded at 0.5 mg/L (Table 4). Using a much earlier version of our system, Miller et al. (1982) were able to detect copper at 18.5 ppb (ug/L) by the hyperactivity of barnacle nauplii. We found statistically significant response in the behavior of D. magna at 7 ug/L copper (Table 3).

The present system also responds faster, with daphnids responding in 2 minutes or less and steelhead responding to 8 mg/L phenol in 7 minutes (Table 1). The MK III alarm criterion is 10 consecutive observations exceeding an alarm threshold, and our criterion for this exercise was the first mean value statistically different from the control values (for the group of test organisms). Even applying the MK III criterion to our system would yield a response time of 17 minutes for steelhead exposed to 8 mg/L phenol (compared to MK III time of 28 minutes). Likewise, the response time of Daphnia magna exposed to 0.43 mg/L phenol would be 6.8 minutes.

Since this project was designed to determine the feasibility of an early warning system based on motion analysis, lower thresholds were not determined. The test concentrations were selected to produce behavioral responses for the purpose of testing the system, and as such were much higher than State and Federal drinking water criteria. Determination of the lower threshold or sensitivity of the system to various toxicants was beyond the scope of this project.

Field Testing

A comparison of the means of the recirculating control and the river water showed no statistically significant difference. Both the control and river water showed tight behavior response patterns. There were no outliers in either sets of data, and when the control data from the recirculating system at the river were compared to the long term control data generated in the laboratory there was no statistical difference.

The equipment and software at the river site need additional modifications based upon the results obtained in this study. Therefore, the following recommendations are presented:

1 - Preliminary plans to condense and streamline the present system of tanks, temperature control units and camera supports should be finalized. The system can be contained in a single unit with heat exchanger (hot and cold water control) and final stage filters stored under the unit supporting the Daphnia tanks and fish chamber. This unit would be about 4 feet long, two feet wide and 6 feet high.

2 - The Venturi filter system would be a separate unit placed in line before the EW test system.

3 - The intake system seemed to function well. If possible, a back flushing system or method of removing fine sediments from the pump unit should be designed.

4 - The basic software design for the field system has been established. This core system now needs the statistical software to operate the early warning alert function. A preliminary program has been started to test each data point as it is collected. This approach and software needs to be laboratory and field tested.

5 - The data from the data-logger needs to be integrated into the statistical evaluation software so that changes in physiochemical data can be evaluated at the same time the behavioral information is being analyzed.

6 - A telecommunication link to the computer from the remote site needs to be developed. This would allow communication and early warning alert information from one or many remote sites to be monitored at a central administrative computer at a water purveyor's headquarters.

7 - The remote site computer hardware needs to be rack mounted and simplified from the present test prototype.

With the design of the new equipment, the selection of the early warning sites will be easier. However, each site will need a 110 volt AC power supply to keep the system running, a vandal-proof unit for the equipment, and telecommunications with the administrative computer. Possible sites based on potential sources of contaminates have been proposed as part of this study (Klamt and Mead, 1988). Any of those sites which satisfy the above criteria could be used for the system.

Alarm Criteria

Progress has been made in the development of alarm criteria. The result of this work is a better understanding of the statistical nature of the observed behavioral data and a clearer understanding of how effective alarm criteria can be developed. This study did not, however, result in selection or development of specific criteria.

For the EWS, alarm criteria depend on detection of change in behavior. Such detection is made possible by various statistical analyses of the behavioral parameters: position (in two dimensions), speed, and rate of change in direction.

The two primary objectives of the alarm criteria are (1) to reliably detect behavioral responses caused by exposure to low levels of chemical stimuli and (2) to detect such responses as quickly as possible. Generally speaking, these objectives are not compatible when collecting data sequentially through time. This is because attaining objective (1) requires increasing the number of data points analyzed, but attaining objective (2) requires minimizing time and, therefore, minimizing the number of data points analyzed. Because this EWS detects response#in minutes, those considerations do not put severe limitations on alarm criteria.

The distribution of the data also influence the selection of statistical tests for alarm criteria. Behavioral data are often analyzed by nonparametric statistical procedures. Such procedures make no assumptions about the distribution of the data. This is appropriate because behavior is not random action (if anything, it is the antithesis of random action) and so behavioral data are typically not normally distributed. (However, very large sample sizes do approach normalcy due to the preponderance of numbers.) But compared to parametric statistics, which assume that data have a normal distribution, there are fewer nonparametric procedures available. Where both a parametric test and its nonparametric analog are both applicable, parametric tests have greater power and reliability in detecting differences.

In our past studies, we often reported results of both parametric and nonparametric tests. For the sake of parsimony and with the objectives of developing alarm criteria that are both quick and reliable for low levels of response, we investigated both the theoretical and practical aspects of using parametric tests.

There is some theoretical basis for using parametric tests. Data used for the alarm criteria at any instant are not the data for each individual organism, but rather a mean for all organisms observed at that time. According to the Central Limit Theory, the distribution of means from a population tends to be normal even when the underlying distribution of the population itself is not normal. Moreover, many parametric tests are robust against violations of initial assumptions.

The distributions of all four behavioral observation parameters for alarm criteria (position in two dimensions, speed, and rate of change in direction) were tested for normality from several experiments. Then the equality of variances under control and test conditions were tested for equality (another assumption of parametric tests).

As a practical basis for selecting tests for alarm criteria, several experiments not directly related to this project, but with similar data, were analyzed by both parametric tests and their nonparametric analogs.

Results (Table 5) were the same for both types of analysis. As a consequence, parametric methods are used for all examples given in this report (although nonparametric tests were also made in a number of cases) and future efforts to develop alarm criteria will focus more on parametric statistical methods than did past efforts.

Comparison of a single observation with the mean of a single sample (mean) was conducted on selected data sets. The experiments using <u>Daphnia</u> exposed to 7.0 ug/L of copper were tested using rate of change of direction. The first 10 means were compared individually to the grand mean of the control and none of the data points were statistically significant from the control. The same data comparing the control with the grouped means from 30 data points were significant. Speed of <u>Daphnia</u> showed mixed results with 5 out of 10 data points showing significance at P = 0.1.

In the case of <u>Daphnia</u> exposed to 1.0 mg/l of phenol, 9 out of the first 20 data points (rate of change of direction) were not significant at P = 0.1. The grouping of the first 15 data points into a data set and then comparing that data set to the grand mean showed more statistical power in determining a difference than using individual data points. However, more data needs to be analyzied to select the final statistical method for the alarm alert detection system.

It should be noted that the intent of such a system is to provide protection from accidental spills and illegal discharges of toxic chemicals. It is not useful for low-level monitoring, and certainly is not intended as a substitute for low-level monitoring as regards concerns of chronic exposure.

Table 5. Summary of statistical comparisons using two parametric tests (ANOVA and Student's t-test) and two nonparametric analogs (Friedman's Two-Way Analysis by Ranks and Kruskal-Wallis test) to analyze the effects of toxicant exposure on the behavior of striped bass (*Morone saxatilis*).

Response and Source	Percent Effluent Ten Percent Friedman	 ANOVA	 Fifty Percent Friedman	 ANOVA
X-coordinate				
effluent	**	**	**	**
replicates	**	**	NS	NS
Y-coordinate				
effluent	**	**	**	**
replicates	**	**	**	**
Speed				
effluent	**	**	**	**
replicates	**	**	**	**
Change of Direction				
effluent	**	**	**	**
replicates	**	**	NS	NS

Response and Source	Percent effluent Ten Percent t-test	 K-Wallis	 Fifty Percent t-test	 K-Wallis
X-coordinate				
effluent	**	**	**	**
replicates	**	**	**	**
Y-coordinate				
effluent	**	**	**	**
replicates	**	**	**	**
Speed				
effluent	**	**	**	**
replicates	**	**	**	**
Change of Direction				
effluent	NS	NS	**	**
replicates	**	**	**	**

** = Highly significant ($P = 0.01$) difference between test and control
NS = No significant ($P < 0.05$) difference

CONCLUSIONS

Task 4 to the section 205(j) project, to evaluate the feasibilityof motion analysis equipment in detecting toxins in water, was satisfied specifically by:

1 - testing the system under laboratory conditions with several species of aquatic organisms exposed to toxicants at several concentrations,

2 - testing the laboratory system in the field situation at a remote site on the Russian River, and

3 - modifying the field system and operating it in the field to collect data as a simulated early warning station.

Task 5 of the project, to develop alarm criteria, was approached from the standpoints of the various statistical methods available to analyze the data and the management/policy concerns of an entity supplying water for domestic use and utilizing such a system. Specific criteria were not presented due to factors that might be site specific, such as the sensitivity of the organisms (not determined in this study) and individual management policies of different water supply entities.

Preliminary laboratory tests with phenol and other toxicants indicate that the approach of following behavioral patterns a video-based computer system holds promise for biomonitoring. Additional testing with other toxicants than those used in this project is suggested. A number of organisms (up to 50) can be followed simultaneously in the same field, which greatly increases the statistical power of a system used for behavioral monitorlng.

Daphnia sensitivity levels and response times to phenol were lower than for the salmonids and fathead minnow. Although the response levels were lower for the gill ventilatory response than for the salmonid overall response, Daphnia and fathead minnows still exhibited faster and lower level responses than the ventilatory measurements reviewed from the literature.

Lower thresholds of response were not determined. However, additional work should be done to determine the lowest concentraitons of particular chemicals which cause a response. Such information would be useful in determining the basic sensitivity of an early warning system.

Seven-day control data for Daphnia indicate a replicable and stable response base with few outliers. Control data for fish need to be expanded. Preliminary results indicate that salmonids tend to be more irregular over time in their control behavioral patterns than Daphnia and fathead minnows. Size, sexual state and number of fish used in the test are factors. Visual sensitivity to outside stimulus is also a factor.

Multiple exposures of the same organism (Daphnia) to phenol resulted in detection of the toxicant by the organism and a statistically significant response. Pre-and post-exposure data were significantly different, even after 24 hours of exposure to control water. However, this fact did not result in a no-detection response.

The video system could encounter difficulties with high turbidity levels in the exposure chambers. This problem can be alleviated with on-line filters to remove particulates.

GLOSSARY OF TERMS

acclimation period - that time required by an organism to resume normal behavior after being disturbed

analog data - continuous data obtained through measurement, e.g. length of a fish

buccal apparatus - the muscular part of a fish's mouth involved with food uptake and swallowing and ventilation

CCD - charge-coupled device; in reference to the video cameras used in this project, the solid-state circuitry responsible for the electronic magnification of optical signals

data logger - electronic instrument that gathers and stores data collected from probes measuring physical, chemical, or electronic conditions for transfer to a computer

diel - the cycle of day and night; as used in this report, those changes in behavior and water quality caused by the cycle of light and dark

digital data - discrete data obtained by counting, e.g. numbers of fish

digitizing - conversion of analog data (typical measurement data) into digital data (discrete data used by modern computers); in reference to motion analysis system, creation of an outline of an object on a video screen from the actual video picture by activating individual pixels or dots of resolution on the video screen

LC_{50} - the concentration of a toxicant at which 50% of the test population dies

Marriotte bottle - a bottle from which a constant head is maintained as the content of the bottle is reduced, providing a constant output of fluid from the bottle regardless of the level of fluid in the vessel

nonparametric - in reference to statistical tests, those tests that are distribution-free, i.e., they may be performed regardless of the distribution of the data

opercular plate - gill cover; the boney plate on the side of a fish's head covering the gills

outlier - data point which is statistically significant from a whole group of points; in any large data set a number of outliers are expected

parametric - in reference to statistical tests, those tests requiring normally-distributed data

peristaltic pump - a pump which moves liquids through flexible tubing by alternately squeezing and releasing the tubing in a circular motion reference toxicant - a toxicant for which the response is known and well-documented for the test species being used

rheotaxis - the aligning of an aquatic organism in response to the direction of a current (positive=facing upstream, negative=facing downstream)

sorbed - physical attachment of a chemical to a substrate such as sediment

speech synthesizer - a device that mimics a human voice by electronic generation of tones; can be used to send voice messages from a computer

standard deviation - a common statistic indicating the amount of variation in a group of numbers; provides information on the variability of data used to calculate a mean

ventilation frequency - rate of "breathing" of fish as measured by the movement of the gill plate; can be measured by the low amplitude electrical impluse

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