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Research and Development

Maximum Utilization of Water Resources in a Planned Community

Chlorine and Ozone Toxicity Evaluation

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August 1979

MAXIMUM UTILIZATION OF WATER RESOURCES IN A PLANNED COMMUNITY

Chlorine and Ozone Toxicity Evaluation

by

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components requires a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This project focuses on methods maximizing the use of water resources in a planned urban environment, while minimizing their degradation. Particular attention is being directed towards determining the biological, chemical, hydrological and physical characteristics of stormwater runoff and its corresponding role in the urban water cycle.

Francis T. Mayo
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ABSTRACT

Relative to the project entitled "Maximum Utilization of Water Resources in a Planned Community" it was essential to study the effect of wastewater disinfection on aquatic organisms. Impoundments in The Woodlands will receive reclaimed wastewater and will provide recreational fishing. To ensure adequate water quality for aquatic organisms the following experiments were conducted.

Flow through bioassays vs. standard static bioassays were run to determine an accurate LC_{50} for chlorine on fingerling channel catfish, Ictalurus punctatus. The 96 hour LC_{50} for chlorine is 0.07 mg/l (total chlorine) from the flow through vs. 0.45 mg/l (total chlorine) from the static bioassay.

The 96 hour LC_{50} for ozone on fingerling channel catfish, as determined from flow through bioassay, is 0.03 mg/l ozone.

Chlorine and ozone exposures had little effect on kidney functions. Exposure to both chlorine and ozone drastically reduced the ability of the gills to actively absorb sodium from the water.

Long term exposure to chlorine drastically reduced both blood pressure and heart rate while exposure to ozone had little, if any, effect. Blood pressure and heart rate are very sensitive physiological parameters and changes are indicative of a stressful environment.

Both chlorine and ozone are extremely toxic to fish at low levels. If detected in receiving waters by present analytical techniques, a toxic condition exists.

This report was submitted in fulfillment of USEPA Grant Number 802433 by Brian R. Hammond and James R. Bishop, Jr. under the sponsorship of the Environmental Protection Agency. This report covers a period from July 1973 to May 1976 and work was completed as of May 1976.

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ACKNOWLEDGMENTS

This project was supported by the U.S. Environmental Protection Agency, The Woodlands Development Corporation and Rice University.

Thanks are due the Texas Parks and Wildlife - Sheldon Fish Hatchery,and Catfish Acres of Winnie, Texas for their donation of fish used in these experiments.

SECTION 1

INTRODUCTION

Non-point source pollution has become of greater concern due to increasingly stringent point source effluent standards and rapid development of urban areas. Urban stormwater runoff is one of the major non-point pollution sources and innovative methods must be developed to minimize the impact of stormwater runoff on receiving waters. One such imaginative water resource plan has been developed for The Woodlands, a planned community in southeast Texas. The U.S. Environmental Protection Agency, Woodlands Development Corporation and Rice University have completed a three year research/demonstration project to evaluate the water resource system at The Woodlands and develop strategies for maximizing the benefits to the community while minimizing the effect on receiving waters.

The hydrological characteristics of a natural watershed change with urbanization. Replacement of flow-retarding vegetation with impervious surfaces, such as roads and buildings, increases the rate and amount of stormwater runoff. Removal of the water is traditionally implemented by the use of an urban drainage system consisting of storm sewers and/or deep, concrete-lined drainage ditches, designed specifically for rapid drainage. Increased runoff volumes and peak flow rates result, creating problems of downstream flooding and channel erosion.

Infiltration of stormwater is a major groundwater recharge source, however emphasis on surface removal minimizes the infiltration rate, resulting in a lowered water table and possible urban land subsidence problems. Water quality deteriorates because natural purification provided by infiltration in compromised.

The urban environment, typified by high population density, provides a major pollutant source for runoff waters. Recent investigations recognize the significance and magnitude of pollution problems from urban stormwater runoff. In terms of specific pollutants, the sediment yield problem is the most dramatic. Due primarily to urban construction, urban sediment loads were found to be as much as 75 times greater than loads in agricultural regions. Other runoff pollutants reported higher in urban regions include dissolved solids, coliform, biochemical oxygen demand and chemical oxygen demand (BOD and COD), polychlorinated biphenyls, heavy metals, pesticides and fertilizers. An increase in bacterial content is also reported. Claudon stated that agricultural and urban runoff regularly contribute *Salmonella sp.* to recreational waters. High fecal coliform and fecal streptococcus numbers have been found in urban runoff. Snowmelt and related agricultural runoff in far northern climes in the continental U.S. have been shown to contribute high densities of indicator bacteria and pathogens to runoff.

The addition of large quantities of inorganic nutrients particularly nitrogen and phosphorous, to freshwater lakes poses a serious problem in lake management. Municipal sewage, agricultural drainage, managed forestland drainage, and fertilization often accelerate the natural process of eutrophication, thus enhancing the growth of bacteria, algae and aquatic vascular plants. Population densities of these organisms often reach nuisance proportions and interfere with the aesthetic qualities and recreational values of lakes. These "blooms" may discolor, impart unsatisfactory tastes and excrete toxins into the water. They can also clog treatment plant filters, and upon decomposition, produce foul odors. Late summer "blooms" can create anoxic conditions and cause the death of fish. Accordingly, values of lake properties may depreciate and there may be increased burdens on municipal water systems due to added costs of filtration and deodorization of the water.

Although literature on eutrophication is extensive, most limnological studies were conducted on lakes that were either eutrophic, or non-eutrophic at the time of study, and few studies were continued long enough to follow changes in the trophic status of lakes. Even fewer studies have been initiated on a drainage system before the construction of lakes. Thus, complete developmental histories of the water resources of particular areas are lacking. Also, it is evident that information on more effective methods of removing mineral nutrients from effluents prior to release into natural waters is badly needed.

OBJECTIVES

The overall goal of this research project was to evaluate the water resource plan for The Woodlands and to make recommendations as necessary to maximize its effective utilization through alternations in design or management.

One of the major objectives was to modify and expand the capabilities of the EPA Storm Water Management Model (SWMM) to apply to the "natural drainage" system designed for The Woodlands. SWMM has been expanded to include the following additional water quality parameters: total COD, total Kjeldahl nitrogen (TKN), nitrates and phosphates. The model has been used to evaluate the effectiveness of the "natural drainage" system in minimizing changes in storm runoff quantity and quality and to assist engineers and planners in designing the drainage system for future development phases at The Woodlands.

Since urban areas are typified by a variety of land uses, including residential, commercial and industrial as the broadest categories, determining effects of land use on stormwater quality was conceived as another major project objective. The Woodlands development plan could not provide sufficiently diverse study areas during the project period. Consequently, urban watersheds of similar physiographic and drainage characteristics in Houston were studied to further relate storm runoff water quality to urban land use.

In order to accomplish these two primary objectives, a massive sampling and monitoring program was established. Rainfall, streamflow and over twenty-five water quality parameters were monitored on a regular basis. Including in the water quality program were intensive programs concentrating on bacteriological water quality, chlorinated hydrocarbons and phytoplankton identification and enumeration.

The sampling program included bacteriological tests to evaluate the traditional relationship between indicator organisms (e.g. fecal coliform, fecal streptococcus) and pathogens in stormwater runoff. Disinfection experiments were conducted to determine relative effectiveness of Cl_2, Br_2, and O_3 in untreated stormwater runoff. Toxicological testing determined maximum tolerable concentrations of disinfectant in the receiving stream for maintenance of fish populations. Algal bioassays were conducted to experimentally determine conditions, including nutrient concentrations, necessary to prevent eutrophication in The Woodlands' lakes.

Finally, the performance of a porous pavement was compared to a conventional pavement with regard to runoff amount and quality, wet skid resistance, hydroplaning and other characteristics related to driveability.

The present requirements for the disinfection of domestic and industrial wastes often requires the use of chlorine and/or ozone in their treatment. In part the study entitled "Maximum Utilization of Water Resources in a Planned Community" was to determine safe environmental conditions for these disinfectants using both flow through 96 hour LC_{50} bioassays and the monitoring of physiological parameters as possible indicators under both static and flow through conditions.

CHLORINE

In a recent review by Brungs (1) entitled, "Effects of Residual Chlorine on Aquatic Life", it is suggested that continuous exposure to 0.002 mg/l or less of residual chlorine would not harm most aquatic organisms, but continuous exposure to 0.01 mg/l would harm some species of fish at some life stages. Present data for trout and salmon would indicate a maximum chlorine exposure of 30 min/day at a concentration of residual chlorine up to, but not exceeding, 0.01 mg/l. Brungs (1) also states that, "No comparable criterion for warm-water fish can be suggested at this time". Considerable work has been done to evaluate the toxic effects of chlorine on a wide variety of aquatic species.

Tsai (2) reported that species shifts occurred following the introduction of chlorine into a Maryland river. Also, it has been reported that the reproduction of fathead minnows is drastically affected by exposure to sub-lethal concentrations of chlorine (3).

An extensive review on chlorine toxicity has been made by Becker and Thatcher (4). However, no acceptable chlorine standard has been established to date.

OZONE

The flow through system was essential for the bioassay of ozone toxicity (96 hour LC_{50}) and for the exposure of adult fish to stable ozone concentrations. The previous early work showed that ozone concentrations of 0.01 mg/l were lethal to fish and invertebrates (5). Ozone has been shown to retard meiosis and cleavage, cause irregular polar bodies and abnormal nuclei cleavage in the eggs of the American oyster (Crassostrea virginica) (6). Although ozone is shown to be toxic to aquatic organisms it has been successfully used in the control of fungal disease in incubating trout eggs (7). It has also been used for the oxidation of dissolved organics and for the reduction of microorganisms in aquaria systems (8). However, its use is not wide spread since following ozone exposure animals show increased disease susceptibility as a result of surface damage, and death if over exposed (9). With these advantages and disadvantages in mind, the following experiments were designed to establish safe environmental conditions for warm water fishes.

SECTION 2

CONCLUSIONS

The environmentally safe surface water concentration for channel catfish (Ictalurus punctatus) exposure to chlorine is 0.007 mg/l. This limit is based on 1/10 the 96 hour LC_{50} value of 0.07 mg/l measured in the water before contacting the fish and the absence of harmful physiological responses at low chlorine exposures.

The channel catfish is sensitive to chlorine at toxic concentrations and would probably avoid exposure. This is evident by observable pulmonary irritation and a significant cardiovascular response to chlorine.

A flow through bioassay compared to a static bioassay produces a lower LC_{50} for chlorine (0.07 mg/l vs. 0.45 mg/l).

The 96 hour LC_{50} flow through bioassay of ozone for catfish fingerlings is 0.03 mg/l ozone measured in the water before contacting the fish. Since this value is the lower limit of analysis it can be concluded that if ozone can be detected, the receiving waters are toxic to fish.

Short term exposure of catfish to both chlorine and ozone did not appreciably affect renal function. No physiological changes were noted in tubular water reabsorption, ion excretion, glomerular filtration rate (GFR) and their corresponding urine flows.

Short term exposure of catfish to both chlorine and to ozone was shown to appreciably reduce the ability of gill epithelium to take up sodium from the external medium. Chlorine is actively taken up by the gills of catfish. Uptake averaged 0.4 mg Cl_2/min/kg.

Long term exposures of catfish to ozone had no significant effect on blood pressure or heart rate. This suggests that fish might not be aware of ozone at low concentrations and would exhibit no avoidance reaction to its presence. This lack of avoidance was actually observed in the bioassay tanks.

SECTION 3

EXPERIMENTAL METHODS

FISH HOLDING AND PREPARATION

Holding

Adult catfish (0.25 - 2.0 kg) were netted and returned to the laboratory. They were held in 150 gal circulating tanks at 22 ± 0.5C. The circulating water was dechlorinated tap water with approximately 6.5 mg/l of dissolved oxygen. The light regime was 12 hours light and 12 hours dark. The fish were fed three times weekly on standard catfish pellets (Purina). All fish were acclimated for two weeks before being used in experiments. Only healthy fish were used for physiological observation.

Cannulation

A single fish was netted and placed in the anaesthetic,"MS 222" (Cresent Research Chemicals, Inc.), dissolved in water from which the animal came. Following the loss of all movement the fish was transferred to a plastic operating box which allowed the gills to be covered and the tail exposed. The procedure for cannulation was as follows and involved the following custom made materials:

a. 2 1/2" long 20 gauge thin wall needle with a specially constructed deflected point,
b. Guide wire with a 4 foot long 0.010" diameter core and a 1/2" long 0.003" diameter felxible end. Over the core and flexible end was attached a wound flexible cover of 0.021" which allowed it to fit down the 20 gauge thin wall needle.
c. Polyethylene cannula with an inside diameter of 0.023" (Clay Adams Intramedic PE 50).

Cannula insertion was as follows: a small hole was made with a sewing needle directly lateral to the haemal arch and approximately 1" before the last caudal vertebrae. Following the removal of the sewing needle, the 20 gauge needle was inserted with the deflection pointed downward. This needle was advanced at a very flat angle until the point slipped between two haemal arches and entered either the dorsal aorta or the caudal vein.

Entrance of a vessel was determined by blood entering the syringe. The needle was then rotated 180° so the deflected opening faced forward. At this point, the fish was heparinized with approximately 0.5 cc of 1000 unit sodium heparin and the syringe removed leaving the needle in place. Immediately the flexible guide wire was introduced down the needle and into the vessel. The needle was then removed leaving the end of the wire several inches up the vessel. The measured cannula was then introduced over the guide wire and the end placed several inches away from the puncture site. The guide wire was then withdrawn and the cannula filled with heparized saline and plugged with a headless stainless pin. Usually a single suture through a caudal ray held the tube in place. Visual observation of the blood pressure in the cannula identified the vessel cannulated. To select the other vessel,be it the vein or dorsal aorta, it was only necessary to enter the haemal arch at the last vertebrae. The needle was inserted along the hypural plate in line with the haemal arch. If the aorta was needed, the deflected point was turned dorsally and ventrally for the vein. The guide wire would then enter the correct vessel and follow it along since they are both enclosed in a bony sheath. With this method, no loss of blood occurred during the operation or following, since the puncture made by the 20 gauge needle was smaller than the outside diameter of the cannula.

Catheterization

While the fish was anaesthetized, a bilumen rubber or polyethylene (Intramedic) heart-shaped catheter was inserted into the bladder through the urogenital papilla. If necessary, the catheter was secured in place with a purse-string suture around the papilla and also a suture through an anal fin ray. To assure complete collection of the urine sample and to remove it as quickly as possible from bladder influences, saturated air was introduced a few seconds before the end of the collection period in order to empty the catheter and collect the complete sample. Using this method, it was possible to use larger sized catheters and thus prevent blockage which occurred with smaller tubes. To prevent evaporation, the tare-weighed collection tubes were covered as the fraction collector rotated.

Experimental Holding

Following the completion of the operation, the anaesthetized fish was placed in a clear plastic box fitted with an inside partition which was adjusted to prevent the fish from turning over and tangling or dislodging the catheter and cannula tubes. These tubes led to the outside through water tight rubber seals in the end of the box. Water was pumped to the anterior end of the box at a rate of 3-5 liters per minute. The fish exhibited only slight opercular movements after complete recovery from the anaesthetic.

Blood Pressure

Following cannulation and catheterization, the fish was transferred to the holding box and allowed to acclimate for several days. Several hours before an experiment, the dorsal aortic cannula was connected to a pressure transducer and a recording physiograph (E and M Instrument). The transducer was calibrated with a mercury manometer and normal blood pressure was recorded.

Gill Sodium Transport

In order to measure sodium uptake by the gills, a fish was placed in a recirculating system containing 10 liters of deionized water, with a known amount of NaCl labelled as ^{22}Na (Amersham/Searle). Removal of ^{22}Na from the circulation tank was measured employing a Beckman LS-133 scintillation counter.

Static Bioassays

Using the procedure described in Standard Methods, static bioassays (96 hour LC_{50}) were run on fingerling channel catfish. Each survival -mortality experiment was repeated three times. For the tests, fingerlings averaging between 6 and 10 cm in length were used. Temperature was maintained at 22 ± 0.5 C. Oxygen was continually monitored and maintained above 4 ppm by the intermittent introduction of air. Chlorine added to the dechlorinated tap water as sodium hypochlorite, was also monitored and was returned to the initial value every 24 hours. In all cases, it was observed that chlorine was rapidly removed from the water of the assay bottles in the presence of respiring fish.

Chlorine Uptake

A single fish was placed in a circulating tank containing 40 liters of water. A calculated dose of chlorine (as aqueous solution of sodium hypochlorite) was introduced and the concentration of chlorine in the tank measured at 5-10 minute intervals until chlorine disappeared.

Continuous Flow or Flow Through Bioassay

Using methods described by Sprague (10) and Standard Methods, 13th Editon (11),continuous flow or flow through bioassays (96 hour LC_{50}) were run on fingerling catfish. Chlorine (sodium hypochlorite) was continually added to the inflow water by a syringe infusion pump, resulting in a constant chlorine level in the water. Ozone was added to the water by aeration at the inflow, using a Sander Ozonisator Model IV and pure oxygen. Ozone concentrations were controlled by regulating the oxygen supply using Kontes flowmeters.

TABLE 1. SUMMARY OF STANDARD CHEMICAL AND PHYSICAL TESTS USED IN THIS RESEARCH

Parameter	Reference (Refer to References Section.)	
Calcium	(12)	pp. 102
Chloride	(11)	pp. 377
Chlorine	(11)	pp. 112
Dissolved oxygen	(12)	pp. 60
Magnesium	(12)	pp. 112
Potassium	(11)	pp. 283
Sodium 22 (Beckman Liquid Scintillation Counter #133)	(13)	pp. 1
Inulin ^{14}C (Beckman Liquid Scintillation Counter #133)	(13)	pp. 1
Sodium	(11)	pp. 317
Statis bioassay	(11)	pp. 569
Flow through Bioassay	(10)	pp. 5
	(11)	pp. 570
Ozone	(11)	pp. 271

SECTION 4

RESULTS

FLOW THROUGH BIOASSAYS

Chlorine

The survival-mortality characteristics of fingerling channel catfish to chlorine were examined and results shown in Table 2 and Figure 1. A flow through bioassay compared to a static bioassay results in a lower 96 hour LC_{50}, 0.07 mg/l chlorine vs. 0.45 mg/l chlorine. The chlorine concentration at which 50% of the fish survived was read directly from Figure 1. A flow through or continuous flow bioassay is a more accurate measurement for 96 hour LC_{50} of a highly reacting toxicant like chlorine (10). The chlorine concentrations in Figure 1 are based on the measured amounts of chlorine added to the inflow and do not represent concentrations during tests with fish. In this report it is shown that the gills of catfish rapidly remove chlorine from the medium. Thus the chlorine concentrations shown are levels that would be representative at treatment outfalls. Samples taken from receiving waters should show chlorine concentrations significantly lower. Based on our 96 hour LC_{50} of 0.07 mg/l chlorine and using the "Aquatic Life Water Quality Criteria" (14) of 1/10 the 96 hour LC_{50}, the final concentration of chlorine in the receiving waters should not exceed 0.007 mg/l. It should be noted that this concentration is below the limits of accurate analyses (11).

Ozone

The flow through bioassay was also used for ozone and the survival-mortality characteristics of fingerling catfish were examined and results are in Table 3 and Figure 2. Ozone proved to be more toxic to fingerling channel catfish than chlorine. The 96 hour LC_{50} for ozone, read directly from Figure 2 is 0.03 mg/l. As in the case of chlorine, the ozone concentrations are based on the amounts of ozone added to the water without fish in the system. Because ozone is highly reactive, it was rapidly decomposed with the presence of organics (fish).

In the presence of fish,ozone could not be detected in the bioassay waters due to the insensitivity of the lower limits of

TABLE 2. NINETY-SIX HOUR LC_{50} BIOASSAY DATA FOR ICTALURUS PUNCTATUS

Initial Chlorine Concen. (mg/l)	STATIC BIOASSAY		FLOW THROUGH BIOASSAY	
	Average % Kill for 3 duplicate experiments	Time Corresponding to average % Kill (hours)	Average % Kill for 2 duplicate experiments	Time Corresponding to average % Kill (hours)
2.0	100%	2-3	--	-
1.0	100	4-8	100%	24
.9	100	36	100	24
.8	100	48	100	24
.7	100	60	100	48
.6	100	60	100	48
.5	63	96	100	72
.4	37	96	100	72
.3	30	96	-	-
.2	13	96	100	72
.1	10	96	95	96
.08	-	-	75	96
.05	-	-	5	96
.03	-	-	0	96
0	0	96	0	96

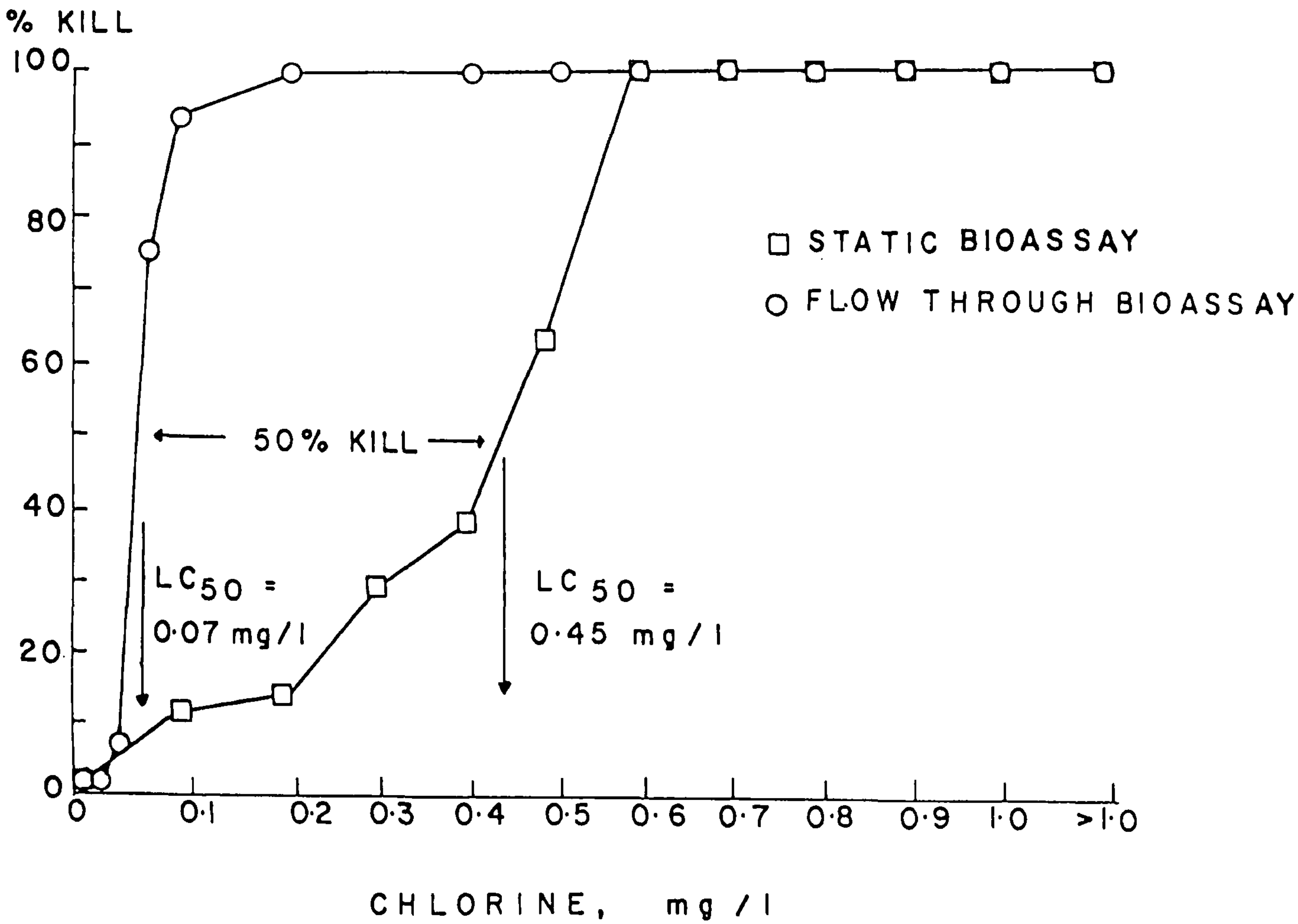

Figure 1. A comparison of survival-mortality characteristics of fingerling catfish to chlorine exposure in a static bioassay and in a flow through bioassay.

TABLE 3. NINETY-SIX HOUR LC_{50} FLOW THROUGH BIOASSAY DATA OF OZONE FOR <u>ICTALURUS</u> <u>PUNCTATUS</u>

Initial Ozone concentration (mg/l)	Percent kill for three duplicate experiments			Average	Time corresponding to average % kill (hours)
	expt. 1	expt. 2	expt. 3		
.20	100	100	100	100	24
.18	100	100	100	100	24
.16	100	100	100	100	24
.14	100	100	100	100	24
.13	100	100	100	100	36-48
.12	80	100	100	93	96
.10	60	100	100	86	96
.05	60	80	80	73	96
.04	60	60	80	66	96
.018	0	0	0	0	96
0	0	0	0	0	96

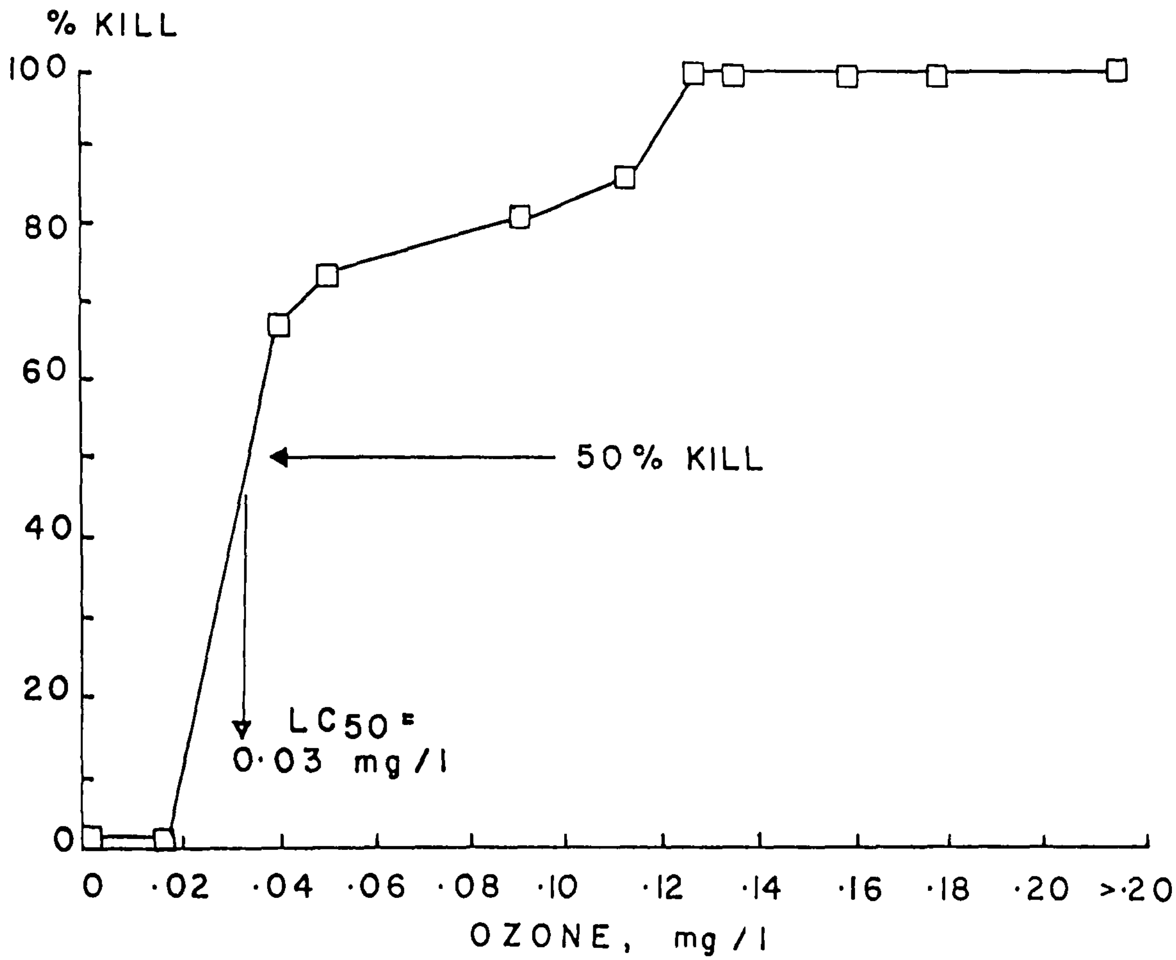

Figure 2. Survival-mortality characteristics of fingerling catfish to ozone exposure in a flow through bioassay.

the analytical technique. However, the presence of ozone was detectable by its characteristic odor. At the lower level ozone concentrations were estimated from the oxygen flow rates (mg/l O_3: l/min O_2). Based on acceptable water quality criteria (1/10, 96 hour LC_{50}), if ozone can be detected either by analytical techniques or by its odor (detectable odor 0.02-0.05 ppm, Feldstein, 15), it can be assumed that its concentration exceeds a safe environmental limit.

PHYSIOLOGICAL OBSERVATIONS

The results of the bioassay experiments established a reference point for the physiological evaluation of the effects of chlorine and ozone exposure on adult fish. Using the LC_{50} as a guide, adult catfish (0.25-2 kg) were exposed to various levels of chlorine and ozone and monitored for those physiological changes which were considered most important. Parameters monitored included: kidney functions (ion excretion, tubular water reabsorption, glomerular filtration rate), gill sodium uptake, blood pressure and heart rate.

Kidney Function

Chlorine -- The use of plasma insulin clearance as a measure of kidney glomerular filtration rate in mammals and fish is well established (16,17). All of the calculations performed on kidney function parameters were done according to Pitts (16). Figures 3 and 4 show typical glomerular filtration rates and urine flows for two different fish, both before and after the addition of chlorine. In these two experiments chlorine was added to the 10 liter reactor 10 hours after the start of the experiment. The concentration of chlorine added was 0.4 mg/l in the experiment shown in Figure 3, and 0.2 mg/l in the experiment shown in Figure 4.

A very high correlation between glomerular filtration rate and urine flow is evident in Figures 2 and 3. This strong correlation indicates that tubular water reabsorption is highly regulated. There is also a significant fluctuation of urine flow and glomerular filtration rate with time. This suggests that there is a high variation in the permeability of water through the gill and body surfaces of the catfish. A corresponding variability in urine ion concentration (Figure 5) and rate of ion excretion (Figure 6) is also observed through time.

After carefully observing the renal function of catfish, before and after exposure to chlorine, it is concluded that exposure of this fish to toxic concentrations of chlorine does not markedly affect flow rate of urine, urine ion concentration, or glomerular filtration rate.

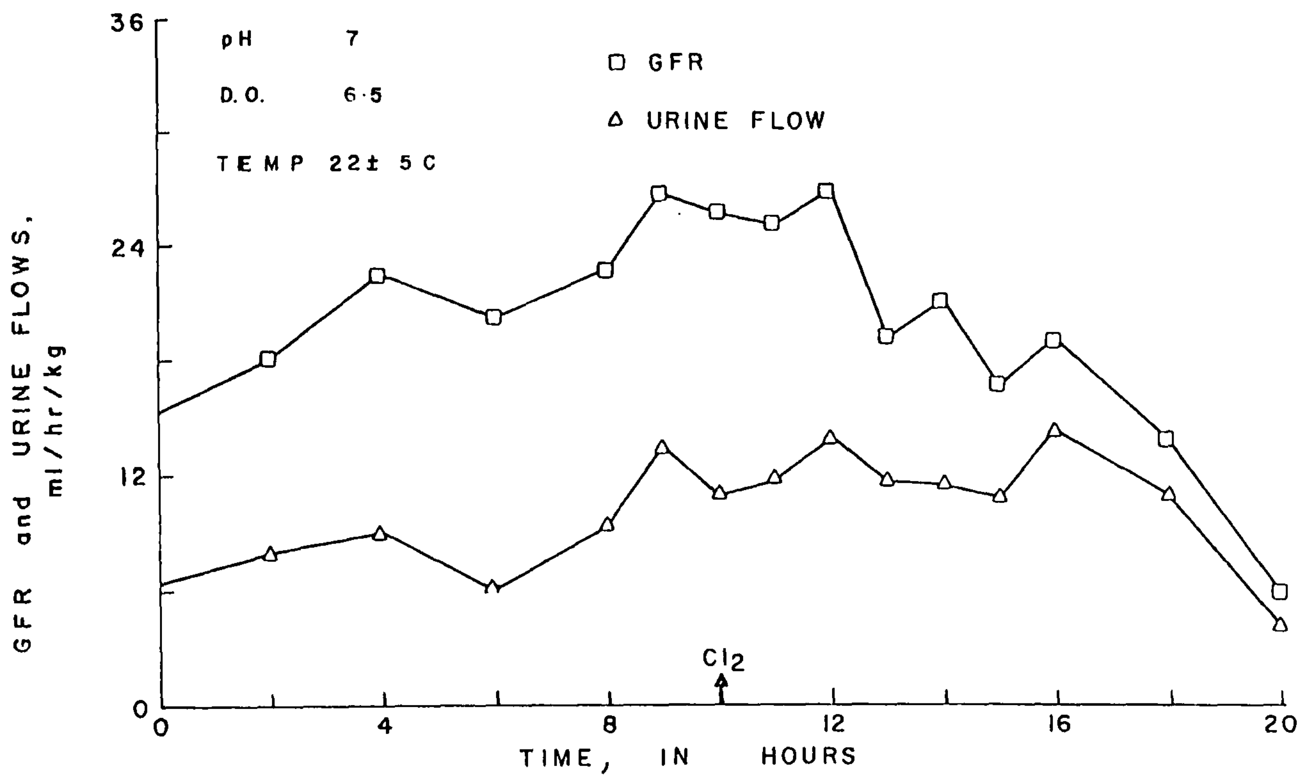

Figure 3. Effect of short term chlorine exposure on the glomerular filtration rate (GFR) and corresponding urine flow of Ictalurus punctatus (Fish #5 wt. 1.155 kg, and 0.4 mg/l of chlorine added 10 hours after start of experiment).

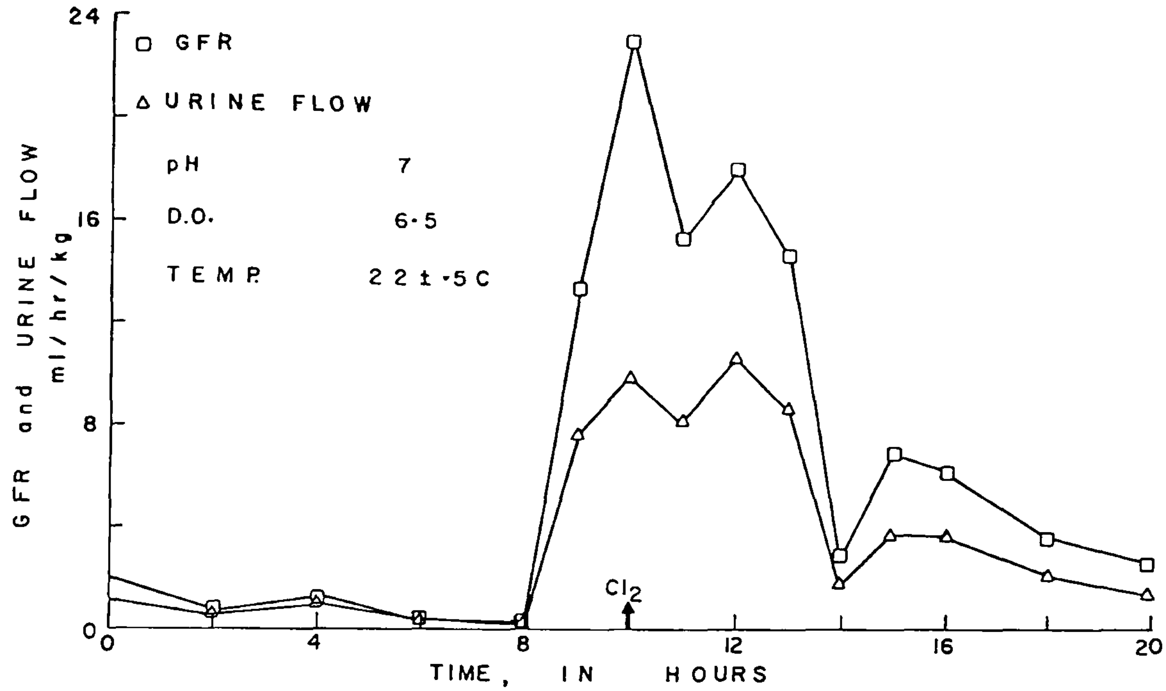

Figure 4. Effect of short term chlorine exposure on the glomerular filtration rate (GFR) and corresponding urine flow of *Ictalurus punctatus* (Fish #16, wt. 0.368 kg, and 0.2 mg/l of chlorine added 10 hours after the start of the experiment).

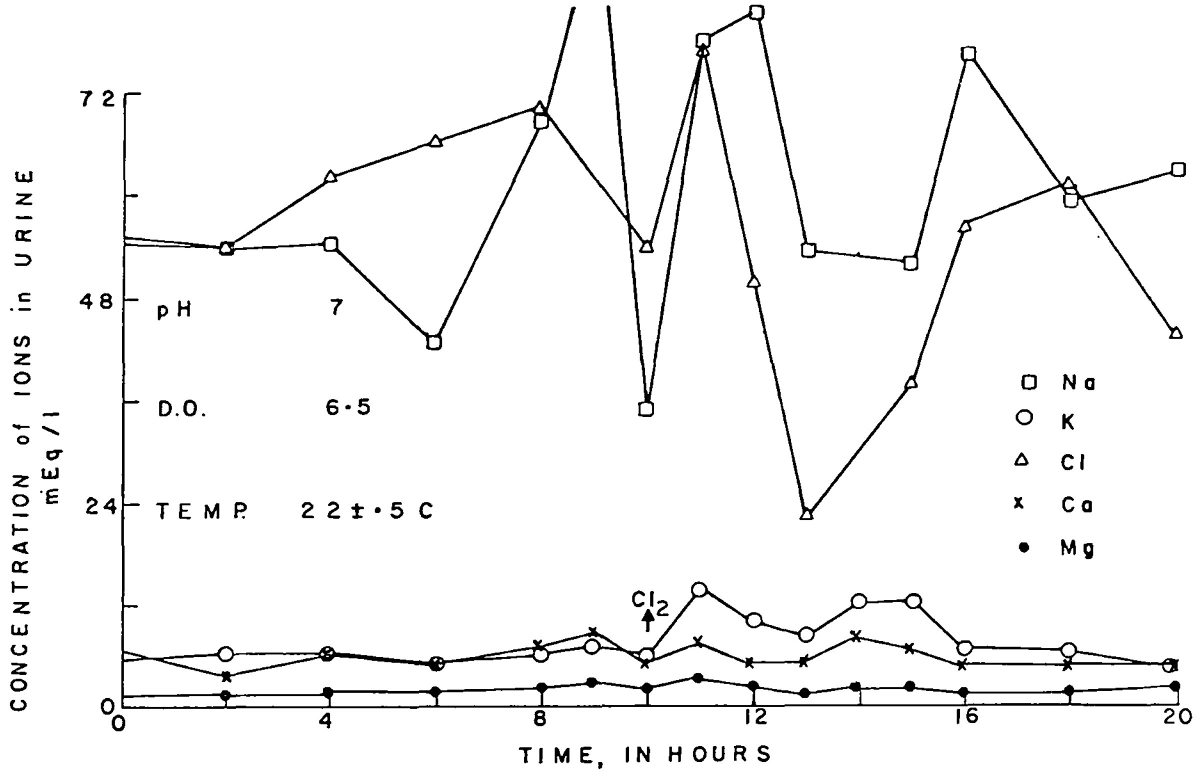

Figure 5. Effect of short term chlorine exposure on the concentration of ions in the urine of <u>Ictalurus punctatus</u> (Fish #16, wt. 0.368 kg and 0.2 mg/l of chlorine added 10 hours after the start of experiment).

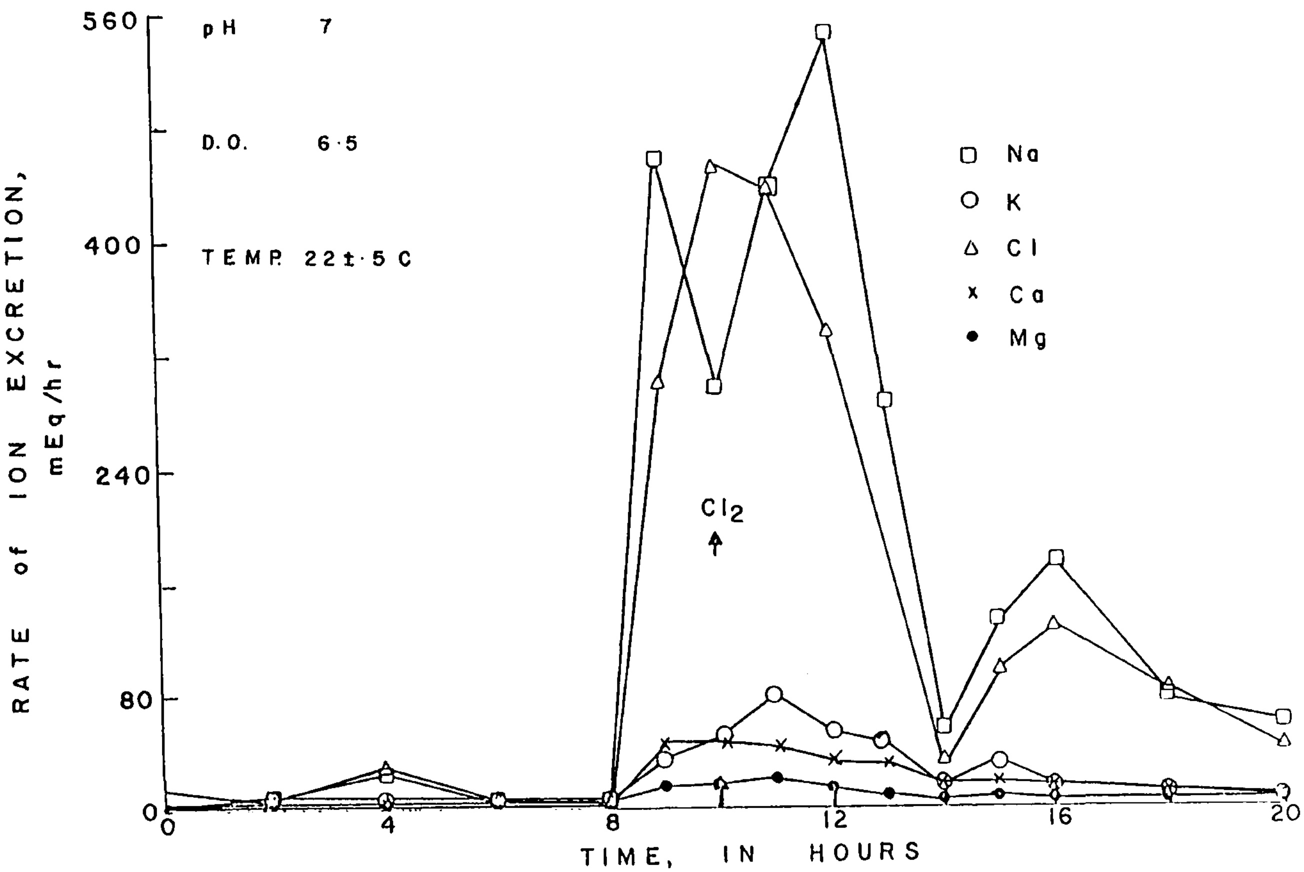

Figure 6. Effect of short term chlorine exposure on the rate of excretion of ions in the urine of *Ictalurus punctatus* (Fish #16, wt. 0.368 kg, and 0.2 mg/l of chlorine added 10 hours after the start of the experiment).

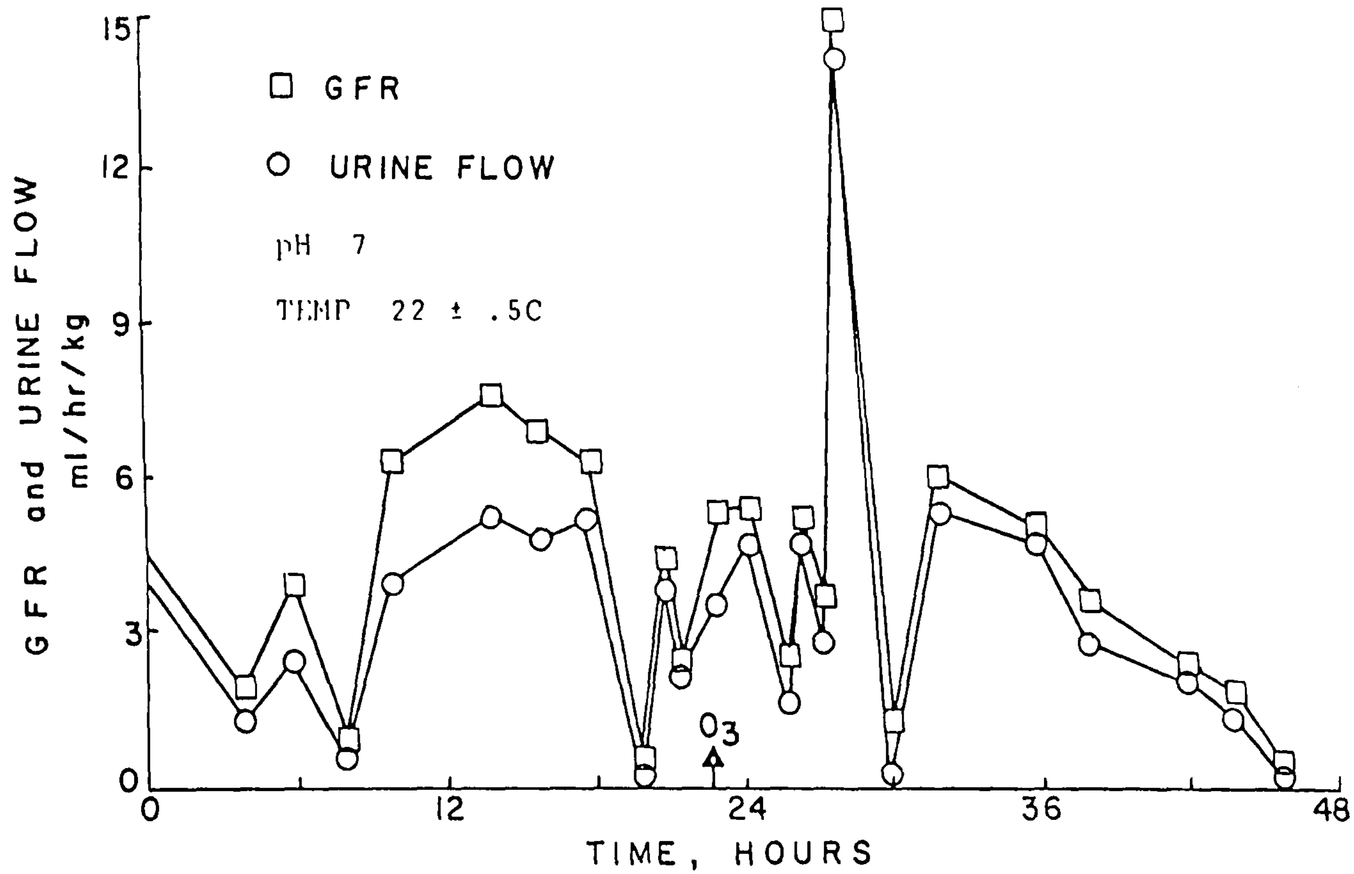

Figure 7. Effect of short term ozone exposure on the glomerular filtration rate and corresponding urine flow of *Ictalurus punctatus* (fish #37, wt. 1.102 kg, 0.36 mg/l ozone).

Ozone -- In these three experiments ozone was added by aeration to the 10 liter flow through system. Figures 7 and 8 show the experiment where concentration of ozone added was 0.36 mg/l. Similar results were obtained using ozone concentrations of 1.8 and 0.9 mg/l. The length of exposure was from 12 to 24 hours at concentrations well above the 96 hour LC_{50}.

A very high correlation between glomerular filtration rate and urine flow is evident in Figure 7. This strong correlation indicates that tubular water reabsorption is highly regulated and that there is also a significant fluctuation of urine flow and glomerular filtration rate with time. This suggests that there is a high variation in the permeability of water through the gills and body surfaces of catfish. A corresponding variability in urine ion concentration (Figure 8) is also observed.

After carefully observing the renal functions of channel catfish before and after exposure to ozone, it is concluded that short term exposure of this fish to toxic concentrations of ozone does not affect urine volume, urine ion concentration or glomerular filtration rate. However, these results do not preclude the possibility of long term ozone exposure affecting renal function.

Gill Function

Uptake of Chlorine by Gills -- Chlorine is rapidly removed from aqueous solution by the gills of fish. This occurrence was deduced from the experimental data presented in Figure 9. In these studies, a different concentration of chlorine (1.5-10 mg/l) was added to the circulating system for each fish. The concentration of chlorine was measured initially and at 5-10 minute intervals until the chlorine had disappeared. The experimental system contained 40 liters of water, in which only slight loss of chlorine was noted when no fish were present (Line A in Figure 9). Similarly, only a small amount of chlorine was removed when an anesthetized fish with no opercular movements was placed in holding tank (Line B in Figure 9). The near coincidence of Lines A and B in Figure 9 indicates that the body surface of the fish does not react markedly with chlorine. However, when a normal respiring fish is present in the system and chlorine is introduced, dramatic changes occur. The fish immediately reacts by significantly increasing opercular pumping rate. Periodically, at this time the fish appears to "cough" in an apparent attempt to clear the gills. Also, the rate of removal of chlorine in the presence of respiring fish (upper four lines in Figure 9) is markedly higher than that for an anesthetized fish. The average rate of uptake of chlorine for a respiring fish is relatively constant at 0.4 mg chlorine per minute per kg of fish and is valid over a wide range of fish sizes (0.37-1.65 kg). The obvious conclusion from the experimental data shown in Figure 9 is that chlorine is rapidly removed by the gills of fish.

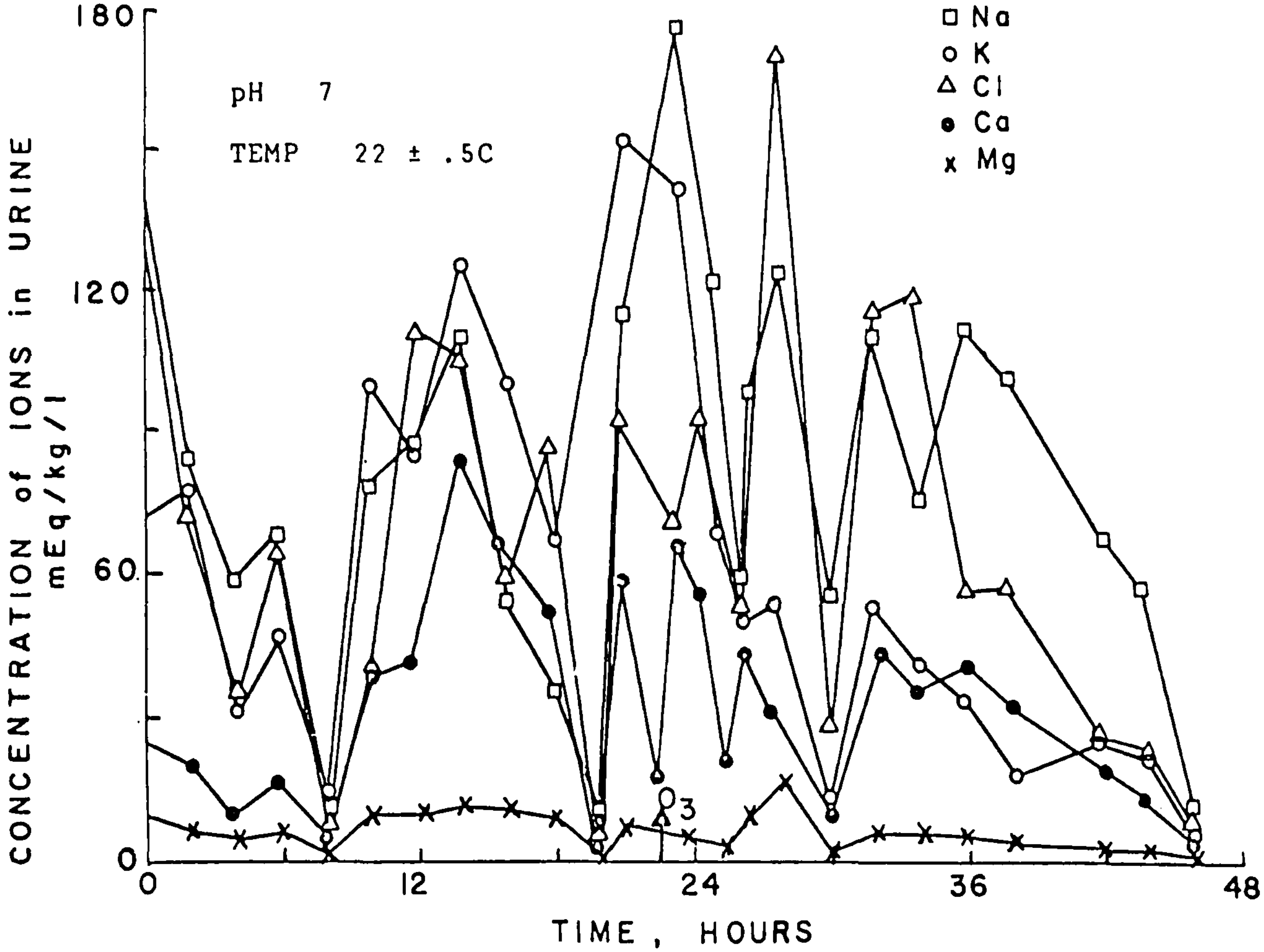

Figure 8. Effect of short term ozone exposure on the rate of excretion of ions in the urine of _Ictalurus punctatus_ (fish #37, wt. 1.102 kg, 0.36 mg/l ozone)

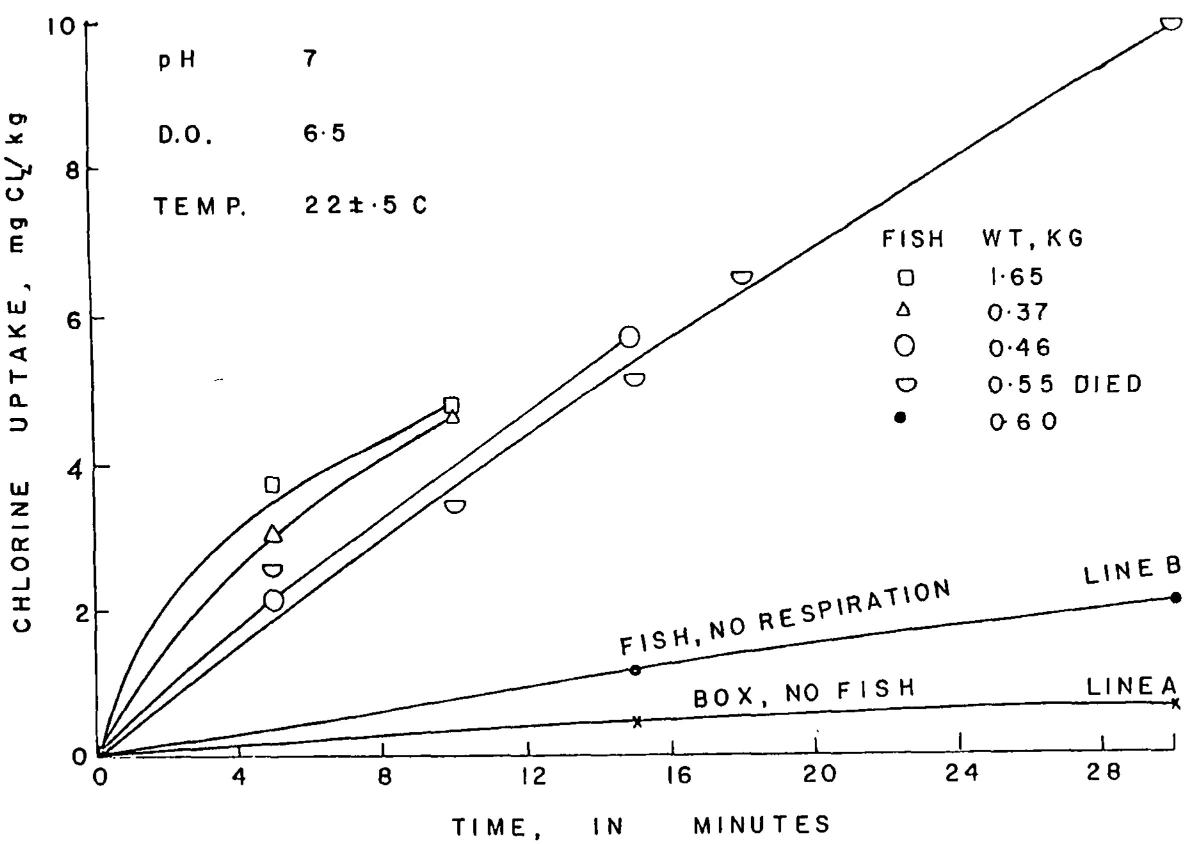

Figure 9. Chlorine uptake by five catfish. Four respiring fish had uptake rates averaging 0.4 mg Cl/min/kg. An anesthetized fish (no opercular movements) had an upatke rate of 0.05 mg Cl/min/kg. Loss of chlorine from the experimental system with no fish present was negligible.

Gill Sodium Transport

Chlorine -- The influence of chlorine exposure on the uptake of ^{22}Na by gills of fish is shown in Figure 10. The disappearance of ^{22}Na from aqueous phase (ordinate axis in Figure 10) is a measure of the removal of ^{22}Na by the gills of fish. The addition of 0.1 mg/l of chlorine at the start of the experiment markdely reduced the ability of the gills to remove sodium from aqueous solution. This reduction in uptake rate following chlorine exposure suggests an impairment of normal physiological function in the sodium transport system.

Ozone --The influence of ozone exposure on the uptake of ^{22}Na by the gills of fish is shown in Figure 11. The disappearance of ^{22}Na from the aqueous compartment is a measure of the removal of ^{22}Na by the gills of the fish. Phase I of the experiment utilized the fish as its own control. A one day recovery period followed. The addition of 0.1 mg/l ozone in the second phase of the experiment markedly reduced the ability of the gills to remove sodium from the aqueous solution. This reduction in uptake following ozone exposure suggests an impairment of normal physiological functions in the sodium transport system of the gill epithelium.

Heart Rate and Blood Pressure

Single Dose Exposure to Chlorine -- The effect of short term exposure to chlorine on the heart rate and blood pressure of adult channel catfish was determined. Blood pressure was continuously recorded prior to, and following, the introduction of chlorine into the 10 liter recirculating system. Figure 12 demonstrates the temporal changes in dorsal aortic blood pressure and heart rate after exposure to 1 mg/l of chlorine, while Figure 13 illustrates the immediate response in the above two parameters after exposure to various concentrations of chlorine. In both experiments, each fish had an initial mean blood pressure of 23 mm Hg, a pulse pressure of 2 mm Hg and a heart rate of 78 to 84 beats per minute. Measureable variations and fluctuations in blood pressure were recorded prior to the addition of chlorine. These variations may be due partially to opercular movements and changes in gill blood vessel resistance. Swimming movements also significantly alter dorsal aorta blood pressure.

The introduction of a single dose of chlorine to the experimental system caused an immediate drop in blood pressure. The mean blood pressure dropped from a pretreatment level of 23 mm Hg to a low of 10 mm Hg between one to five minutes after chlorine was added. Blood pressure did not return to pretreatment levels in the 30 minute period following initial exposure. Generally the recovery time and change in blood pressure was increased with increasing chlorine concentration. It was also observed that rhythmic changes in mean blood pressure correlated

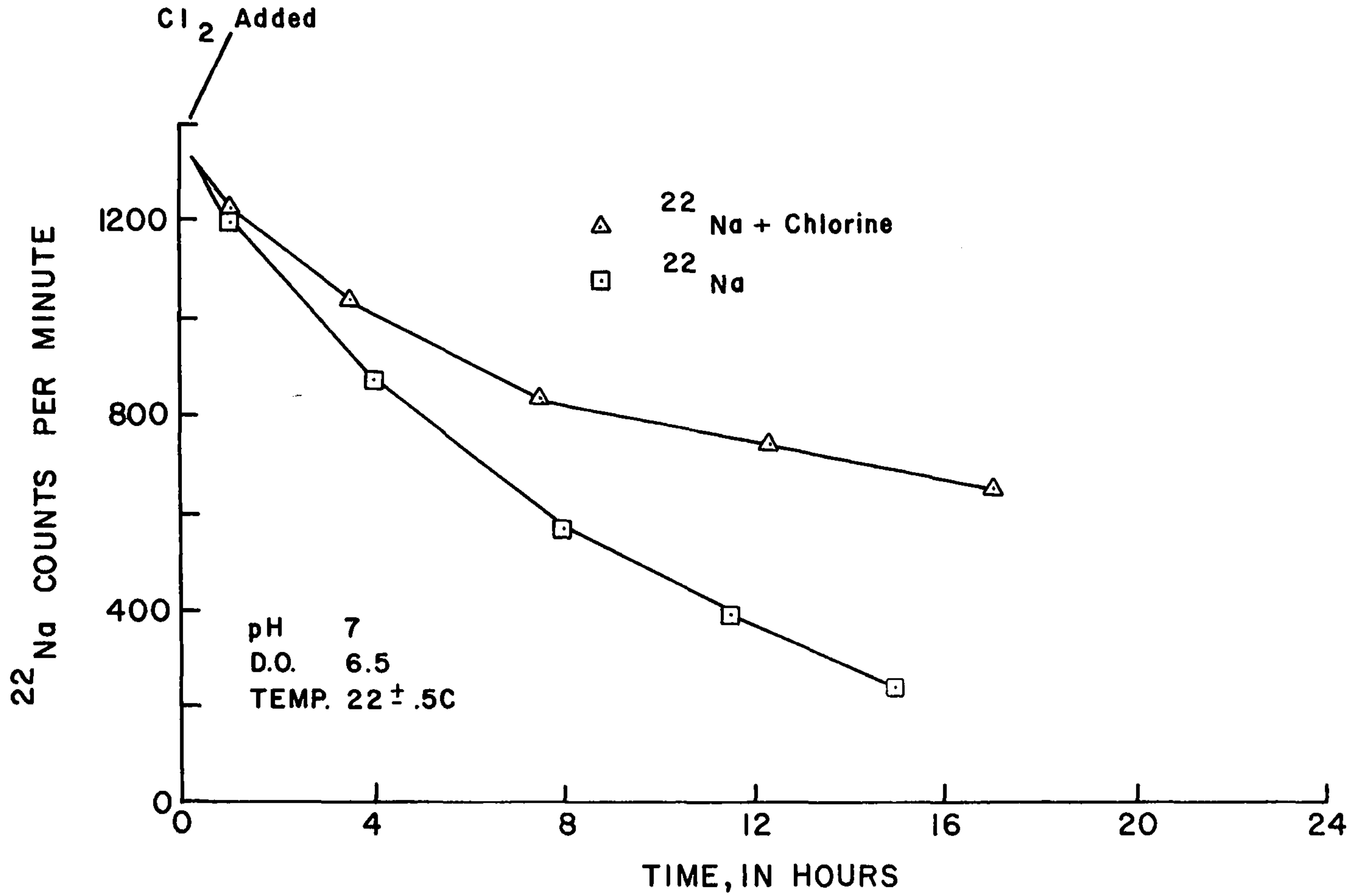

Figure 10. Effect of short term chlorine exposure on the uptake of ^{22}Na by the gills of <u>Ictalurus punctatus</u> (Fish #34, wt. 1.119 kg, and 0.1 mg/ℓ of chlorine added at start of experiment).

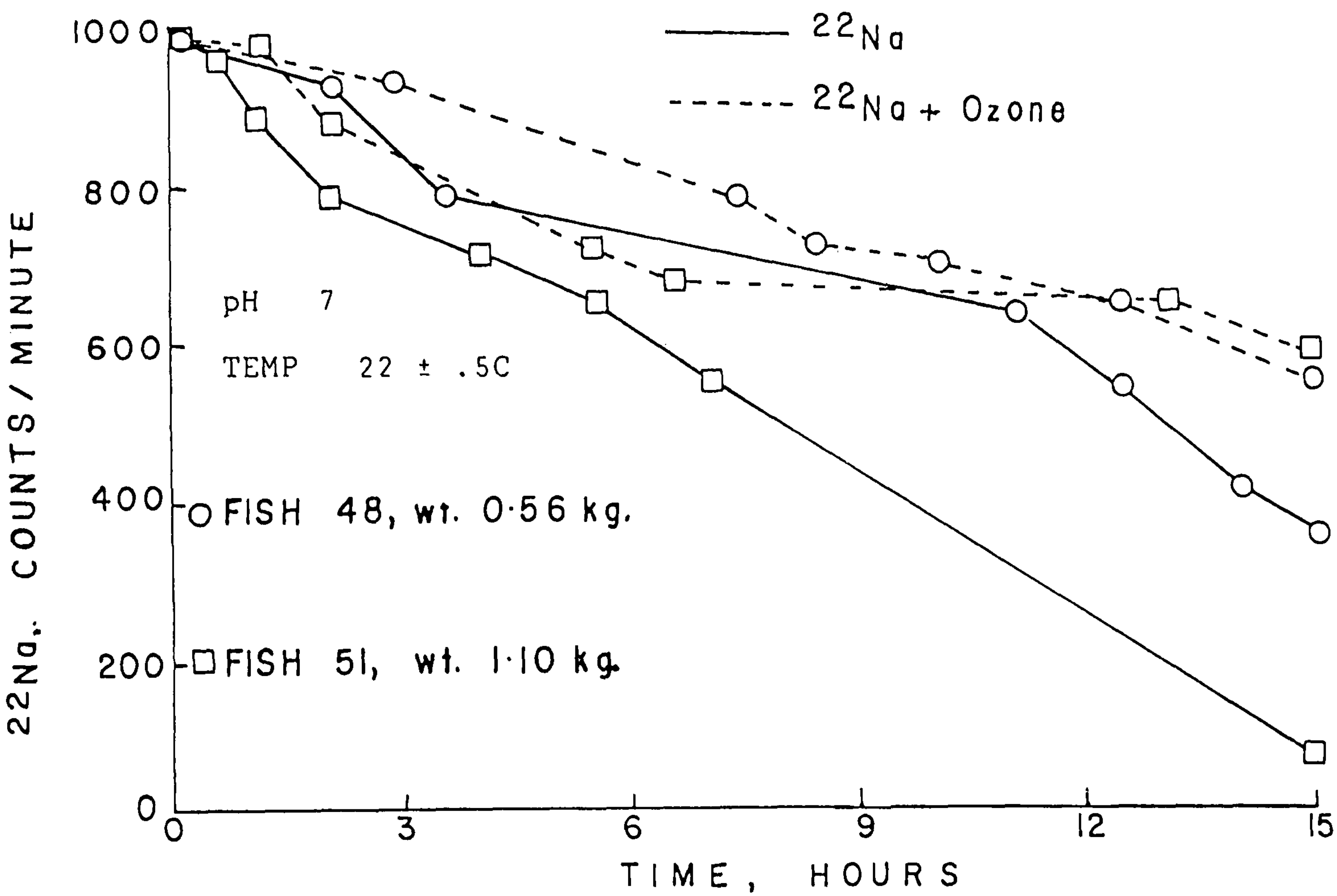

Figure 11. Effect of short term ozone exposure on the uptake of ^{22}Na by the gills of <u>Ictalurus punctatus</u> (0.1 mg/l ozone).

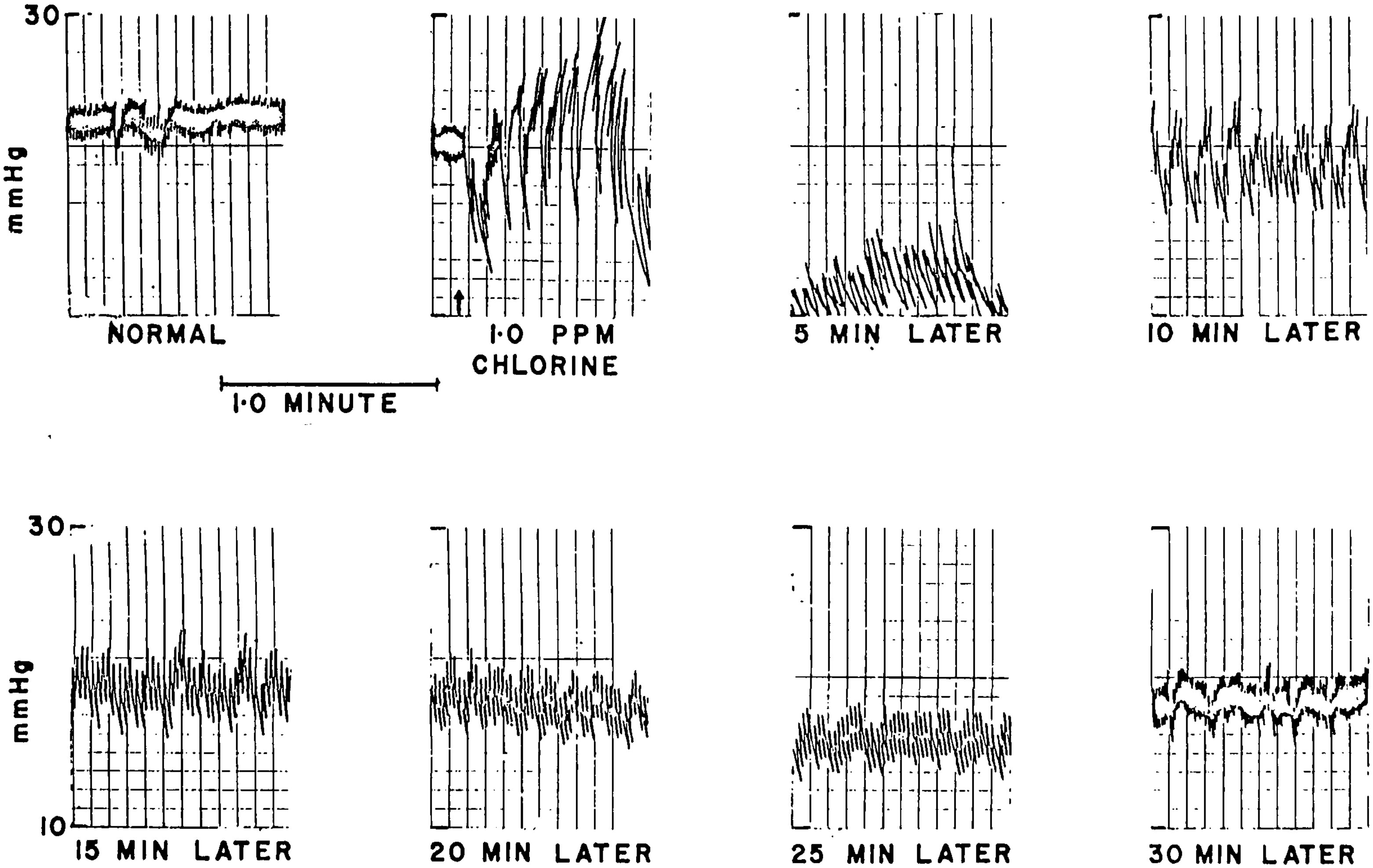

Figure 12. Influence of short term chlorine exposure on the heart rate and blood pressure of Ictalurus punctatus (fish #26, wt. 1.036 kg, and 1.0 mg/l of Cl_2 added at start of experiment).

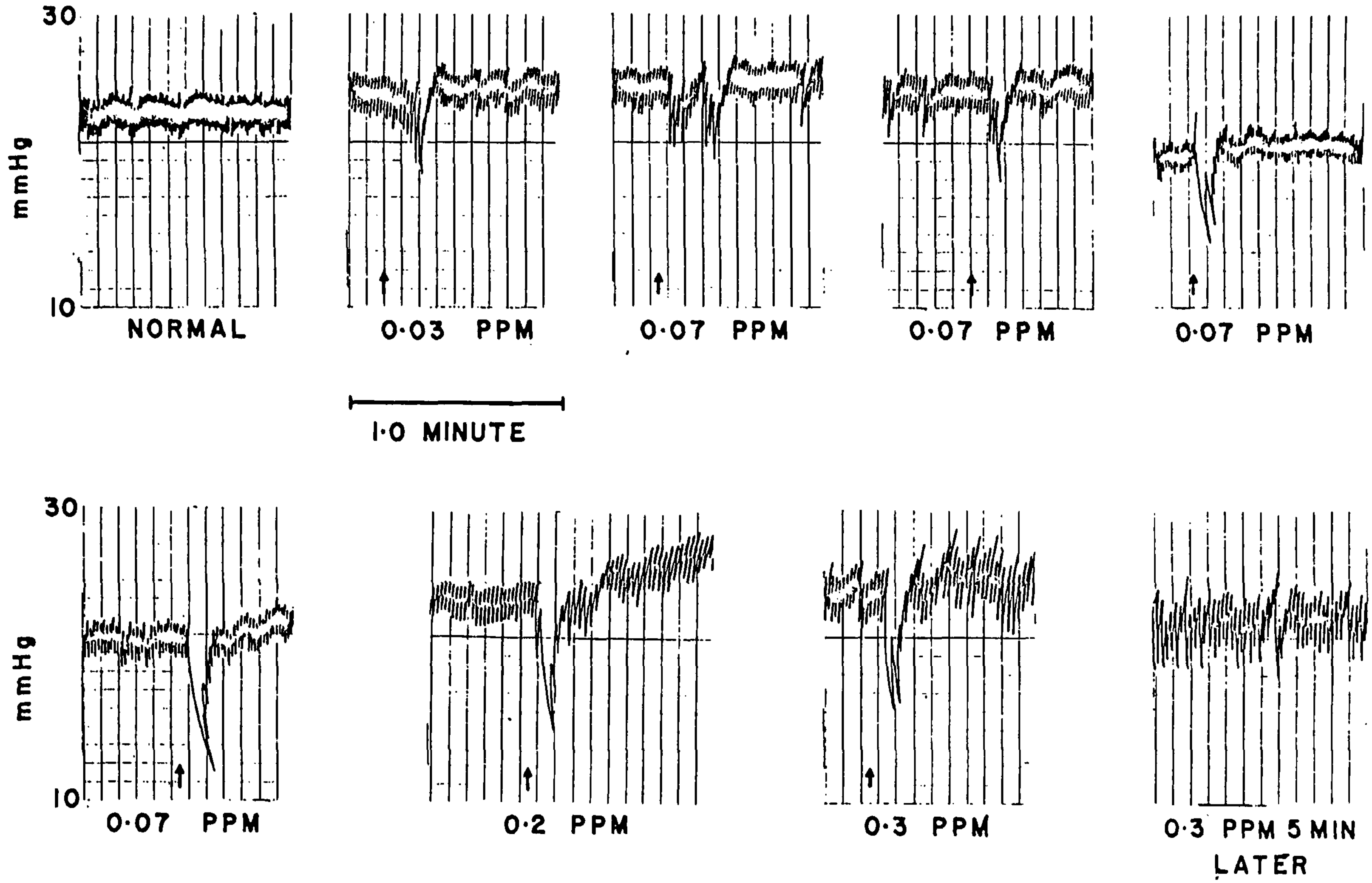

Figure 13. Heart rate and blood pressure of *Ictalurus punctatus* immediately following exposure to different concentrations of chlorine (fish #30, wt. 0.985 kg).

with opercular movements. Severe opercular movements were associated with drastic fluctuations in mean pressure.

The heart rate of the fish was also affected by short term exposure to chlorine. Heart rate declined from a normal value of 78 beats/min to 42 beats/min, 20 minutes after initial exposure.

The most significant physiological effect of single dose chlorine exposure were changes in dorsal aortic blood pressure. Figure 14 summarizes the changes in blood pressure and heart rate following exposure to 1.0 mg/l of chlorine. The change in blood pressure is dramatic (vagal inhibition) and the time for recovery increases with increasing chlorine exposure.

Continuous Exposure to Chlorine -- The effect of long term exposure to low levels of chlorine on the heart rate and blood pressure of adult channel catfish was determined. Blood pressure was continually recorded prior to and after introduction of chlorine to the flow through system. Figure 15 demonstrates the change in dorsal aortic pressure and heart rate after a 7.5 hour exposure to 0.22 mg/l chlorine. Figure 16 demonstrates the change in dorsal aortic blood pressure and heart rate after a 5 hour exposure to 0.17 mg/l chlorine.

Fish #38, Figure 15, had an initial mean blood pressure of 27 mm Hg, a pulse pressure of 3 mm Hg, and a heart rate of 55-60 beats per minute. The introduction of 0.22 mg/l chlorine caused an immediate drop in mean blood pressure, from 27 mm Hg to 17 mm Hg, an initial decrease in heart rate of 50% but an increase in the pulse pressure. The initial response is probably due to vagal inhibition. During the 7.5 hours of chlorine exposure blood pressure dropped to 16 mm Hg, pulse pressure to 2 mm Hg and heart rate decreased to 40-45 beats per minute.

Fish #39, Figure 16, had an initial mean blood pressure of 27 mm Hg, a pulse pressure of 2 mm Hg, and a heart rate of 65-70 beats per minute. The introduction of 0.17 mg/l chlorine caused a gradual drop in blood pressure from 27 mm Hg to 20 mm Hg. There was no apparent change in pulse pressure or heart rate. During 5 hours of chlorine exposure, blood pressure dropped to 21 mm Hg, pulse pressure dropped to 1 mm Hg and heart rate decreased to 55-60 beats per minute.

Figure 17 is a condensed summary of blood pressure fluctuations (Fish #38 and Fish #39) due to chlorine exposure over an 8 hour period.

Low Level Chlorine Exposure -- The exposure of fish to chlorine at levels approaching the 96 hour LC_{50} was immediately detected by the pronounced drop in blood pressure (Figure 18). Continued exposure for 5 hours resulted in an increase in heart

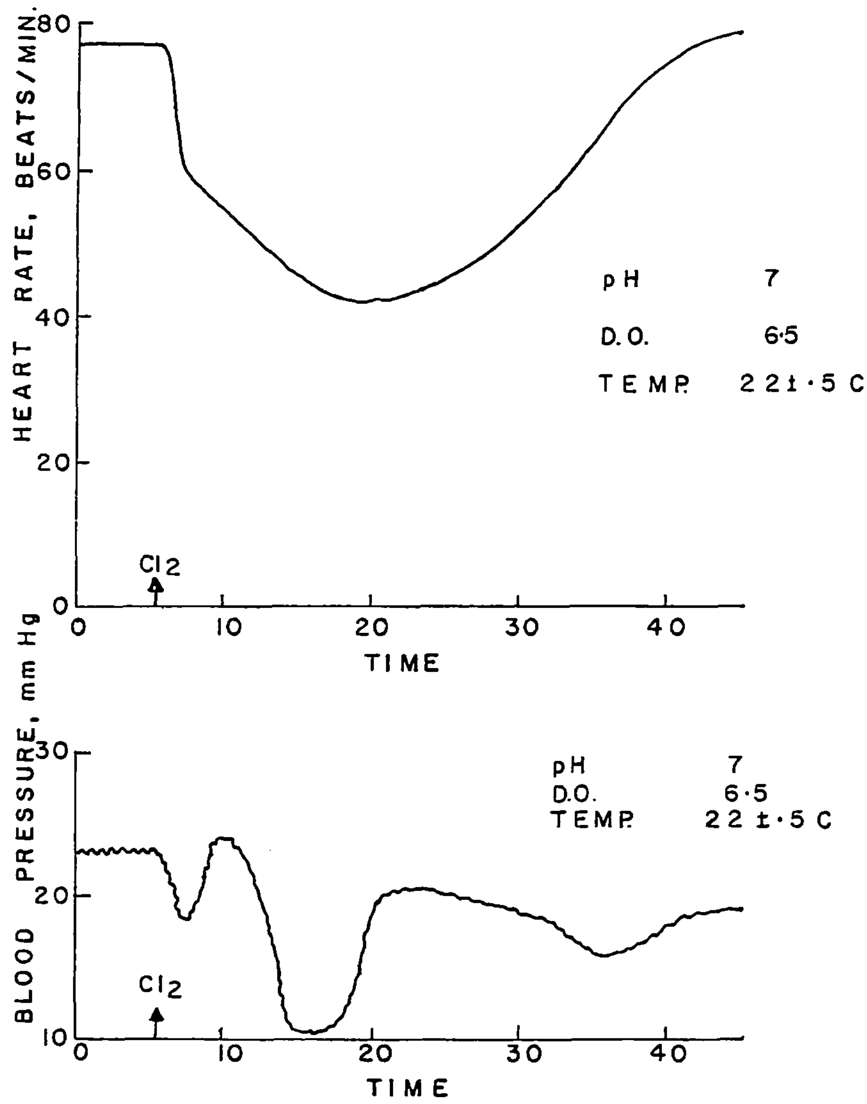

Figure 14. Summary of changes in blood pressure and heart rate following exposure to 1.0 mg/l of chlorine (Fish #26, wt. 1.036 kg.).

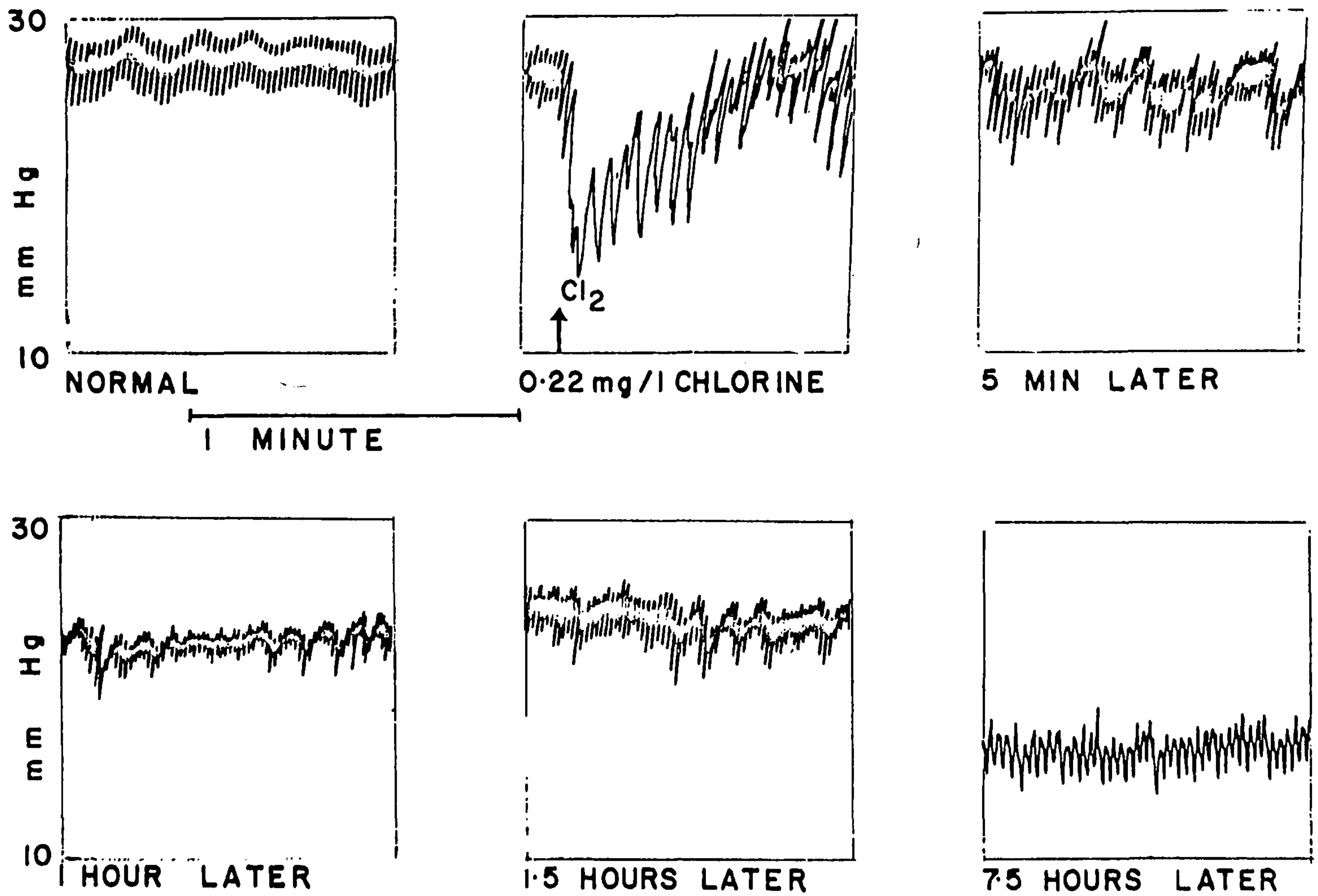

Figure 15. Influence of long term chlorine exposure on the blood pressure and heart rate of _Ictalurus punctatus_ (fish #38, wt. 0.832 kg).

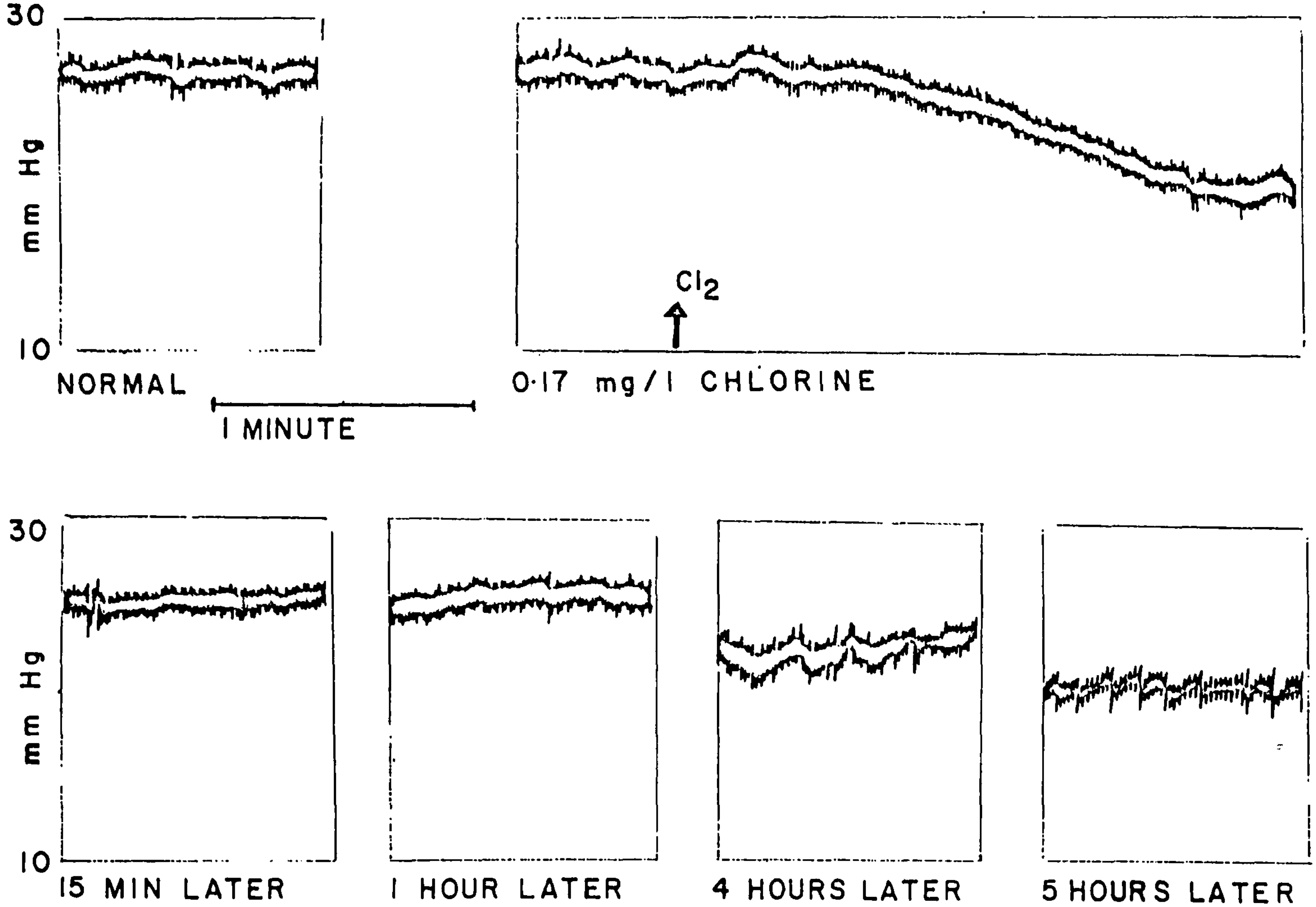

Figure 16. Influence of long term chlorine exposure on the blood pressure and heart rate of *Ictalurus punctatus* (fish #39, wt. 0.854 kg).

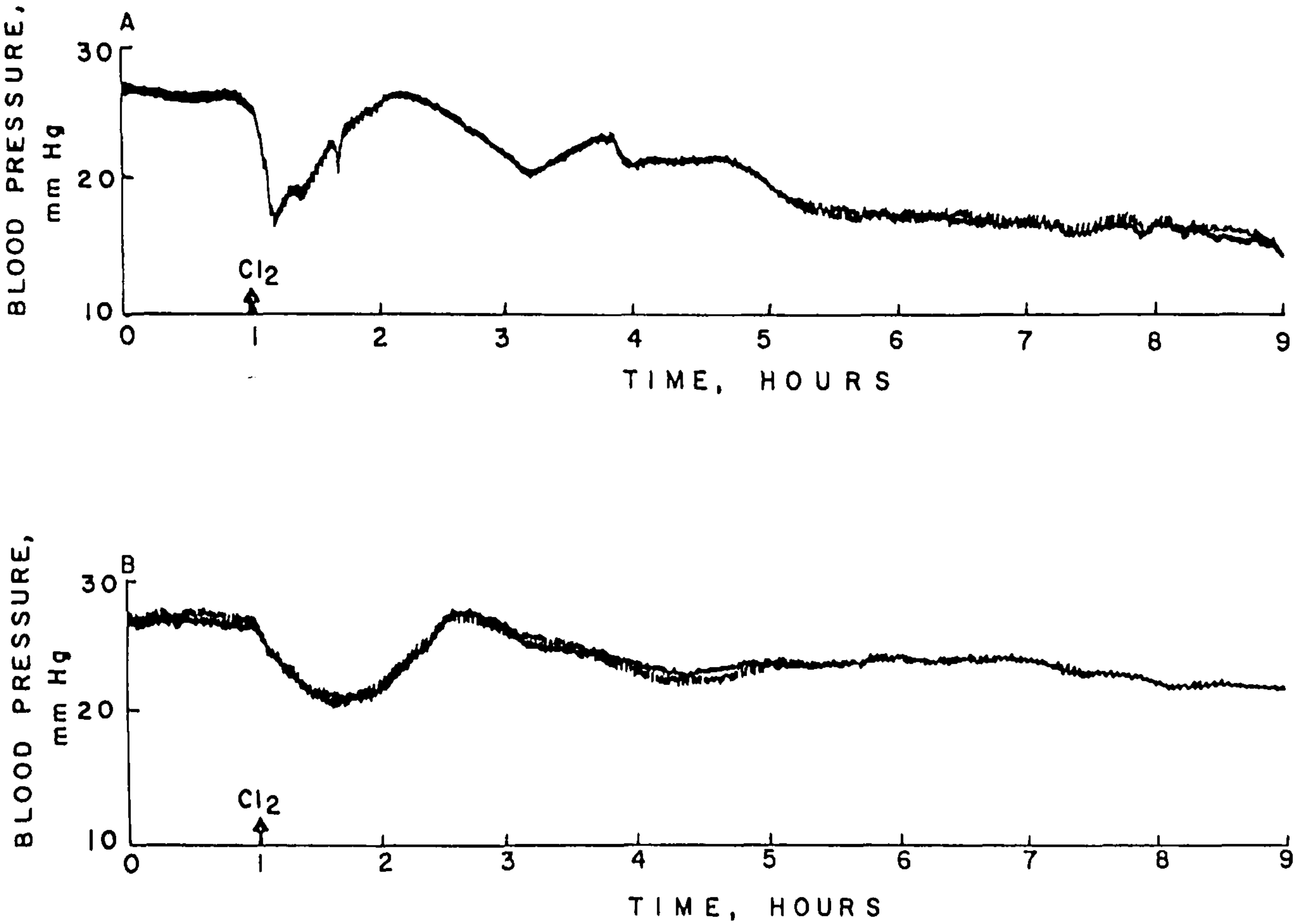

Figure 17. Summary of changes in blood pressure following long term exposure to chlorine (A- fish #38, wt. 0.823 kg, 0.22 mg/l chlorine, B- fish #39, wt. 0.854 kg, 0.17 mg/l chlorine).

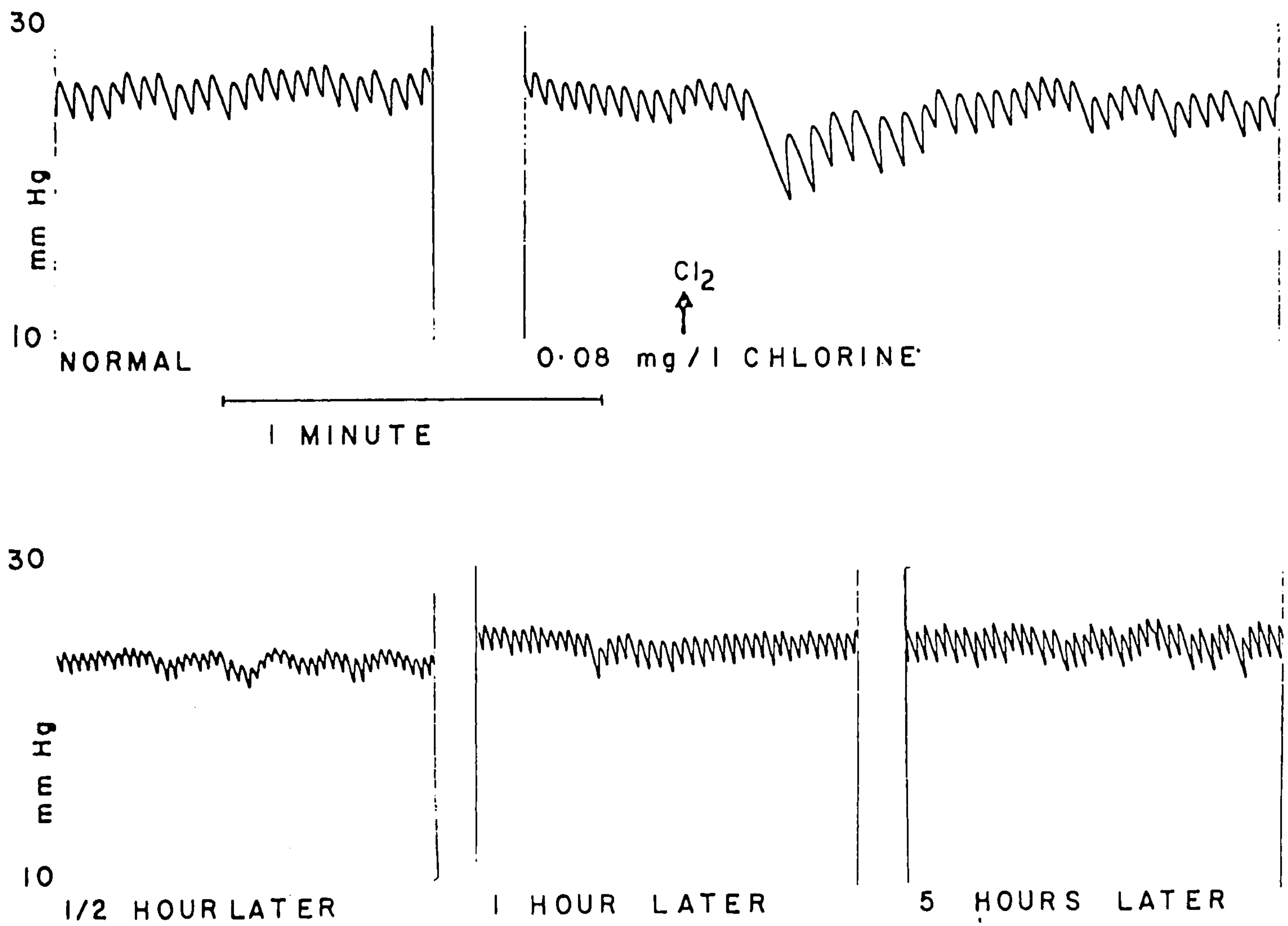

Figure 18. The influence of chlorine at a dose approximating the 96 hour LC_{50} on heart rate and blood pressure of *Ictalurus punctatus* (fish #54, wt. 1.544 kg.)

rate from 22 beats/minute to 45 beats/minute. At an exposure of 0.03 mg/l chlorine there was no initial reaction shown by blood pressure or heart rate. After two hours the fish appeared normal with no apparent disfunction (Figure 19). Thus it can be concluded that on exposure to a chlorine concentration of 0.007 mg/l chlorine (1/10 of 96 hour LC_{50}) fish should show no physiological response and the environment should be safe for their survival.

Ozone

Continuous Exposure to Ozone -- Tests to determine the effects of ozone on blood pressure, pulse pressure and heart rate were run. Levels of ozone from 0.1 to 0.5 mg/l for 10 hours had no apparent effect on these parameters. This leads to the conclusion that ozone has no effect on gill vascular resistance. It was also noted that the pronounced vagal inhibitions associated with chlorine exposure did not occur with ozone. The absence of this drop in blood pressure normally attributed to vagal inhibitions induced by external stimuli leads to the conclusion that fish may be insensitive to the presence of ozone at low levels in the water and any avoidance reaction would not take place. Thus, it can be concluded that since ozone is so extremely toxic to fish and that they apparently lack the ability to sense its presence at low levels in their environment, the area immediately adjacent to treatment outfalls poses a threat. Measures should therefore be introduced to assure that no ozone reaches the aquatic environment.

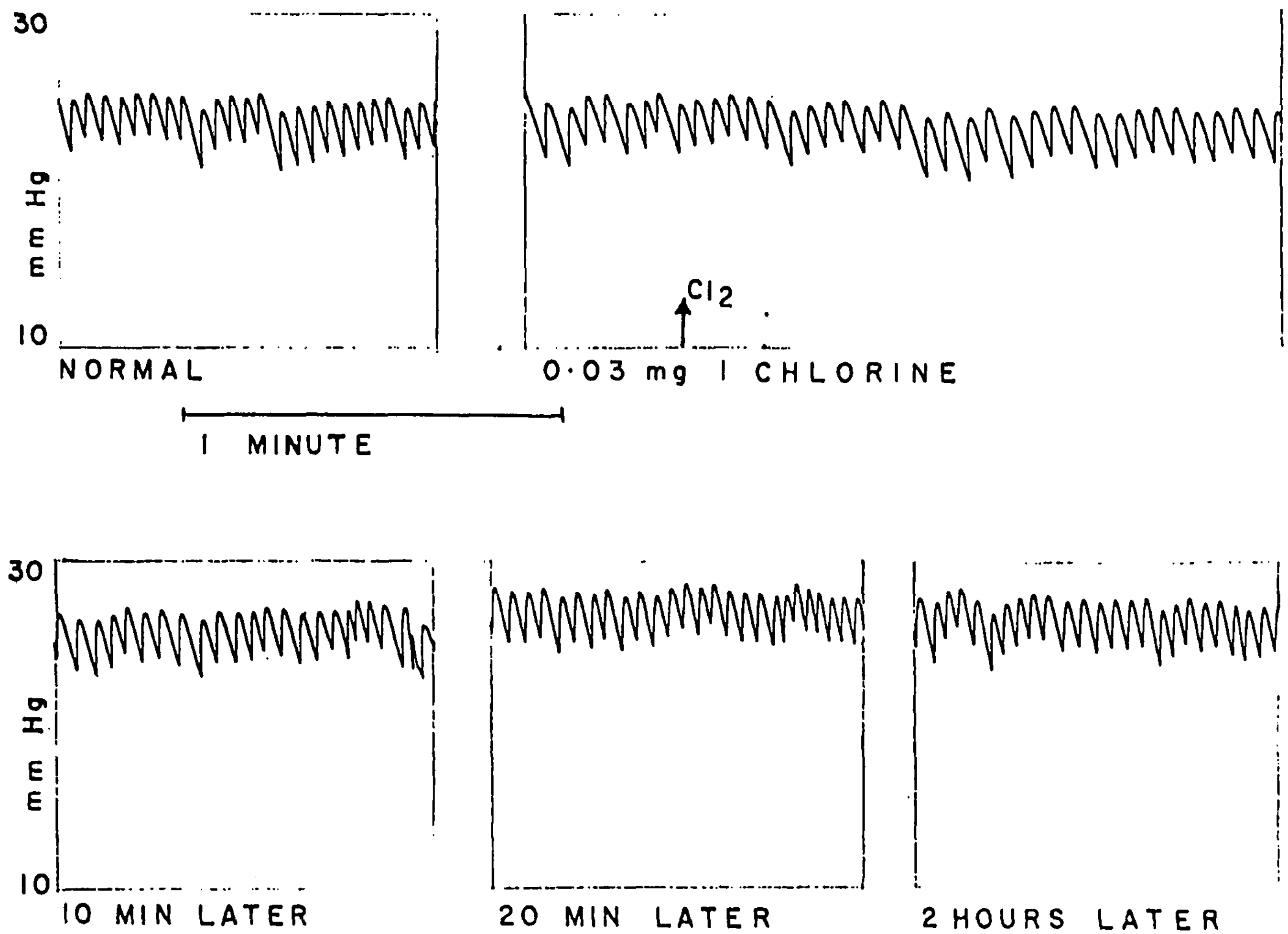

Figure 19. The influence of chlorine at a dose less than half of the 96 hour LC_{50} on heart rate and blood pressure in Ictalurus punctatus (fish #54 wt. 1.544 kg).

REFERENCES

1. Brungs, W.A. Effects of residual chlorine on aquatic life. Jour. Water Poll. Control Fed., 45, 2180-2193, 1973.

2. Tsai, C., "Effects of Chlorinated Sewage Effluents on Fish in Upper Patuxent River, Maryland." Chesapeake Sci. 9, 2, 83 (1968).

3. Arthur, J.W. and J.G. Eaton, "Chloramine Toxicity to the Amphipod, Gammarus Pseudolimnaeus, and the Fathead Minnow, Pimephales promelas" Journ. Fish. Res. Bd. Can., 28, 1841 (1971).

4. Becker, C.D. and T.O. Thatcher. Toxicity of Power Plant Chemicals to Aquatic Life. U.S. Atomic Energy Commission. Report, Wash-1249, UC-11, June 1973.

5. Hubbs, C.L. High Toxicity of Nascent Oxygen. Physiological Zoology 3(4):441-460, 1969.

6. MacLean, S.A., et al. The Effects of Ozone-treated Seawater on the Spawned, Fertilized, Meiotic, and Cleaving Eggs of the Commercial American Oyster. Mutation Research. 2:283-285, 1973.

7. Benoit, R.F., N.A. Matlin. Control of Saprolegnia on Eggs of Rainbow Trout (Salmo gairdneri) with ozone. Trans. Amer. Fish. Soc. 95(4):43-432, 1966

8. Spotte, S.H. Fish and Invertebrate Culture. Wiley-Interscience, Inc., New York. 1970.

9. Adger, S., Hawaiian Marine Imports, Houston, Texas. Personal communication.

10. Sprague, J.B. The ABC's of Pollution Bioassay using Fish. A paper presented at The Symposium on Environmental Monitoring, Los Angeles, California. June 27-28, 1972.

11. Standard Methods, 13th Edition. American Public Health Assoc., New York, N.Y. 1971.

12. Methods for Chemical Analysis of Water and Wastes. U.S. EPA Report No. EPA-625-/6-74-003, 1974.

13. Beckman Instruments, Inc. Fullerton, California. Bulletin 800-C.

14. Horton, R.K. Aquatic Life Water Quality Criteria. Environ. Sci. Technol, (11):888,1967.

15. Feldstein, M. "Air Pollution" in Handbook of Analytical Toxocology, ed. I. Sunshine. The Chemical Rubber Co. 708, 1969.

16. Pitts, R.F. Physiology of the Kidneys and Body Fluids. Amer. Public Health Asso., New York, N.Y. 1971.

17. Hickman, C.P., Jr., B.F. Trump. The Kidney, in Fish Physiology, Vol. 1, ed. W.S. Hoar, D.J. Randall, New York Academic Press. 91-239, 1969.

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16. ABSTRACT

To ensure adequate water quality for impoundments receiving disinfected wastewater in The Woodlands, Texas the following experiments were conducted. Using fingerling channel catfish, Ictalurus punctatus the 96 hour LC_{50} for chlorine is 0.07 mg/1 (total chlorine) and 0.03 mg/1 for ozone in flow through bioassays.

Chlorine and ozone exposures had little effect on kidney functions. Exposure to both chlorine and ozone drastically reduced the ability of the gills to actively absorb sodium from the water.

Long term exposure to chlorine drastically reduced both blood pressure and heart rate while exposure to ozone had little, if any, effect. Blood pressure and heart rate are very sensitive physiological parameters and changes are indicative of a stressful environment.

Both chlorine and ozone are extremely toxic to fish at low levels. If detected in receiving waters by present analytical techniques, a toxic condition exists.

17. KEY WORDS AND DOCUMENT ANALYSIS

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