United States
Environmental Protection
Agency

Environmental Research
Laboratory
Duluth MN 55804

Research and Development

EPA-600/S3-81-003 Apr. 1981

EPA

Project Summary

Effects of Fluctuating, Sublethal Applications of Heavy Metal Solutions Upon the Gill Ventilatory Response of Bluegills *(Lepomis macrochirus)*

John Cairns, Jr., Kenneth W. Thompson, and Albert C. Hendricks

This study demonstrates the use of minicomputers for continuously observing the ventilatory behavior of fish in order to monitor the quality of the water passing through fish sensing chambers. The water source could be an industrial effluent, agricultural runoff, or a carefully controlled laboratory source as in this study.

Situations of complex, fluctuating toxic effluents were simulated in order to describe responses that could be expected. These results were then compared with those of control fish which were not exposed to toxic effluents.

Not only did ventilatory rates significantly increase in response to laboratory effluents (sublethal concentrations of heavy metal solutions), but also the amplitude of the ventilatory signal was reduced considerably. This is a promising system that could be developed into a useful, on-line environmental monitor for industrial, agricultural, or other purposes.

This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN and it's Newtown Fish Toxicology Station, Cincinnati, OH, 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

This project was undertaken to investigate the feasibility of using ventilatory behavior as a monitor of environmental quality. The weak electrical signals produced by the movement of the muscles in the branchial region as a fish ventilates its gills can be detected by submerged non-contact electrodes placed at either end of a holding tank (Figures 1 and 2). The primary objectives were to describe the average ventilatory behavior as well as the response to sublethal amounts of various heavy metal solutions.

Most previous studies of the effects of environmental changes on the ventilatory behavior were carried out by visual observation of short, intermittent strip chart recordings. Interfacing with a minicomputer allows the continuous accumulation of data and, thus, a more complete picture of natural variability as well as of the response to toxicants.

In this study, the ventilatory signals from 16 fish at a time were amplified

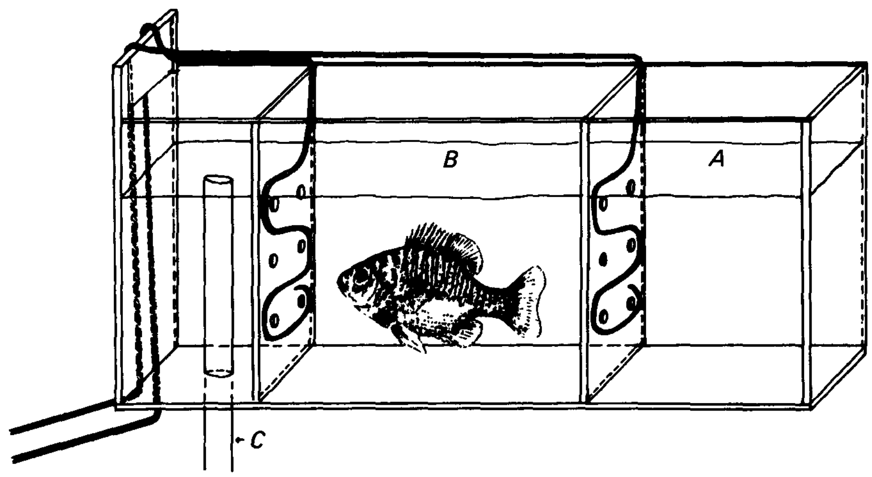

Figure 1. A bluegill in a ventilatory sensing chamber. Water flows into the receiving chamber (A), through the sensing chamber (B), and out the drain (C). The elctrodes are the S-shaped wires at either end of the sensing chamber. The signal is carried to an amplifier (AM).

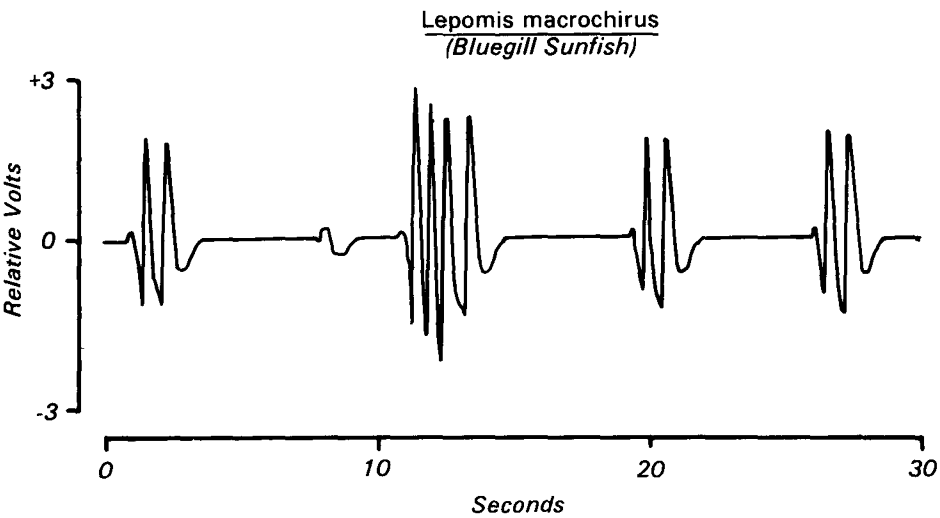

Figure 2. An example of the amplified ventilatory signal of a bluegill ventilating at approximately 20 times per minute.

using high-grain amplifiers which were interfaced to a PDP8/E minicomputer. These data were accumulated continuously, and both the ventilatory signal and the average amplitude of the ventilatory signal were recorded on magnetic tape for later analysis.

Most fish toxicity data are based on 96-hour, constant concentration laboratory tests, usually for a single toxicant at a time. Actual industrial or agricultural effluents are usually complex mixtures that vary in composition and concentration, and the present study investigated the effects of fluctuating as well as constant toxicant applications.

The fish were exposed to sublethal concentrations of divalent zinc, divalent copper, to mixtures of zinc and copper, or to mixtures of zinc, copper, divalent nickel, and hexavalent chromium. Median lethal concentrations (LC50) were determined with 96-hour continuous flow toxicity tests (Table 1). The toxicity of the two mixtures was found to be additive.

Table 1. *Ninety-six Hour Median Lethal Concentrations (LC50) of the Various Toxicants Used in This Study*

Toxicant*	LC50 (mg/l)	95% Confidence Limits
MixA		
Zn^{+2}	3.2	2.1- 4.6
Cu^{+2}	1.0	0.9- 1.2
Ni^{+2}	21.2	18.1- 25.5
Cr^{+6}	132.9	113.6-155.7
Zn^{+2}	1.4	1.0- 2.0
Cu^{+2}	0.4	0.3- 0.6
MixB		
Zn^{+2}	0.9	0.7- 1.3
Cu^{+2}	0.5	0.2- 1.2
Ni^{+2}	10.0	4.6- 23.1
Cr^{+6}	61.6	47.6- 82.4

**Only zinc, copper, mixA, and mixB were used in ventilatory testing.*

Three different exposure patterns were used for each of the various toxicant solutions. Exposure was for 96 hours at constant concentration, or for one or the other of two intermittent patterns during which the concentrations fluctuated (Figure 3). All the fish were acclimated to continuous light conditions to minimize diurnal variation, and temperature was maintained at $22.5 \pm 1°C$.

A total of 55 control and 125 treated fish were monitored during the 12 ventilatory tests. Average rates and amplitudes were calculated for each fish for the pre-exposure, the exposure and the post-exposure intervals. The average ventilatory rates of the control fish varied only slightly and at random throughout the 9 day (216 hours) recording periods (Figure 3). On the other hand, fish that were exposed to the various toxicant solutions increased their rates by an average of 54% when exposed to toxicant. The rates of these individuals then decreased to near normal levels when the toxicant exposure ceased. Correspondingly, the ventilatory amplitude of the exposed fish was reduced by an average of 36% while the controls varied at random. As found for rate, the amplitude of the treated fish returned to near normal levels during the post-exposure recovery period.

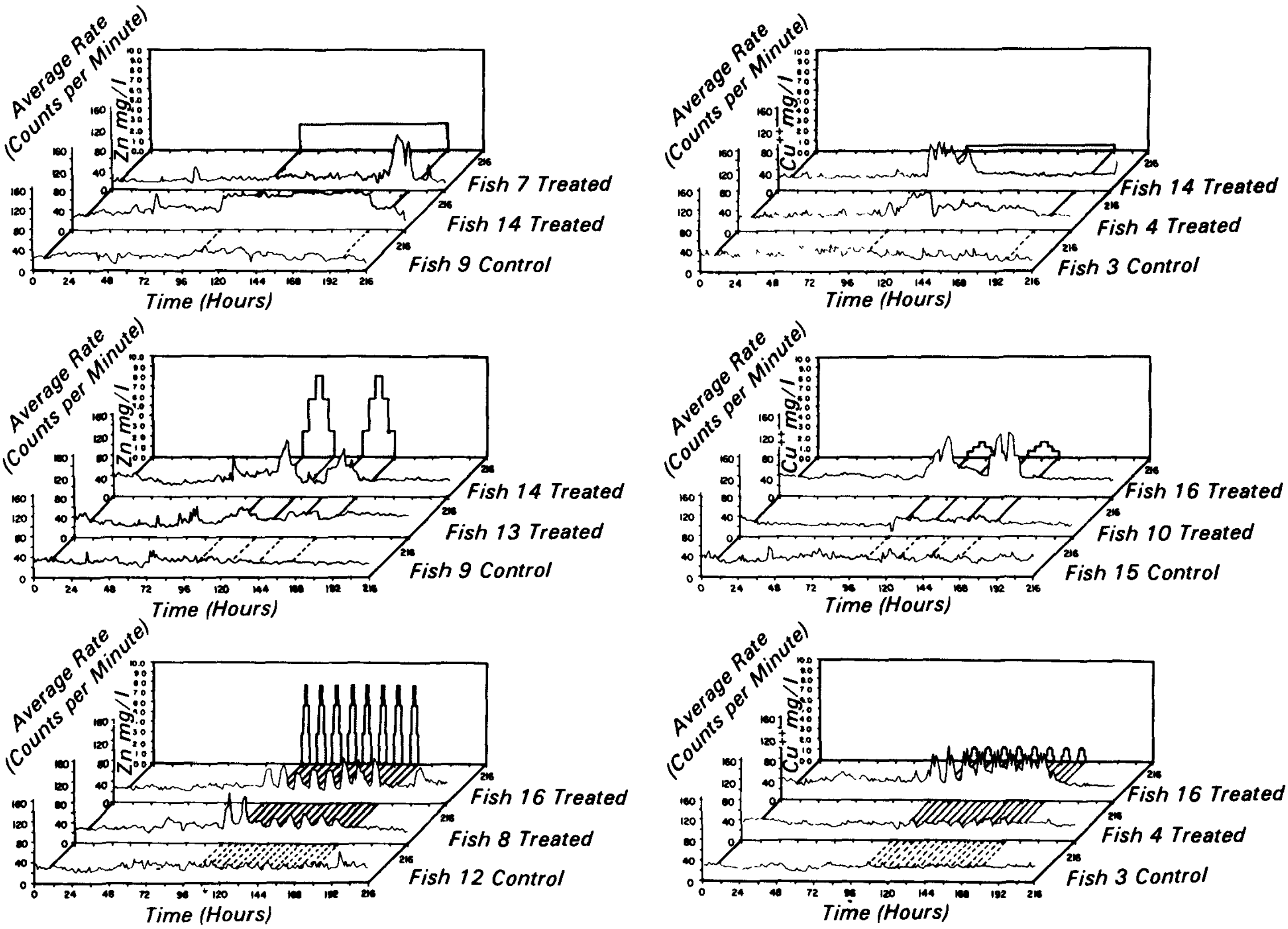

Figure 3. *Examples of the variability seen in the ventilatory rate response of the bluegill to solutions of Zn^{++} and Cu^{++}. The first fish in all six cases are untreated controls while the remaining two were chosen to give examples of the range of the variability of response to the toxicant solutions. The solid line on the fourth vertical plane in each case indicates the shape, duration, and concentration of the toxicant application.*

The addition of dissolved salts to the water in the holding chamber caused a reduction in the electrical resistance and this, when carried to extremes (i.e., sea water), can cause a large reduction in the signal amplitude. However, none of the toxicant concentrations used in this study could be shown to cause more than a 10% reduction in amplitude, and most would cause less than 2% reduction. Thus, the observed amplitude reduction indicates a significant response to the toxicant solutions—a response that can be used to monitor water quality, or more significantly, to enhance the use of ventilatory rate for that purpose.

Chi squared goodness of fit tests and Cochran's C statistic indicated that within each interval (pre-exposure, exposure, post-exposure) the average rates and amplitudes were normally distributed and the variances were homogeneous. Thus, it was appropriate to compare the results between fish using an analysis of variance (ANOVA). The indication from these tests was that there was little or no statistical difference between the fish before or after exposure, but, during the exposure period, a considerable difference existed between the exposed and control fish. This was true for both rate and amplitude studies. Statistically differentiating between the responses to the various patterns of application was difficult due to the extreme smoothing of data. However, it is significant to note that the fish are capable of responding in similar manner to repeated exposures to toxic effluents (Figure 3).

Conclusions

1. The ventilatory response of the blue gill was shown to be measurably affected by sublethal concentrations of zinc, copper, and complex mixtures of heavy metals.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1981-757-012/7055

2. The typical response of this species was an elevated ventilatory rate and a reduced ventilatory amplitude in the presence of sublethal concentrations of the various toxicant solutions.
3. Fish are capable of responding similarly to repeated exposures of toxicant solutions, indicating the feasibility of an environmental monitoring system based on ventilatory behavior.
4. The amplitude response was primarily due to a real change in ventilatory behavior of the fish, not to a change in electrical properties of the water due to the addition of the toxicant solutions.
5. The ventilatory data met the requirements for parametric statistical analyses when comparing individuals. Thus, when sample size is large enough, conventional methods such as the analysis of variance can be employed.

John Cairns, Jr., Kenneth W. Thompson, and Albert C. Hendricks are with the University Center for Environmental Studies, Virginia Polytechnic Institute and State University, Blacksburg, VA.
***William B. Horning, II,** is the EPA Project Officer (see below).*
The complete report, entitled "Effects of Fluctuating, Sublethal Applications of Heavy Metal Solutions Upon the Gill Ventilatory Response of Bluegills (Lepomis macrochirus)*," (Order No. PB 81-150 997; Cost: $11.00, subject to change) will be available only from:*

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

The EPA Project Officer can be contacted at:

Newtown Fish Toxicology Station
Environmental Research Laboratory—Duluth
U.S. Environmental Protection Agency
Cincinnati, OH 45244

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Postage and
Fees Paid
Environmental
Protection
Agency
EPA 335

Official Business
Penalty for Private Use $300

RETURN POSTAGE GUARANTEED