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Project Summary

An Automated Monitoring System for Fish Physiology and Toxicology

Richard W. Carlson, Gregory J. Lien, and Bruce A. Holman

This full report describes a data acquisition and control (DAC) system that was constructed to manage selected physiological measurements and sample control for aquatic physiology and toxicology. Automated DAC was accomplished with a microcomputer running menu-driven software developed with an extended BASIC. An interface module was built that connected standard sensors and controls to the computer. Digital I/O signals for sample device control and analog signals from sensors were multiplexed through the interface module. Time intervals for automated DAC were user defined, and test data were displayed on a monitor, printed, stored on disk, and transferred to a minicomputer for analysis. Automated measurements were made of temperature, ventilation volume, oxygen content of exposure (inspired) and expired water, and pH of both waters from four *in vivo* rainbow trout *Salmo gairdneri* preparations. Oxygen uptake efficiency and oxygen consumption were calculated. Urine and expired water samples were also collected from all fish.

Non-automated sampling included ventilation frequency, cough frequency, the electrocardiogram, and aortic blood from an implanted canula. Sampled blood was analyzed for oxygen, carbon dioxide, pH, hematocrit, and hemoglobin. The respiratory-cardiovascular data gathered with this system were used to define fish acute toxicity syndromes (FATS) specific to known modes of toxic action.

This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN. to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

One approach to understanding causal relationships between chemicals and their effects was developed recently and is termed fish acute toxicity syndromes (FATS). These are collections of direct and indirect measures of effect, or clinical signs, manifested in the animal upon exposure to chemicals that are unique and specific to a common mode of action. Based on a group of measurable toxic signs involving the respiratory-cardiovascular system in rainbow trout *Salmo gairdneri*, FATS have been defined for narcotics, oxidative phosphorylation uncouplers, acetylcholinesterase (AChE) inhibitors, respiratory membrane irritants, and the pyrethroid insecticide fenvalerate.

FATS testing required data acquisition on 11 respiratory-cardiovascular variables and the capability to monitor more if necessary. Monitoring was performed manually during all previous FATS tests and consumed the full attention of at least three people along with the part-time help of several others during both a seven hour control period and for up to 48-h during the acutely lethal exposure period. Only two fish could be prepared and tested at one time, and two tests were therefore required to gather suf-

ficient information on four fish to reliably define a FATS. Although measurements were made often enough so that statistical relevance was established, a higher sampling frequency was desirable for greater confidence.

The objective here was to develop an automated system to efficiently quantify some of the physiological functions of a whole fish preparation exposed to acutely lethal chemical concentrations. It was desired that the system provide for data gathering on at least four fish, and to do so at predetermined intervals throughout the test including periods of unattended operation. Another requirement was that it remain flexible enough so that sampling and measurement regimes could be changed, singly or collectively, during a test. With these in mind, a system was designed that could be constructed from commercially available sensors, control valves, a personal computer, a specially constructed interface, and menu-driven software to coordinate system activities.

Procedures

The system performed physiological monitoring on rainbow trout *Salmo gairdneri* that weighed between 0.6 and 1.0kg and were exposed to a lethal concentration of an organic chemical. This required the integration of several subsystems that included: (1) exposure apparatus that provided water and toxicant delivery; (2) automated sampling and measurement circuits and devices that provided automatic data collection and sampling of physiological functions; (3) non-automated circuits and devices that provided monitoring of those physiological functions that defied automation at this time; (4) a microcomputer system that controlled all aspects of automated monitoring; and (5) an interface that provided all necessary interconnections and switching between the computer and external devices.

The exposure system was the same as that described by McKim and Goeden (1982) except that the toxicant delivery apparatus and fish chambers were enclosed in a specially constructed and vented enclosure to minimize human exposure to potentially hazardous chemicals. Each of the four respirometer-metabolism chambers (Figure 1) was modified so that water overflow from the A compartment on fish chamber one and from all B compartment standpipes was directed into flow measuring devices; other A compartment and all C compartment overflow went directly to drain. From ports located on the sides of the chambers, water was directed to the different sensing electrodes without aeration.

The interface fulfilled three important needs. First, information in the form of analog signals, or varying voltages, from the meters measuring dissolved oxygen concentration, pH, temperature, and from the pressure transducers for water flow rate must be read into the computer.

Secondly, the computer was required to control the operation of solenoids and motors used in making flow measurements and water samples. Additionally, the toxicant pumps were controlled and the urine fraction collector was advanced through the interface interconnections. Lastly, the interface provided power to operate or control the operation of the different sensors and devices attached to it. An internal power supply provided + 12, + 15, and -15 volts DC to the system, while connections to power supplies external to the interface supplied + 5, -5, and + 24 volts DC.

The microcomputer was an IBM PC/XT specially manufactured for Analog Devices and included Concurrent CP/M-86 as its operating system, a multitasking system that managed the I/O of all devices attached to the computer, provided file management, and loaded and ran the operational program for DAC. The operational DAC program was written in-house and named "TEST." TEST contained 1083 lines of source code and required 55 KB of memory for the undocumented source code or 48 KB for compiled object code. TEST consisted of a short main program to begin and direct program execution, a timing task and an interrupt task running concurrently, and 25 subroutines that performed all the functions required by the main and task portions of the program.

Figure 2 shows the overall layout of the data acquisition and control (DAC) system that performed sampling, measurement, and calculation of selected physiological functions. Individual components and their operation are discussed in the research report. The DAC system monitored pH, dissolved oxygen (DO), temperature, and flow rate of both the incoming and expired water in which the fish resided during a test. This was accomplished by monitoring the expired water (B compartment) of up to four fish chambers and the incoming water (A compartment) of fish chamber one .(Figure 1) Additionally, samples of both waters and urine fractions from each fish were collected automatically and held for chemical analysis. A single water sample was taken from the A compartment whenever any or all of the B compartmer were so scheduled. Also, whenever a fish chamber was monitored for pH, D or temperature, the A compartment w sampled immediately afterward so th the samples of inspired and expir water were as close in time as possib This was necessary because t calculations for oxygen uptake efficien (U_E) and oxygen consumption (VO involved the difference in DO content both waters at that moment.

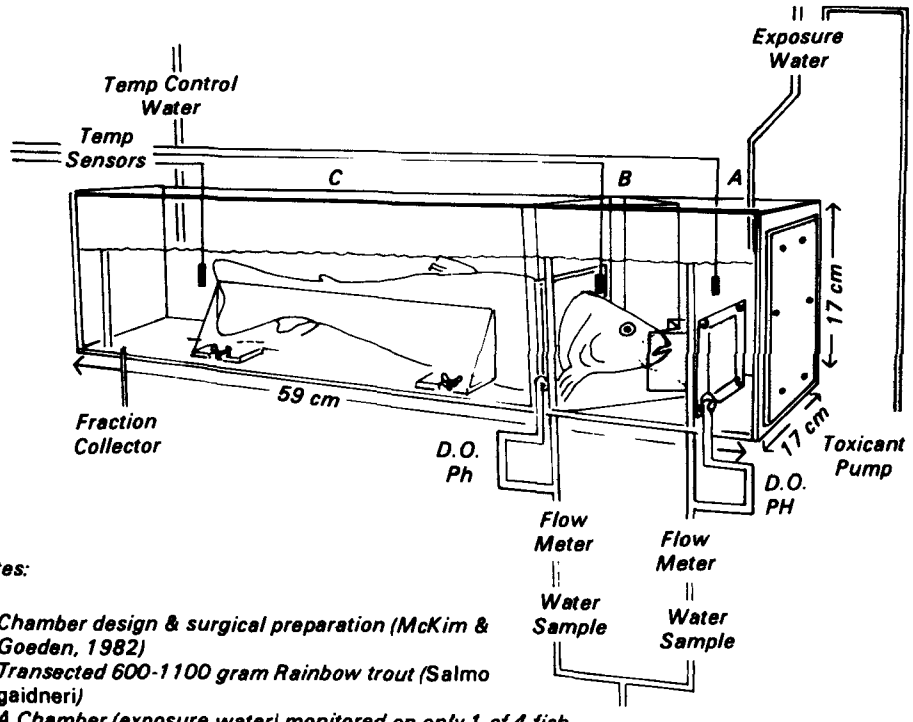
After each measurement or samplir operation the computer monitor scre was updated to show the results for th time interval, a continuous hard copy w appended and all data were appended a file residing on the hard disk.

During each FATS experiment mea urements were made on the physiologic variables shown in Table 1. Ventilatic volume (V_G), VO_2, and U_E wer monitored automatically while the remai der were done manually. Ventilato frequency (f_v) and cough frequency (f were determined from portions of stri chart recordings made of the tro ventilatory patterns. These were mo itored from non-contact stainless ste wire electrodes placed in the B and compartments of each fish chamber.

Spinally-transected rainbow trout we each fitted with a latex rubber membrar that separated expired water from incon ing water, a dorsal aortic cannula f blood sampling, copper wire electrod for monitoring the EKG, and a urina catheter. After surgery the fish we placed in individual respirometer chan bers, the electrode connections mad and the urinary catheter was connecte to the C compartment port.

Results and Discussion

To date 17 tests involving 68 fish hav been completed using the syster Testing included three freshwater contr runs, two control tests on a carrier solve used to aid dissolution of some te chemicals, and 13 tests with organi chemicals used in describing fish acut toxicity syndromes (FATS). Collectivel the results from these tests showed th the system performed as designe despite some sporadic electronic ma functions and problems with sensor cal bration during some tests. The firs chemical tested with the automate system, 2,4-dinitrophenol, was in th group of uncouplers originally tested, an the results obtained using the automate system were consistent with thos obtained manually. This verified that th automated system was suitable for FAT



Notes:

1. *Chamber design & surgical preparation (McKim & Goeden, 1982)*
2. *Transected 600-1100 gram Rainbow trout (*Salmo gaidneri*)*
3. *A Chamber (exposure water) monitored on only 1 of 4 fish*
   B Chamber (expired water) (Vg) ventilation volume
   C Chamber (fresh water) input for maintaining temp

Figure 1. *Schematic diagram of respirometer-metabolism chamber. Connections for ventilatory pattern and EKG are not known.*

testing as well as showing that the responses used to define a FATS were reproducible.

The main advantage gained by using the automated system for FATS testing was that four fish could be monitored simultaneously for their cardio-respiratory responses. When done by the automated system, measurements of V_G and DO were rendered effortless and it became possible to monitor more than two fish per test. This at least doubled the number of FATS tests that could be done in the same time frame.

Also, round-the-clock monitoring was now possible and this greatly increased the number of measurements done for V_G, U_E, and O_2. This ensured data gathering throughout a test for every fish and increased the confidence that data were not missed for periods of critical change.

Another advantage to using the automated system was that certain judgments concerning the course of an experiment could be made while it was in progress. For instance, it is characteristic of narcosis-inducing chemicals that their effects on an organism are reversible even at the point of apparent death, usually defined as respiratory arrest in aquatic toxicology, whereas effects induced by chemicals with more specific modes of toxic action are irreversible. By following V_G and VO_2 on the computer printout as well as locomotor activity, ventilation, and the EKG on those recordings, the fish could be revived at various stages of intoxication with toxicant-free water and recovery monitored if it were necessary to demonstrate that certain chemicals were narcotic.

Conclusions

1. Automated monitoring of respiratory-cardiovascular variables from fish resulted in a considerable savings of time and effort when compared to manual data gathering methods.
2. Automated monitoring provided continual data collection during periods of unattended operation, thus ensuring that data were collected during times when critical changes may have occurred.
3. The real-time sampling and calculation of vital signs permitted judgments on the course of an experiment.
4. Automated monitoring allowed rapid data collection at shorter time intervals than manually possible. A greater number of samples provided for greater statistical reliability.
5. Data were easily manipulated and transferred between computers because they were immediately stored in computer files.
6. Less manual sampling reduced human exposure to potentially hazardous chemicals.

References

McKim, J.M. and H.M. Goeden. 1982. A Direct Measure of the Uptake Efficiency of a Xenobiotic Chemical Across the Gills of Brook Trout (*Salvelinus fontinalis*) Under Normoxic and Hypoxic Conditions. Comp. Biochem. Physiol. 72C: 65.

Table 1. *Physiological Variables Monitored in Rainbow Trout to Define the Toxic Responses Associated with Fish Acute Toxicity Syndromes (FATS)*

Variable		Units
Ventilation Volume	(V_G)	ml/min
Total Oxygen Consumption	($V0_2$)	mg/kg/h
Gill Oxygen Uptake Efficiency	(U_E)	%
Ventilation Frequency	(f_V)	no./min
Cough Frequency	(f_C)	no./min
Heart Frequency	(f_H)	no./min
Total Blood Oxygen (arterial)	(TaO_2)	g/100 mL
Total Blood Carbon Dioxide (arterial)	($TaCO_2$)	mmol.L
Blood pH (arterial)	(pHa)	pH units
Hematocrit	(Hct)	%
Hemoglobin	(Hb)	g/100 mL

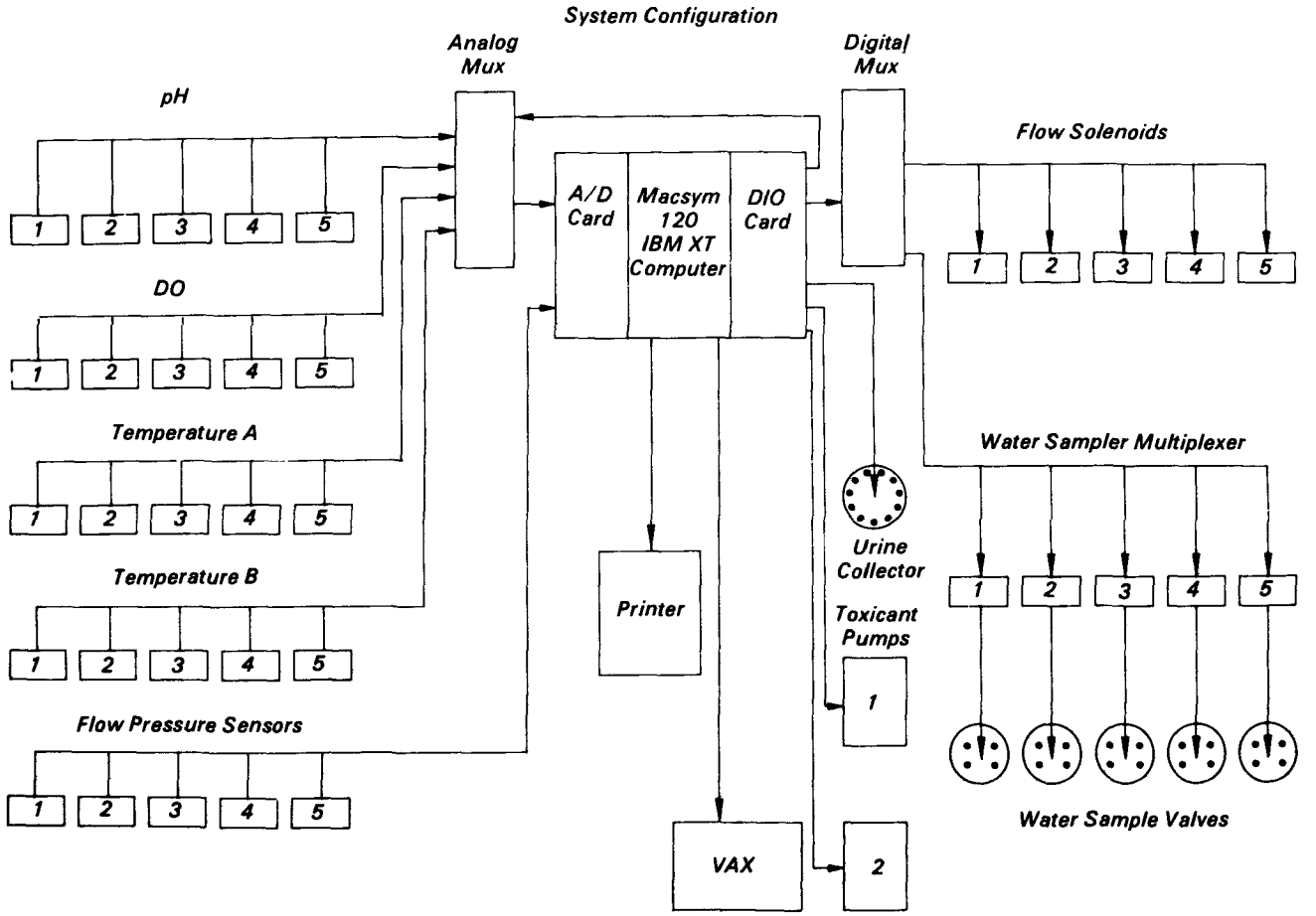


Figure 2. Block diagram of the automated system.

The EPA authors, ***Richard W. Carlson*** *(also the EPA Project Officer, see below),* ***Gregory J. Lien,*** *and* ***Bruce A. Holman,*** *are with the Environmental Research Laboratory, Duluth, MN 55804.*

The complete report, entitled "An Automated Monitoring System for Fish Physiology and Toxicology," (Order No. PB 89-155 212/AS; Cost: $15.95, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

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