CARDIAC, VENTILATORY AND METABOLIC RESPONSES OF TWO ECOLOGICALLY DISTINCT SPECIES OF FISH TO WATERBORNE CYANIDE

by

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Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Zoology

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May, 1986

Blacksburg, Virginia

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(ABSTRACT)

Changes in heart rate, ventilatory activity and oxygen consumption were determined in trout (Salmo gairdneri) and brown bullhead catfish (Ictalurus nebulosus) during exposure to a steadily increasing concentration of waterborne cyanide selected to produce death in 8-9 hours for each species. The lethal cyanide concentration for the bullheads was an order of magnitude higher than for trout. Trout developed an immediate and gradually increasing bradycardia throughout the exposure period. Cyanide produced tachycardia in the bullhead followed by a gradual onset of bradycardia as the concentration of cyanide was raised. Pericardial injection of atropine (a muscarinic cholinergic antagonist) indicated that bradycardia in the trout was due initially to increased vagal tone but later due to the direct effect of cyanide on the heart.

Hyperventilation in the trout persisted throughout the exposure period, although the rate and amplitude fluctuated

and was variable between individual fish. During the last hour of exposure (highest cyanide concentration), ventilation was characterized by rapid, shallow breaths with a sudden respiratory arrest. The bullheads showed hyperventilation during the first 3 hours of exposure followed by a gradual, linear drop in ventilation rate and amplitude until death occurred. Cardiac and ventilatory responses in both species were attributed to stimulation of central and peripheral chemoreceptors by cyanide. Evidence is presented which suggests the initial response in the bullheads was due, at least in part, to gustatory stimulation by the cyanide. Oxygen consumption of the trout remained above pre-exposure levels for the majority of the test period. Oxygen consumption in the bullhead paralleled the changes in heart and ventilatory rates.

Whole-body lactate and pyruvate levels of fingerlings of both species during cyanide exposure were measured to estimate the extent of anaerobiosis. Whole-body lactate levels were greater in the bullheads than the trout, indicating a higher capacity for anaerobiosis, probably due to a greater fuel supply. Whole-body pyruvate levels in both species did not change from control levels until the last hour of cyanide exposure.

ACKNOWLEDGEMENTS

I would like to offer my thanks and appreciation to the following people:

Dr. F. M. Anne McNabb and Dr. Albert G. Hendricks for their guidance and expertise.

Dr. Alan G. Heath for his endless patience, invaluable advice and friendship; and to his family for their warm hospitality.

Connie DiAngelo and Alex Grissom for their friendship, help and humor.

Dr. Theodore Lidsky and Dr. Corinne Manetto for their support and encouragement during the preparation of this manuscript.

And most of all, my wife, Pat, and daughter, Kate, for never losing faith in me and for keeping the home fires burning.

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INTRODUCTION

Cyanide is a widespread chemical found in surface waters. It originates not only from direct industrial wastes but often from biological sources (Krutz, 1981). Whatever the source, cyanide ionizes in water to release cyanide ion (CN^-) which, depending on the pH, hydrolyzes to form hydrocyanic acid (HCN). At pH 7 about 99% of the cyanide is in the form of HCN while at pH 9.3 equal quantities of HCN and CN^- exist. The acute toxicity to fish of solutions of simple cyanides has been found to decrease with an increase in the pH of the solutions, and it has been concluded that molecular HCN is more toxic than the CN^- ion (Bridges, 1958).

The toxicity of cyanide to fish has long been known, and is attributed to the inhibitory effects on the electron transport system of the mitochondria, such that oxygen cannot be biochemically utilized. A number of studies have found that the lethal level to various species of fish is in the range of 30 to 150 ug/l as HCN (reviewed by Doudoroff, 1976). With a few exceptions, cardiovascular and respiratory responses of fish during cyanide exposure have been little investigated.

Fish exposed to a highly lethal level of cyanide exhibited an initial hyperventilation followed by a declining ventilation rate ending in death (Jones, 1948). Morgan and Kuhn (1974), using sublethal cyanide concentrations, demonstrated a dose dependent increase in ventilation rates over the first two days of exposure, followed by a return to normal rates after the fifth day of an eight day exposure period. The authors attributed the return to normal rates in spite of continuous cyanide exposure to either detoxification of the cyanide, the activity of cyanide as a respiratory depressant overcoming the initial stimulation, or both. Eclancher and Dejours (1975) injected NaCN (10 ug/1) into the ventral aorta of trout and carp and observed an immediate increase in ventilation and bradycardia. From this, they concluded that control of cardiac and ventilatory rates in fish is influenced by stimulation of arterial chemoreceptors analogous to those found in higher vertebrates, and both respond in a similar manner to cyanide. In the only other study involving the effects of cyanide on the cardiac rate of fish (Bahr, 1973), bradycardia and an electrocardiogram identical to that seen during exposure to environmental hypoxia were observed in the trout.

Metabolic responses of intact fish (as indicated by changes in oxygen consumption) demonstrate the respiratory

depressant effect of cyanide. Studies by Jones (1947, 48), Carter (1962), Negilski (1973) and Dixon and Leduc (1981) show a decrease in oxygen consumption during cyanide exposure, with the degree and rate of inhibition depending on the cyanide concentration. Whether there was any anaerobic contribution to meet the metabolic demand during the decreasing oxygen consumption of the fish in these studies was not investigated.

The most significant factor affecting the relative sensitivity to cyanide of different fish species appears to be their ability to tolerate environmental hypoxia. A review of cyanide toxicity tests shows that salmonids (an hypoxia-sensitive group) are highly sensitive to cyanide, while cyprinids and ictalurids are relatively insensitive to cyanide, and are also tolerant of low oxygen levels (Doudoroff, 1976; Davis, 1975; Blazka, 1958; Marvin and Heath, 1968).

The objectives of this study were to measure and compare the cardiac, ventilatory and metabolic responses to waterborne cyanide of rainbow trout and brown bullhead catfish, two species with divergent sensitivities to cyanide and to environmental hypoxia. The anaerobic contribution to total energy production during cyanide exposure in each spe-

cies was investigated by measuring whole body lactate and pyruvate levels.

LITERATURE REVIEW

Cyanide from biological sources enters the water as HCN, while most of the cyanide used in industrial processes is in the form of technical cyanide salts such as potassium (KCN), sodium (NaCN) or calcium cyanide ($Ca(CN)_2$), all of which are soluble in water. Whatever the source, cyanide ionizes in water to release cyanide ion (CN^-) which, depending on the pH, hydrolyzes to form hydrocyanic acid (HCN), the more toxic of the two forms. The greater toxicity of the molecular form is attributable to the relative ease with which small, uncharged molecules penetrate into the blood and other tissues of organisms, whose external membranes are relatively impermeable or less permeable to the charged ions (Doudoroff and Katz, 1950).

Cyanide presumably enters the fish through the gills, digestive tract and by absorption through the skin, however, the actual partitioning has not been determined. Once in the general circulation, cyanide produces tissue anoxia in such vital organs as the heart, brain and breathing muscles by inhibiting aerobic oxidative respiration. This inhibition is due to the binding and inactivation by cyanide of

cytochrome oxidase, the terminal component of the mitochondrial electron transport chain (Solomonson, 1981).

Powers (1917) appears to have been the first to test the toxicity of cyanide to fish. Since that time an extensive literature on the toxicity of cyanide to fish under various environmental conditions has accrued (see Doudoroff, 1976, for review). However, with a few exceptions, cardiovascular and respiratory responses of fish during cyanide exposure have been little investigated.

This is not the case with mammals. Studies on the effects of cyanide on the regulation of breathing and blood flow in intact animals, and the effect of cyanide on tissue respiration in various species and organs were reported about 50 years ago (Gesell and Hertzman, 1927; Dixon and Elliot, 1929). Later, attention focused more on the effects of cyanide on chemoreceptor reflexes for control of ventilatory and cardiovascular performance (Brodie, 1959; Calvelo et al., 1970). These studies showed that during cyanide exposure, hyperventilation in mammals is caused by cyanide stimulation of extracranial, carotid and aortic chemoreceptor mechanisms, and bradycardia in the dog caused by cyanide has been attributed to the activation of the chemoreceptors in the carotid body (Jacobs et al., 1971).

Although cyanide affects the utilization, rather than the availability of oxygen, the ventilatory, cardiac and metabolic responses are similar to those seen in fish exposed to environmental hypoxia. Thus, a discussion of these parameters in fish exposed to hypoxia is in order.

An increase in gill ventilation volume is employed by fish to bring more water over the gills in response to decreases in the oxygen content of the blood (Smith and Jones, 1978). The receptors that detect this change are believed to be located in the post gill arterial complex (Randall, 1982). The gill ventilation volume is the product of the stroke volume (depth) and the breathing frequency. An increased ventilation volume can be obtained by an increase in either (or both) of these variables and appears to be species specific. Trout (Hughes and Saunders, 1970) and brown bullhead catfish (Marvin and Heath, 1968) rely on an increase in breathing depth more than frequency, while the bluegill increases the breathing frequency rather than depth in response to environmental hypoxia (Heath, 1973). The tench increases both depth and rate of breathing equally when exposed to low oxygen levels (Randall and Shelton, 1963).

It is reasonable to assume that an increase in ventilation volume would result in more efficient oxygen uptake and delivery to the blood, however just the opposite is true.

Utilization is the ratio between oxygen extracted from the ventilation stream and oxygen contained in the inspired water. Saunders (1962) showed that the utilization of oxygen is inversely proportional to the respiratory volume, thus quiet breathing is more "efficient" in extracting oxygen from the water than is heavy, rapid breathing. This is due to a decreased diffusion time at the gill surface and altered gill geometry resulting from the increased water flow (Hughes, 1966). This decrease in utilization may not have affected the survival times of the fish used in this study since the water was well aerated, but could account for the decreased tolerance to cyanide at low oxygen levels reported by Cairns and Scheier (1958).

In addition to changes in ventilation, environmental hypoxia also causes changes in cardiac performance. In teleosts, the cardiac innervation consists of one pair of cardiac branches from the vagus (X cranial) nerve, which enters the sinus venosus bilaterally (Laurent, 1983). The heart is under double antagonistic control with both a vagal cholinergic inhibitory pathway producing negative inotropic and/or chronotropic effects via cholinergic receptors, and an adrenergic excitatory effect mediated by beta adrenoceptors (Gannon, 1971; Holmgren, 1977). Some teleosts, such as plaice, lack an adrenergic innervation to the heart

(Santer, 1972) and may rely solely on circulating catecholamines for adrenergic control.

Bradycardia in fish occurs as a response to environmental hypoxia (Randall and Shelton, 1963; Farrell, 1984), and toxicants which damage the gills and thus lower the arterial Po_2 (Skidmore, 1970; Bass and Heath, 1977; Holeton and Randall, 1967). This cardio-inhibition in response to changes in arterial Po_2 is mediated by oxygen receptors located on the surface of the anterior pair of gill arches in the region of the efferent vessel (Daxboeck and Holeton, 1978). Stimulation of these receptors increase the activity of the cardiac branch of the vagus nerve causing the release of the neurotransmitter substance acetylcholine into the heart tissue, which then acts via muscarinic acetylcholine receptors to slow the heart rate (Butler and Metcalfe, 1983). It was initially thought that bradycardia represented a fall in cardiac output (Randall and Shelton, 1963), and was a mechanism to conserve cardiac energy expenditure during a time of reduced oxygen availability (Heath, 1964). However, Holeton and Randall (1967) have shown in trout that hypoxia produced no substantial change in cardiac output although there was a shift from high rate - low stroke volume to low rate - high stroke volume. It is now thought that

bradycardia serves to improve the pattern of gill blood flow for a more efficient gas transfer (Wood and Shelton, 1980).

Cardioacceleration in fish can result from: (1) a decrease in vagal cholinergic tone, (2) by direct adrenergic nerve action via the vagosympathetic nerve trunk (Gannon and Burnstock, 1969) - the latter being relatively more important at high temperatures (Wood et al., 1979), or (3) from humoral adrenoceptors present in the heart responding to increased blood catecholamine levels. Catecholamines are released into the circulation from chromaffin tissue located in the kidney in response to exhaustive exercise or harsh stress such as handling, hemorrhage or hypoxia (Mazeaud, Mazeaud and Donaldson, 1977). Excitatory adrenergic regulation of heart rate is probably secondary to the inhibitory cholinergic vagal tone as indicated by bradycardia during hypoxia, in spite of an increase in circulating catecholamines. Instead, circulating catecholamines may exert a positive inotropic effect (improve stroke volume), which would offset the reduced heart rate to insure an adequate cardiac output (Pettersson and Nilsson, 1980).

The overall response of fish to environmental stresses can be interpreted by observing changes in oxygen consumption. Oxygen consumption in fish is customarily measured under one of three conditions: active, standard, or routine

(Fry, 1971). Active oxygen consumption is measured while the fish is forced to swim at a maximum rate. Standard oxygen consumption is measured when the fish is completely at rest. Since this does not occur under natural conditions the activity must be measured and the oxygen consumption extrapolated to zero activity. Routine oxygen consumption is measured with the fish in a rested condition, housed in chambers just large enough to permit fin movement but not large enough to allow free swimming, and no effort is made to measure spontaneous activity of the fish in the chamber. The oxygen consumption measurements in this study can be classified as routine.

The metabolic rate in fish, as measured by oxygen consumption, can be influenced by a large number of internal and external factors, among them being temperature, activity level, and size (see Fry, 1971, for review). An increase in ventilation volume results in an increased oxygen consumption (Saunders, 1962) which can be explained by the increased metabolic cost of breathing which, under resting conditions, represents from 10 to 20% of the total energy demand of the fish (Hughes and Saunders, 1970; Hughes and Shelton, 1962). Hyperactivity induced by a pollutant, whether by its irritant effect or by direct stimulation of the CNS will also produce

an increase in oxygen consumption (Waiwood and Johansen, 1974).

The ability of a fish to maintain relatively normal oxygen consumption depends on coordinated adjustments in the functioning of the respiratory and cardiovascular systems. If the degree of environmental hypoxia, pollution or exercise exceeds the ability of these mechanisms to provide adequate oxygen to the tissues, it becomes necessary for the cells to derive part, or all, of their energy form the anaerobic metabolism of glycogen (Heath and Pritchard, 1965).

In many organisms this is accomplished by anaerobic glycolysis, where glycogen, the storage form of energy, is metabolized to the level of lactate, utilizing the Embden-Meyerhof pathway. Useful energy production in the form of ATP occurs in two reaction steps, therefore, per mole of glucose, a net of two moles of ATP are synthesized (Hochachka et al., 1973). Anaerobic glycolysis, as indicated by an increase in lactic acid in the blood or muscle tissues, is the predominate pathway for ATP production under anaerobic conditions in the majority of fish and has been found to occur in trout (Heath and Pritchard, 1965) and in brown bullhead catfish (Burton and Spehar, 1971).

A number of invertebrates have special anaerobic pathways which result in higher phosphorylation efficiency than

obtained from the Embden-Meyerhof pathway (Hochachka, 1980), and several workers have tried to determine the invertebrate type anaerobic endproducts in fish, however, their role in fish metabolism appears to be of minor importance (Van den Thillart, 1982; Johnston, 1975; Smith and Heath, 1980).

MATERIALS AND METHODS

SOURCES AND MAINTENANCE OF EXPERIMENTAL FISH

Adult and fingerling rainbow trout (Salmo gairdneri) were obtained from the state trout hatchery at Wytheville, Virginia. Adult brown bullhead catfish (Ictalurus nebulosus) were netted from the Gaithright Reservoir, Bath County, Virginia; and bullhead fingerlings were obtained from Zett's Fish Farm, Drifting, Pa.

A total of 64 brown bullhead catfish were used in this study; 19 adults with a mean weight of 204.6 grams and a range of 171 to 267 grams, and 45 fingerlings with a mean weight of 3.6 and a range of 2.3 to 5.2 grams. Fifty-five rainbow trout were also used; 19 adults having a mean weight of 172 grams with a range of 135 to 219 grams, and 36 fingerlings with a mean weight of 5.75 grams, ranging from 2.3 to 8.3 grams.

In the laboratory, the fish were held in holding tanks supplied with a continuous flow of aerated, dechlorinated Blacksburg tapwater. The fish were acclimated to the test temperatures (15°C. for trout, 25°C. for bullheads) for a

minumum of two weeks prior to use. All fish were fed Purina Trout Chow ad. libitum at random times every two days. Feeding schedules were arranged so that no fish were fed 24 hours prior to experimentation. A constant photoperiod (14L:10D) was maintained at all times, with no attempt to alter the photoperiod with the changing seasons.

Prior to placing the bullheads in the holding tanks, 3-5 mm. of their dorsal and pectoral spinous rays were clipped to facilitate handling. During the acclimation period the bullheads underwent prophylactic drug treatment for prevention of disease consisting of alternate weekly doses of oxytetracycline hydrochloride (15 mg/1) and acriflavin hydrochloride (10 mg/1) (Burton, 1970).

EXPERIMENTAL APPARATUS

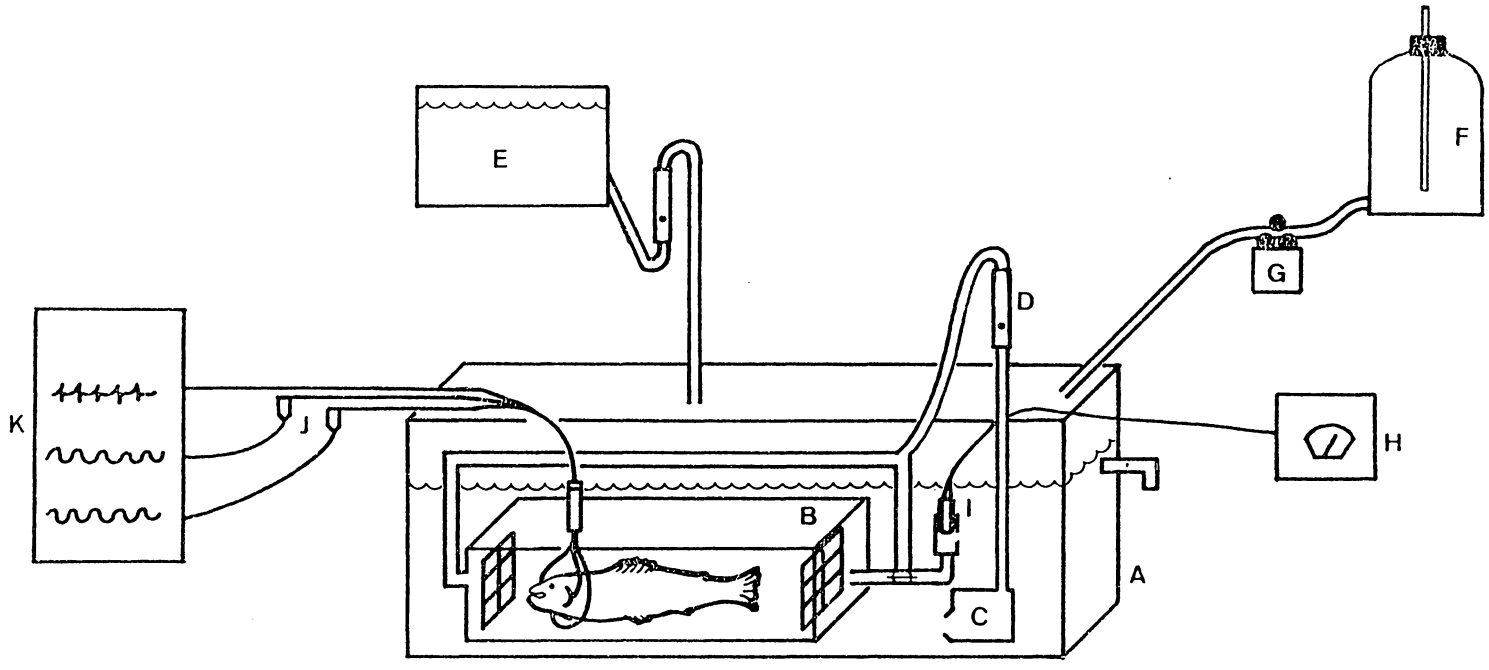
Five series of experiments were conducted in this study: (1) preliminary determination of circadian rhythms, (2) measurement of oxygen consumption, heart rate and ventilatory activity during an increasing concentration of cyanide, (3) measurement of oxygen consumption, heart rate and ventilatory activity during a constant concentration of cyanide to confirm dose dependency, (4) measurement of cardiac and

ventilatory activity during gustatory stimulation, and (5) measurement of whole body lactate and pyruvate levels during cyanide exposure. Adult fish were used in experiments (1) through (4) and fingerlings were used in (5). The apparatus shown in Fig. 1 was used for the work involving adult fish. A modification of the apparatus was used for fingerlings and will be discussed later.

For each run, the test fish was housed in a flow-through test chamber submerged in a 30 liter glass aquarium. The chamber was an epoxy-coated wooden box measuring 30 x 7.5 x 7 cm. This was of sufficient size to permit free fin movement but still prevent the fish from turning around in the box. Holes at each end of the box accommodated inflow and outflow water lines. Heavy wire screens were placed in front of the holes to prevent blockage by the fish. A glass tube, which entered the box through the lid and projected above the waterline of the aquarium, provided passage for catheters and ECG leads from the fish to the outside of the chamber.

A head tank provided the aquaria with a constant flow of dechlorinated tapwater, controlled by a flow meter to produce 90% water replacement every 8 hours (Sprague, 1969). This constant flow, plus charcoal filters in the aquaria, eliminated the buildup of metabolic byproducts during the acclimation and test periods. Airstones provided >90% oxygen

Figure 1. Diagram of experimental apparatus. A, glass aquarium; B, continuous-flow respirometer; C, pump; D, flow meter; E, water supply; F, Marriotte bottle containing cyanide solution; G, peristaltic pump; H, oxygen meter and (I) electrode; J, pressure transducers; K, polygraph. Temperature of the aquarium water was maintained by a water-bath (not shown).


E
F
G
D
K
J
H
B
I
A
C

saturation at all times. A constant water temperature (±0.5°C) for each species was provided by a waterbath. Aquarium water was circulated through the fish chamber by a pump, at a flow rate of 12.5 1/hr.

A stock potassium cyanide solution (1 ml = 0.1 mg KCN), made by dissolving 0.254 g KCN per liter distilled water, was contained in a Mariotte bottle and delivered to the test aquarium by a peristaltic pump. The pyridine-barbituric acid colorimetric method (APHA, 1980) was used for determination of the cyanide concentration. Cyanide measurements were made every 30 minutes throughout the period of exposure.

SURGICAL PREPARATION OF ADULT FISH

Fish were anesthesized in a Benzocaine solution (100 mg/1) and placed in a sling on an operating table equipped with two reservoirs, one containing dechlorinated tapwater and the other containing dechlorinated tapwater with Benzocaine (50 mg/1). Water from the reservoir containing the anesthetic was pumped into the fish's mouth and over the gills during the surgical procedures. Upon completion of the surgery, water was pumped from the reservoir containing the

fresh water to allow the fish to partially recover from anesthesia before being placed in the test chamber.

The opercular and buccal cavities were catheterized using the method of Saunders (1961). Electrocardiographic (ECG) leads consisted of two enamel coated stainless steel wires, bared at the tip. In the trout, these were inserted ventrally on either side of the heart and sutured into place.

Due to the anterior position of the heart and the surrounding bone in bullheads, a modified approach was used for this species. The wires were bilaterally inserted directly into the pericardial cavity through the membrane separating it from the opercular cavities. These wires exitted the opercular cavities dorsally and were secured by sutures anterior to the dorsal fin. Preliminary experiments showed that the wires did not interfere with ventilatory movements, and continuous ECG recording over three day periods showed no changes with time in heart rate or ECG pattern.

Following catheterization and insertion of ECG leads, the fish were allowed to recover from anesthesia and placed, head upstream, in the flow-through chamber. The opercular and buccal catheters were connected to Statham pressure transducers and monitored on a Grass Model 5D six-channel polygraph to record pressure changes associated with ventilation. The ECG leads were attached to the polygraph pream-

plifiers to record cardiac rate and pattern. The dissolved oxygen content (mg O_2/l) of the inflow and outflow water from the chamber was monitored by a YSI Model 54A oxygen meter and electrode, air calibrated prior to each run. The fish were left undisturbed for a minimum of 18 hours to allow acclimation to the chamber before beginning a test.

EXPERIMENTAL PROTOCOL FOR RESPIRATORY AND CARDIAC MEASUREMENTS

Oxygen consumption was calculated using the following formula: $(DO_{in} - DO_{out})/W \times F = V_{02}$ expressed in milligrams oxygen consumed per kilogram body weight per hour (mg O_2/kg/hr) where $(DO_{in} - DO_{out})$ is the difference in dissolved oxygen concentration (mg/l) between the inflow and outflow water, W is the wet weight of the fish (in kg.), and F is the flow rate through the chamber (l/hr).

During the first hour of each cyanide experiment (following the 24 hour acclimation period) baseline data were gathered by recording ventilation, heart rate and oxygen consumption for a one minute period, every 15 minutes. The mean of the four recordings was used as "normal" values for that fish. Initial recordings were made at the start of the

cyanide exposure and then every thirty minutes throughout the period of exposure. The flow of cyanide into the aquarium was adjusted to provide a continually increasing cyanide concentration which reached a lethal level in 7-9 hours for each species. Death was defined as no ventilatory activity for one minute for the trout and three minutes for the bullheads. The latter show extended periods of apnea during severe hypoxia (Marvin and Heath, 1968), thus the different criterion for death.

In order to ascertain whether the responses observed in the increasing cyanide concentration were dose-dependent or merely the result of cyanide accumulation, a group of each species were subjected to an increasing cyanide concentration for 3 1/2 hours (the point at which the ventilatory rate for both species had peaked and began to decline) and then held at this constant level throughout the remaining exposure period. Cardiac and ventilatory activity were monitored and the data were then compared to those obtained in experiment 2.

ESTIMATION OF ANAEROBIC METABOLISM DURING CYANIDE EXPOSURE

Fingerlings were used for this experiment because the large size of the adults made homogenization of the entire fish difficult. Preliminary tests showed that the fingerlings of each species had the same survival times and behavioral responses to cyanide as the adults. Temperature control, water supply and cyanide delivery were identical to the adult studies. The test aquarium contained four Plexiglas cylinders enclosed by fine mesh screens at both ends; the control aquarium contained one cylinder. Six fingerlings were housed in each cylinder, each group visually isolated from the others by black plastic sheeting. A small pump assured even mixing of the cyanide in the test aquarium.

Sampling times for this phase of the study were determined by the oxygen consumption data from the adult fish of each species. Thus, cyanide-exposed fingerlings were sampled when adult oxygen consumption was maximal, -25%, -50% and -75% of baseline values. At each of the above times a cylinder was randomly chosen, quickly lifted from the water, the screen removed and the fingerlings dropped into liquid nitrogen to halt enzymatic activity. This procedure took less than 5 seconds.

After approximately one minute in the liquid nitrogen, each fingerling was weighed and homogenized for one minute in a Waring blender containing six times its weight of 0.6 N perchloric acid. Two ml of the slurry was then contrifuged at 12,000 X g for 15 minutes to precipitate the protein. Lipids were removed by mixing 1 ml of supernatant with 2 ml ethyl ether and mixing with a Vortex mixer for 1 minute followed by centrifugation at 1000 X g for 1 minute. The aqueous phase was removed and the sample frozen at -70°C. until analyzed.

Lactate and pyruvate were measured using an anion exchange high performance liquid chromatography column (Biorad HP-87H) heated to 38°C. The column was used in conjunction with a Varian model 5000 chromatograph with a Varian model 2050 variable wavelength detector (set at 214 nm) and a Varian model 4270 integrator. The solvent front was 0.013 N sulfuric acid in 5% acetonitrile.

RESULTS

EXPERIMENT 1 - DIURNAL CYCLES

In a preliminary investigation, four fish of each species were examined for the presence of diurnal cycles. Following surgery and acclimation, ventilation rate, heart rate and oxygen consumption were monitored hourly for a 12 hour period beginning at 8 a.m. All measured parameters of both species fluctuated somewhat throughout the day, with ventilation rate being the most variable (changing as much as 5% from the mean), however, no consistent cycles relating to time of day were observed.

EXPERIMENT 2 - EXPOSURE TO AN INCREASING CYANIDE CONCENTRATION

Behavior changes

An increase in cyanide concentration of 0.02 mg/l per hour resulted in a mean survival time of 7 3/4 hours for the

trout with the earliest death occurring at 7 hours and the longest survival time being 8 1/2 hours. Trout were able to maintain equilibrium for the first three hours of exposure, but all had turned over onto their sides after 3 1/2 hours. Violent struggling, which generally started after the first hour of exposure, continued up to the fifth hour, even though the trout were lying on their sides. When the cyanide concentration exceeded 0.1 mg/l (5 hours) the struggling ceased and the trout showed no body movement except those associated with ventilation.

To achieve a lethal level in approximately 8 hours for the bullheads, the cyanide concentration was increased to 0.2 mg/l per hour, ten times the dose required for the trout. At the end of the first hour the bullheads had already been exposed to a cyanide concentration greater than the lethal dose for the trout. Even at this high concentration the earliest death occurred at 8 hours, and 3 of the 7 bullheads survived for 9 hours.

The most violent struggling in the bullheads occurred within the first 1/2 hour of exposure. Following this initial period of excitation the bullheads settled to the bottom of the chamber where they remained motionless, with only occasional displays of activity. Few bullheads were observed lying on their sides, however this may be due in part to the

difference in body shape of the two species. The trout has a laterally flattened body with a high center of gravity, whereas the bullhead has a dorsoventrally flattened body. This lower center of gravity, plus laterally protruding pectoral spines could easily prevent the bullhead from lying on its side in the narrow respirometer.

HEART RATE AND ECG PATTERN

Because of variability in pre-exposure values for heart rate, ventilation rate and oxygen consumption between individual fish, the data from each fish were calculated as percent change from pre-exposure values recorded for that fish. Exposure to cyanide produced consistent patterns for each species, however onset and duration differed among individuals.

The mean pre-exposure heart rate for the trout was 79 beats per minute with a range of 74 to 93 beats per minute. Trout developed bradycardia (slowing of the heart rate) as the cyanide concentration began to rise, and the extent of the bradycardia increased in a linear fashion with the rising cyanide dose, resulting in a -67% rate after 4 1/2 hours (Fig. 2). Following this maximum inhibition of the heart,

the rate gradually rose to 57% of the pre-exposure values and this general level persisted throughout the exposure period and, in some fish, even continued after all ventilation had ceased. The hearts that continued to beat after ventilation stopped averaged 32 beats per minute. In 2 of the 7 trout, following the increase in heart rate, the rate once again decreased and then stopped in conjunction with ventilation.

The decrease in heart rate of the trout was due to a decreased pacemaker rhythm, as evidenced by an increase in the interval between consecutive P-waves. In all fish, the T-wave was elevated during cyanide exposure (Fig. 3). In the two trout in which the heart stopped, there were missed beats and decreased pacemaker rhythm. During the hour preceeding cardiac arrest the ECG pattern was one of several evenly spaced beats interrupted by periods of several seconds with no electrical activity, then 2 or 3 more beats.

The mean pre-exposure heart rate for the 7 bullheads was 50 beats per minute, with a range of 39 to 58. Cyanide exposure produced bradycardia in all bullheads during the first 30 minutes which was coincident with the maximum period of struggling. In 6 of the 7 fish tested, a tachycardia (increase in heart rate) of +60% after 3 hours of exposure was seen (Fig. 4), even though the fish were calm. Heart rates did not drop to pre-exposure levels until after approximately

Figure 2. Changes in heart rate of seven rainbow trout exposed to a linearly increasing cyanide concentration

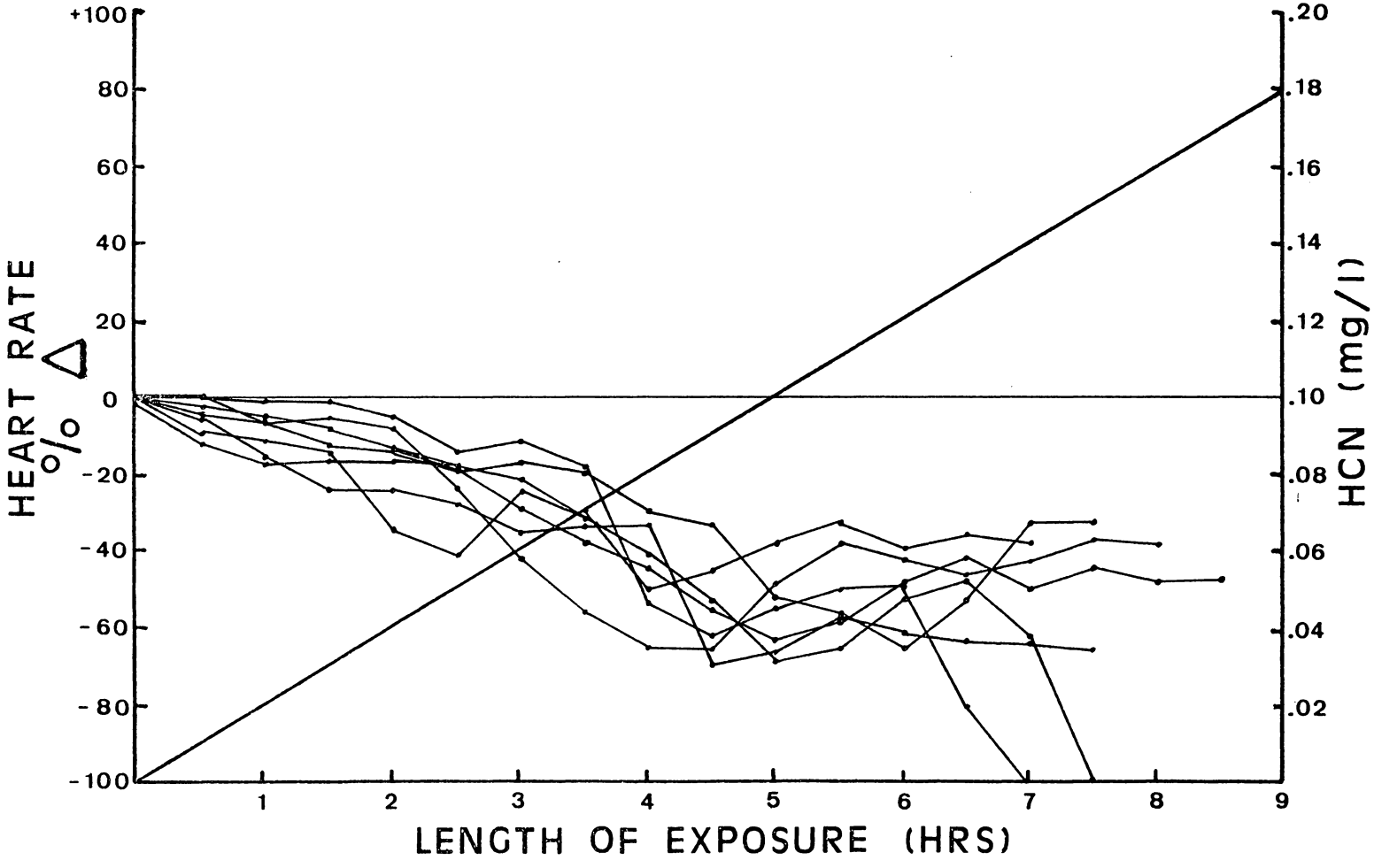

+100
80
60
40
20
0
-20
-40
-60
-80
-100
HEART RATE
% Δ
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

Figure 3. Changes in heart rate and ECG pattern of a rainbow trout exposed to a linearly increasing cyanide concentration

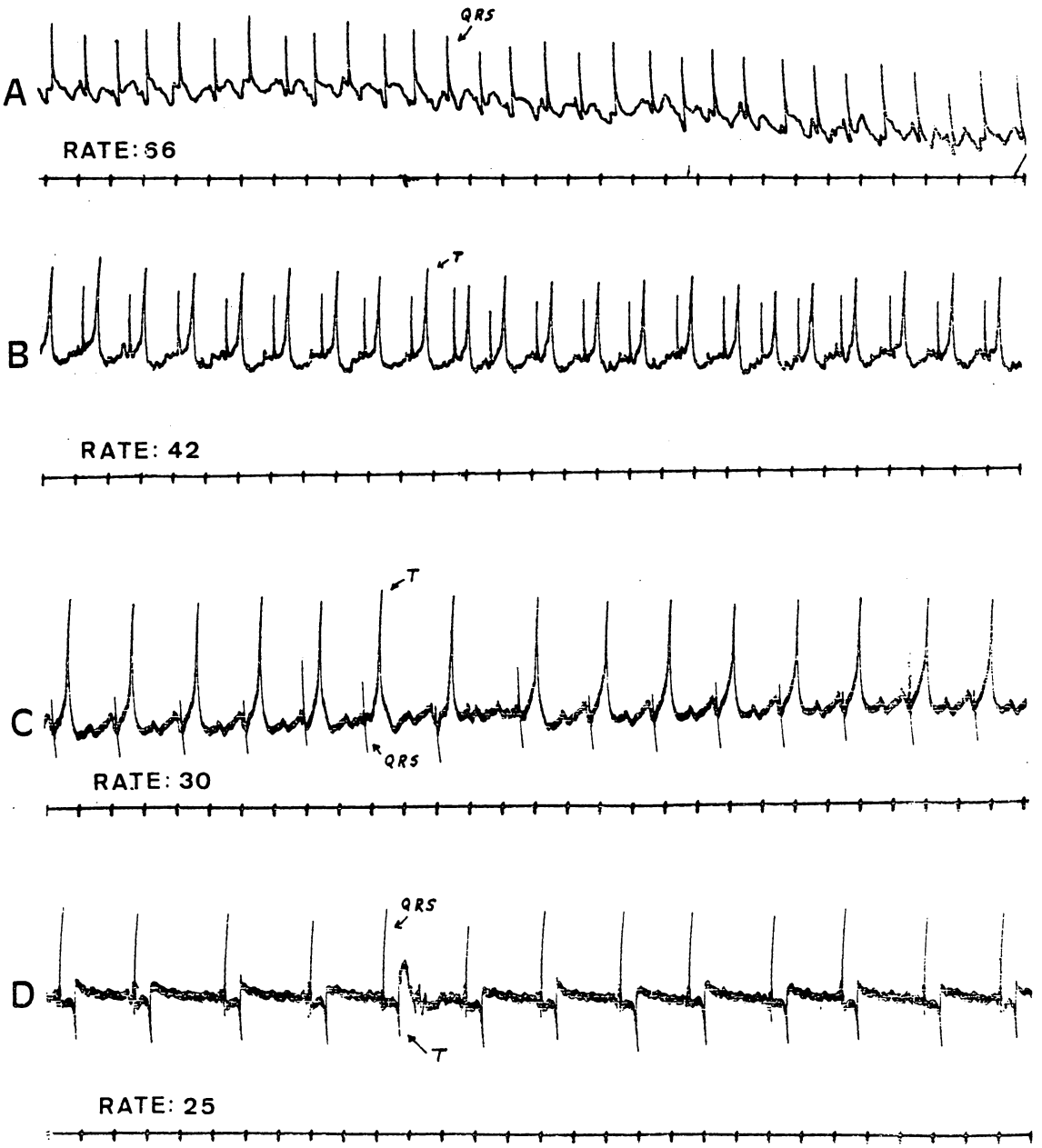
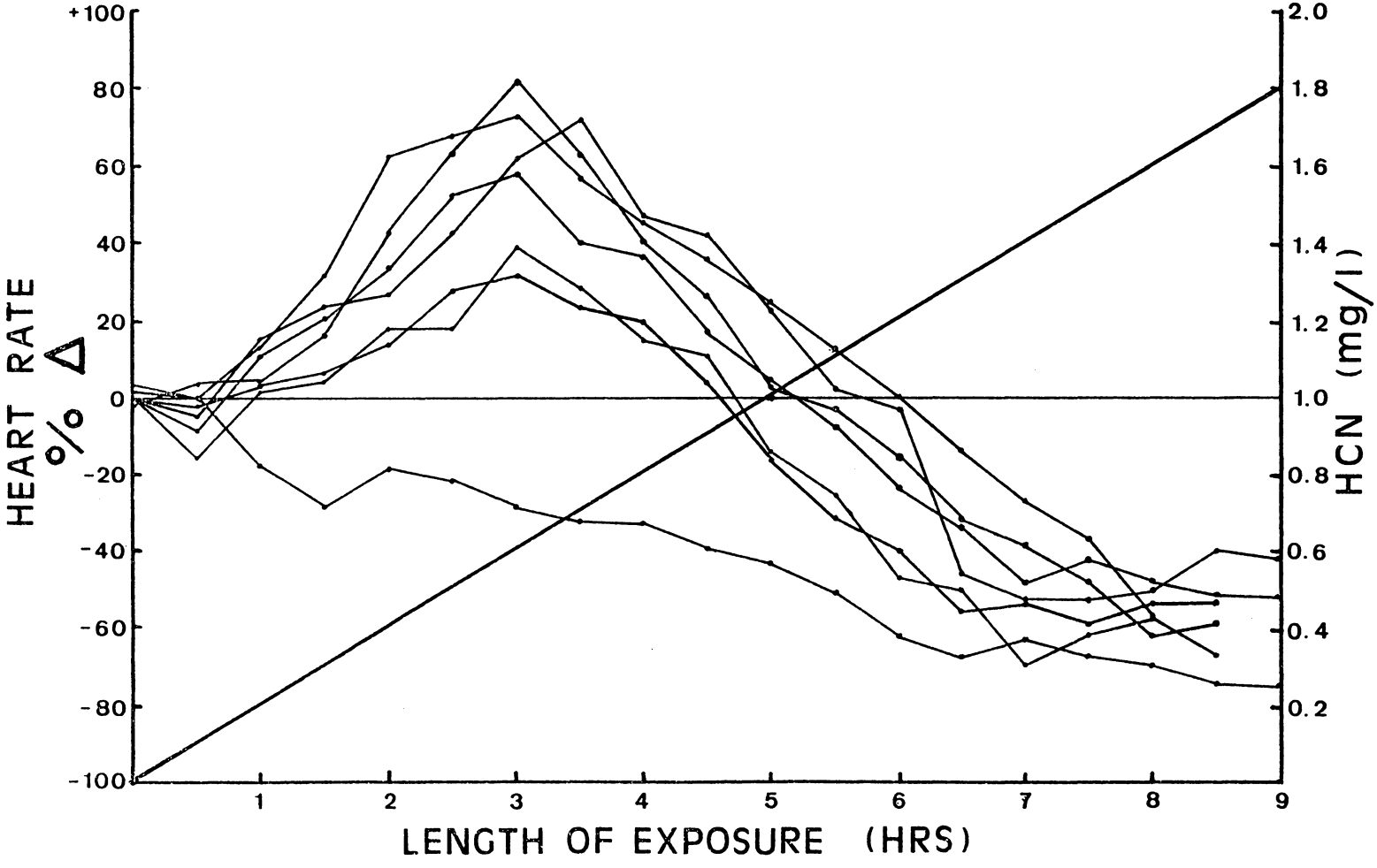

A
QRS
RATE: 56
B
T
RATE: 42
C
T
R
QRS
RATE: 30
D
QRS
T
RATE: 25

Figure 4. Changes in heart rate of seven brown bullhead catfish exposed to a linearly increasing cyanide concentration


+100
80
60
40
20
0
-20
-40
-60
-80
-100
HEART RATE
% Δ
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

5 hours followed by gradually increasing bradycardia until the end of the experiment. All bullhead hearts continued to beat after ventilation stopped, averaging 44% of normal values (22 beats per minute).

Cyanide exposure produced alterations of the ECG patterns in the bullheads identical to those seen in the trout.

Bradycardia due to vagal (cholinergic) inhibition was evaluated by injecting atropine (.5 mg/ml), dosage 0.14 ml/100 g, directly into the pericardial cavity of both fish species during bradycardia and recording changes in heart rate. In preparation for this, fish were fitted with a fine (PE 50) catheter, which entered the pericardial cavity after passing through the membrane separating the cavity from the gill chambers. Fish used in this experiment were exposed to an increasing cyanide concentration identical to that used in experiment 2. For the trout, atropine was injected 30 minutes after bradycardia appeared, and at hourly intervals throughout the exposure period. For the bullheads, atropine was injected 15 minutes after the initial bradycardia first appeared, and again, following an extended period of tachycardia, 30 minutes after the bradycardia reappeared and at hourly intervals thereafter.

Atropine injection during initial stages of bradycardia in the trout (heart rate -10 to -15%), resulted in an in-

crease in heart rate to above pre-exposure values. As the exposure period and degree of bradycardia increased, atropine had a diminishing effect. When the heart rate was less than 60% of normal values (after approximately 4 hours of exposure), atropine had no chronotropic effect (Table 1).

Atropine injection during initial bradycardia in the bullheads (first hour of exposure) raised the heart rate to, but never above, pre-exposure levels. Atropine injection during bradycardia after 5 hours of exposure had no effect, even though the degree of bradycardia was at approximately the same level (-10%) as those recorded during the first hour of exposure (Table 2).

Regardless of the type and extent of cardiac rate changes seen in the trout and bullhead during cyanide exposure, the hearts continued to beat after ventilation ceased. Cardiac fibrillation was not observed and the heart, although operating at rates different from pre-exposure levels, remained synchronized and able to pump blood.

VENTILATION RATE AND AMPLITUDE

Cyanide exposure produced an increase in ventilation rate in both species, however a difference in rate of in-

Table I. Changes in heart rate (HR) following pericardial atropine injection in trout exposed to waterborne cyanide. Number in parentheses indicates baseline (BL) heart rate.

Fish #	Time of inj (hr)	Pre-inj HR	% decrease from BL	Post-inj HR	% change from pre-inj HR
1 (83)	1	69	16%	86	25%
	2	63	24%	78	23%
	3	54	35%	60	11%
	4	50	40%	50	0%
	5	44	49%	44	0%
2 (78)	1	72	8%	86	20%
	2	70	10%	70	0%
	3	54	31%	60	11%
	4	40	49%	40	0%
	5	40	49%	40	0%
3 (68)	1	64	6%	68	6%
	2	59	13%	68	15%
	3	42	38%	50	19%
	4	38	44%	40	5%
	5	30	56%	30	0%
	6	33	53%	33	0%
4 (84)	1	80	5%	88	10%
	2	75	11%	80	7%
	3	48	43%	48	0%
	4	42	50%	42	0%

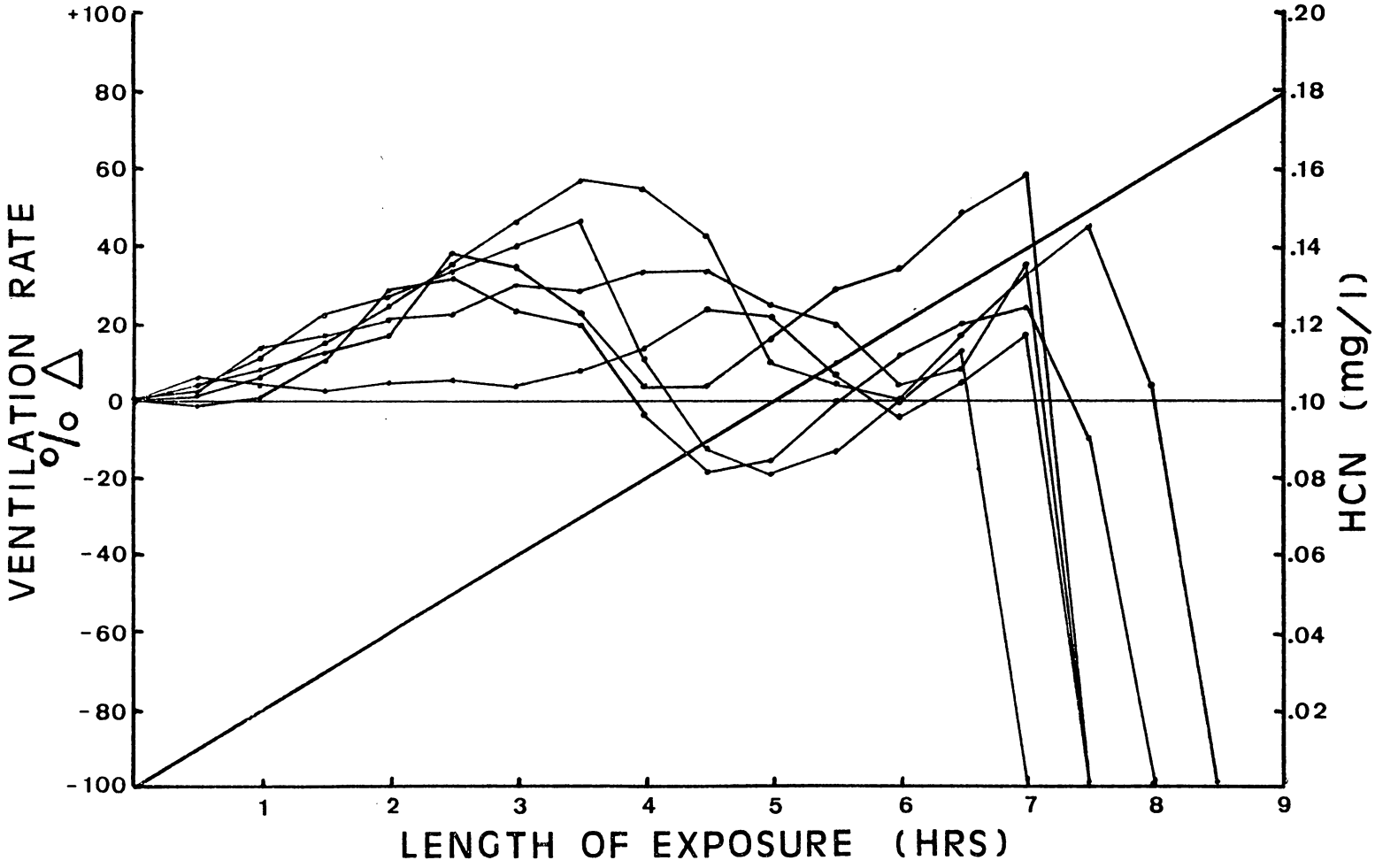
Table II. Changes in heart rate (HR) following pericardial atropine injection in brown bullhead catfish exposed to waterborne cyanide. Number in parentheses indicates baseline (BL) heart rate.

Fish #	Time of inj (hr)	Pre-inj HR	% decrease from BL	Post-inj HR	% change from pre-inj HR
1 (56)	0.5	50	11%	56	12%
	1.0	45	20%	50	11%
	1.5	59 - tachycardia:		no injection	
	6.0	44	21%	44	0%
2 (39)	0.5	35	10%	38	9%
	1.0	40 - tachycardia:		no injection	
	6.0	35	10%	35	0%
3 (43)	0.5	35	18%	42	20%
	1.0	46 - tachycardia:		no injection	
	6.0	39	7%	39	0%
4 (56)	0.5	52	7%	56	8%
	1.0	44	21%	54	23%
	1.5	56 - tachycardia:		no injection	
	5.0	50	11%	50	0%

crease and pattern were seen. The mean pre-exposure ventilatory rate for trout was 85 cycles per minute. Ventilation frequency peaked at +35% after 3 hours and subsequently declined to values approaching, and often falling below, normal rates (Fig. 5). Regardless of the extent and duration of the period of decreasing ventilation, all trout showed an increase in ventilation to above normal rates during the latter stages of the exposure period. Death was characterized by an abrupt drop in ventilation rate from above normal levels to zero within a 30 minute period.

The pattern for changes over time in trout ventilation amplitude, although quite variable, appeared to be independent of the ventilation rate. In several fish the amplitude peaked (+350%) after 4 1/2 hours of exposure; 1 1/2 hours after ventilation had peaked and a time in which the rate had dropped to values approaching pre-exposure levels. Following its peak, the amplitude decreased continually until the end of the experiment. In all cases, the buccal amplitude decreased at a faster rate than did the opercular amplitude, and even though buccal pressure changes were evident throughout the exposure, it is felt that these changes were due to the action of the opercular pump rather than those associated with movement of the lower jaw, as visual observations of several fish revealed no jaw movement even though

Figure 5. Changes in ventilation rate of six rainbow trout exposed to a linearly increasing cyanide concentration


+100
80
60
40
20
0
-20
-40
-60
-80
-100
VENTILATION RATE
% Δ
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

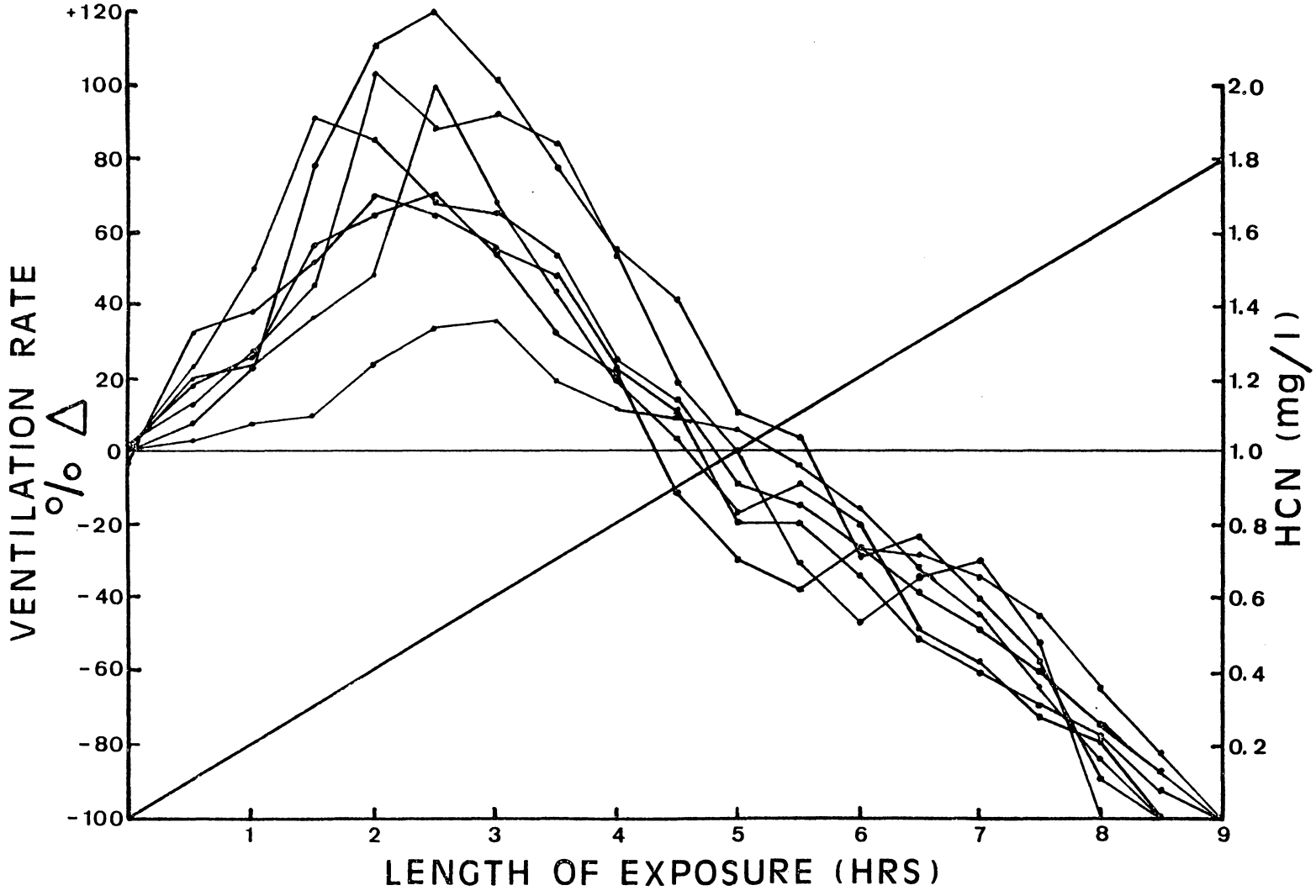
buccal pressure oscillations were being recorded. In the latter stages of the experiment the depth of opercular ventilation became very irregular, fluctuating several times during a 30 second recording period.

The pre-exposure ventilatory frequency of 51 per minute for the bullheads was affected by cyanide to a greater degree than the trout. Ventilation peaked at +91.5% after 2 hours, followed by a gradual linear decline which persisted throughout the exposure period (Fig. 6). Ventilatory pattern during the last hours of exposure was characterized by several shallow respirations with intermittent periods of apnea. As the exposure increased, the number of consecutive respirations became fewer and the periods of apnea became longer, often lasting 30 to 45 seconds.

Cyanide exposure of bullheads produced a 12-15 fold increase in opercular and a 10-12 fold increase in buccal amplitude. Both peaked in conjunction with ventilation rate and followed the same linear decrease during the exposure period. Unlike the trout, buccal and opercular amplitude declined at equal rates. During the last hours of exposure the amplitude was stable over the 30 second recording periods and averaged 20% of normal values.

The relationship between the cardiac and ventilatory frequencies during cyanide exposure was examined in both

Figure 6. Changes in ventilation rate of seven brown bullhead catfish exposed to a linearly increasing cyanide concentration


+120
100
80
60
40
20
0
-20
-40
-60
-80
-100
VENTILATION RATE
% Δ
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

species. Synchrony (a heart beat during a particular phase of the ventilatory cycle) was not evident in either species during any phase of the cyanide exposure. However, during the latter stages of cyanide exposure the bullhead heart rate slowed in conjunction with periods of apnea (Fig. 7).

OXYGEN CONSUMPTION

Oxygen consumption of the trout, which averaged 94 mg O_2/kg/hr prior to exposure, began to increase as soon as cyanide was introduced into the respirometer and reached maximum levels (averaging +57.5%) within 2 hours (Fig. 8). This increased rate of oxygen consumption remained at consistently high levels through most of the exposure period and then fell rapidly during the last hour. The prolonged period of high oxygen consumption paralleled the increased ventilation and overall motor activity.

The oxygen consumption of the bullheads (mean pre-exposure value of 84.73 mg O_2/kg/h) increased to +49.2% after 3 hours (Fig. 9). Within 30 minutes of this peak, the oxygen consumption began to decline and continued in a linear fashion until the end of the experiment.

Figure 7. Relationship between cardiac and ventilation (opercular and buccal) rates in a brown bullhead catfish during periods of apnea during cyanide exposure

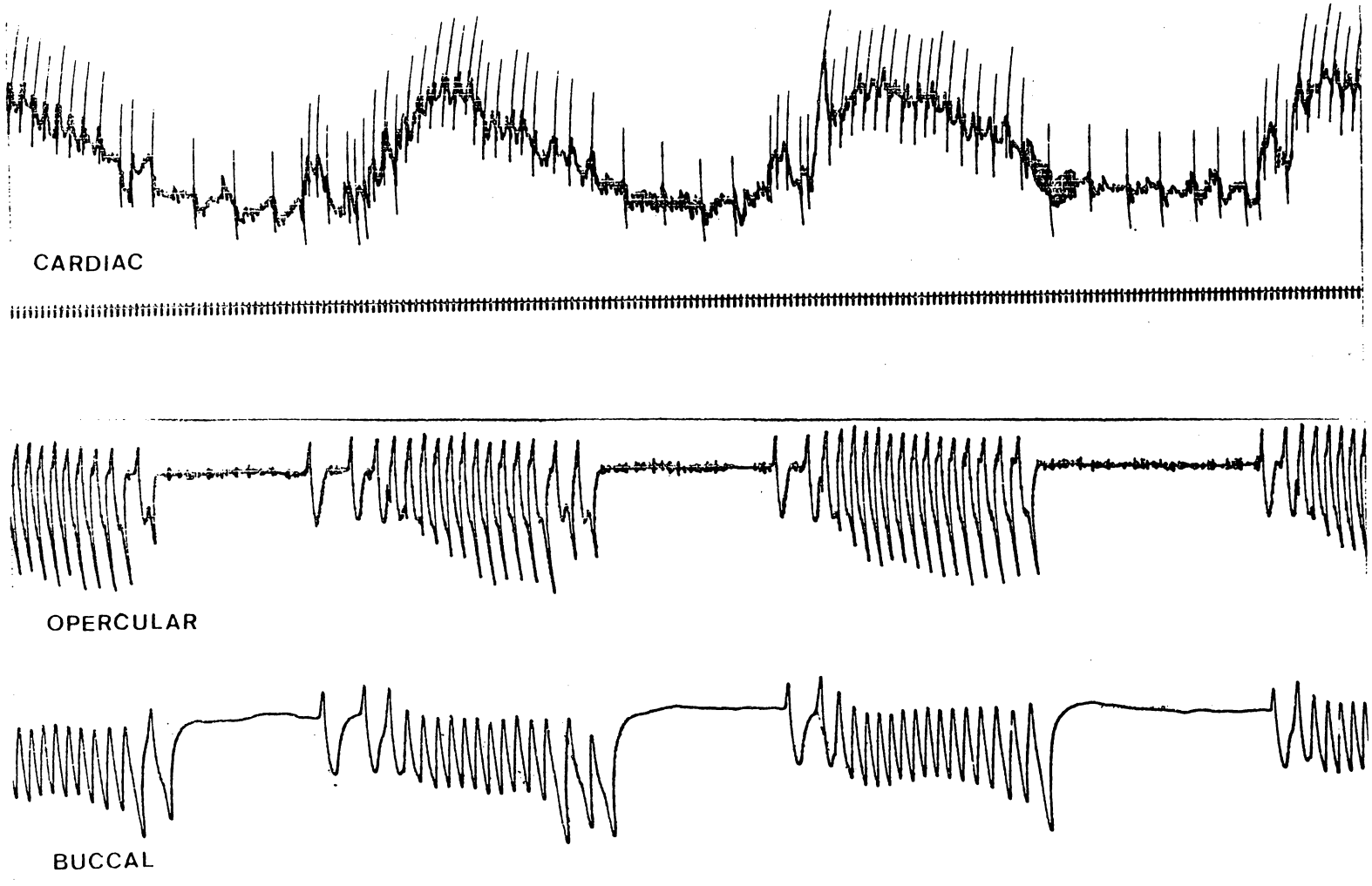

CARDIAC
OPERCULAR
BUCCAL

Figure 8. Changes in oxygen consumption of seven rainbow trout exposed to a linearly increasing cyanide concentration

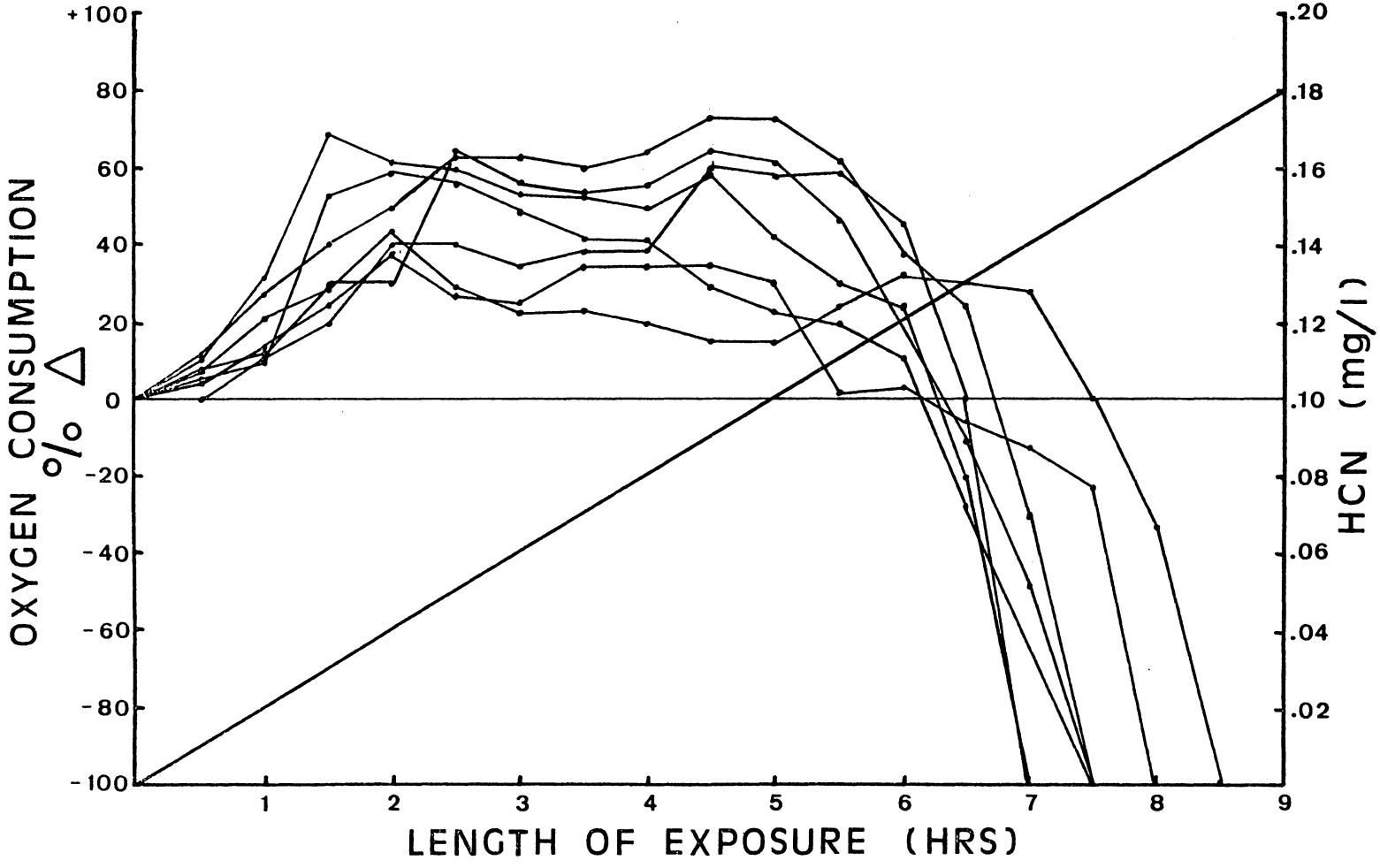

+100
80
60
40
20
0
-20
-40
-60
-80
-100
OXYGEN CONSUMPTION
% Δ
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

Figure 9. Changes in oxygen consumption of seven brown bullhead catfish exposed to a linearly increasing cyanide concentration

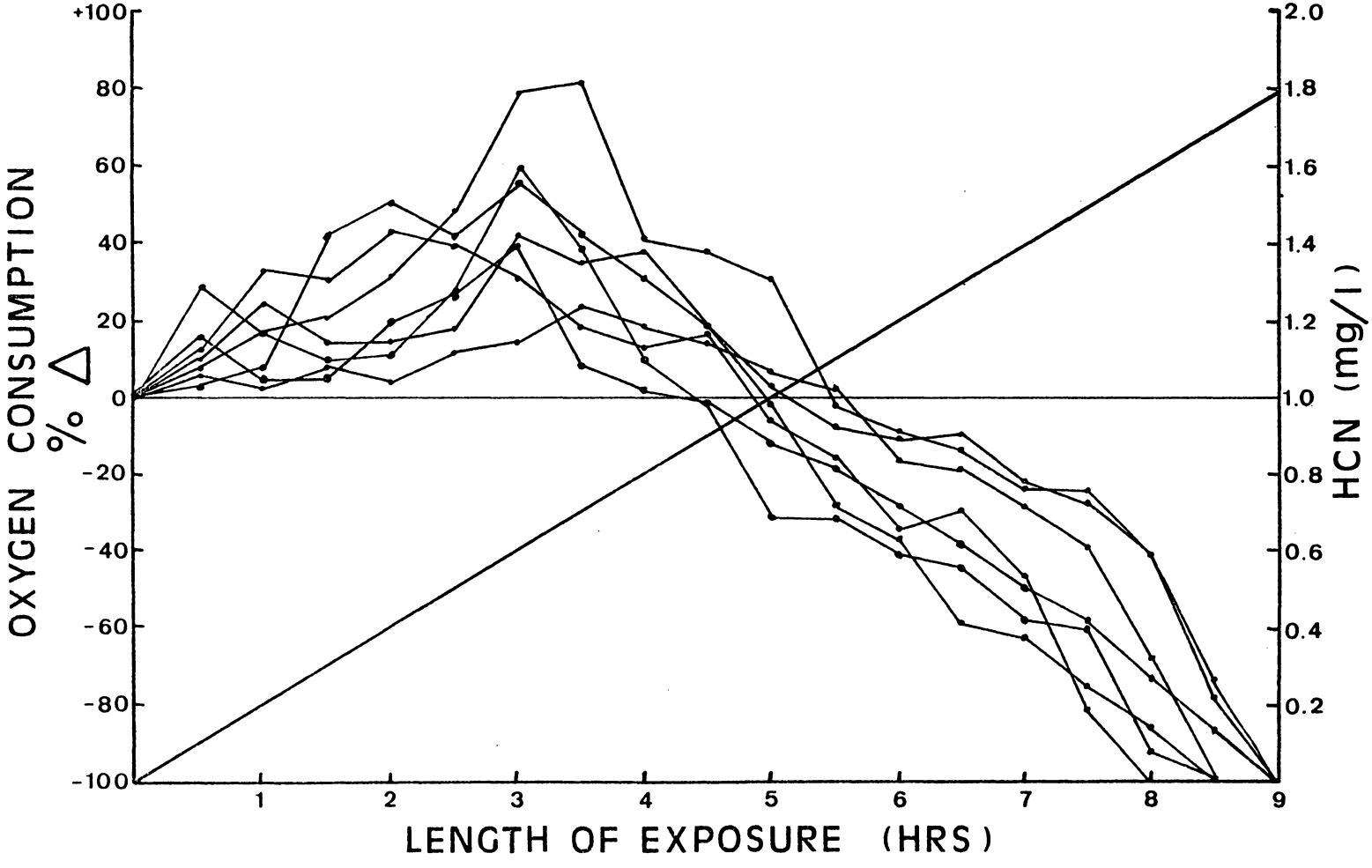

OXYGEN CONSUMPTION
% Δ
+100
80
60
40
20
0
-20
-40
-60
-80
-100
HCN (mg/l)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

Figure 10. Relationship between heart rate (HR), ventilation rate (VR) and oxygen consumption (Vo2) of rainbow trout exposed to a linearly increasing cyanide concentration

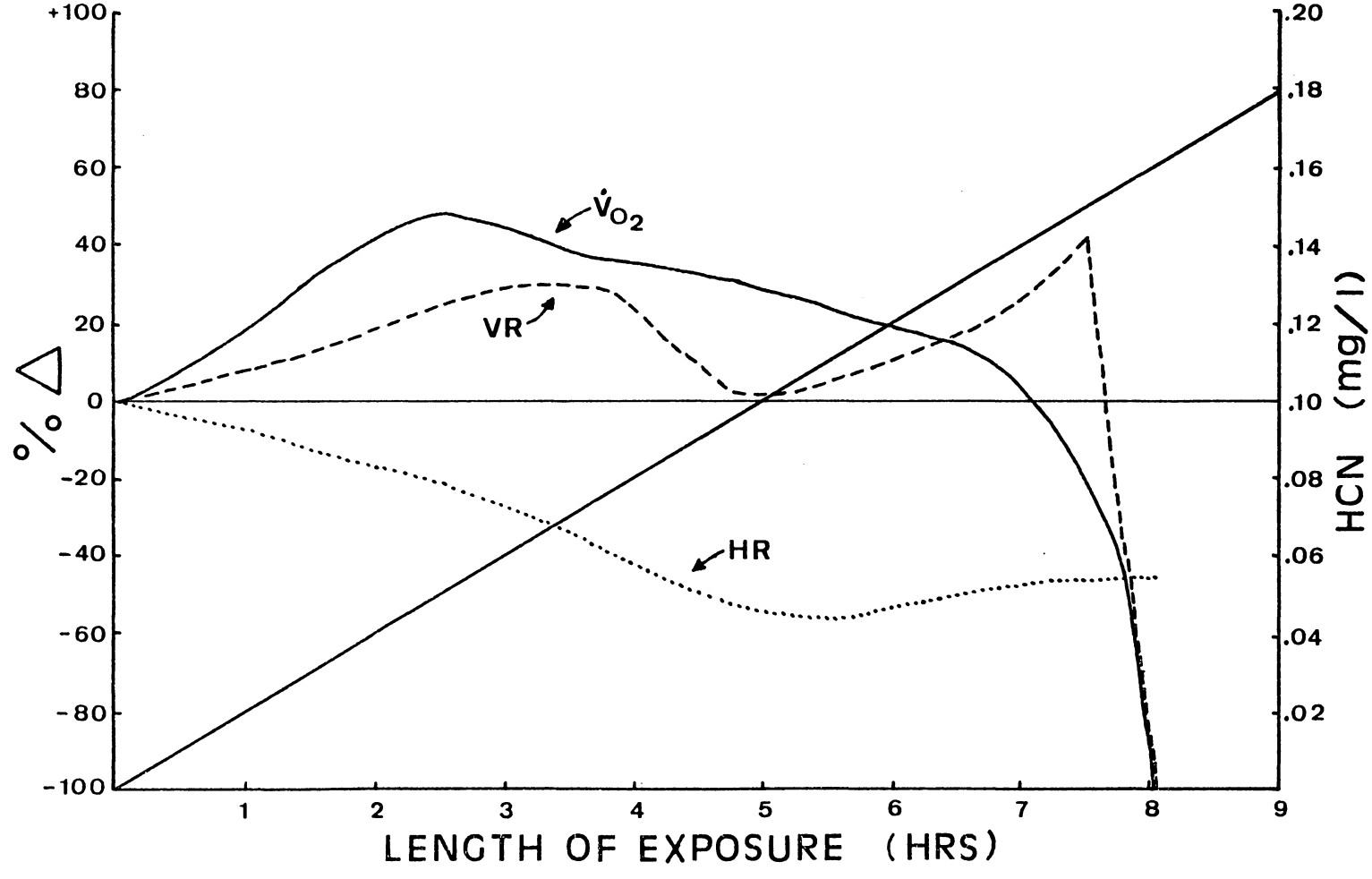
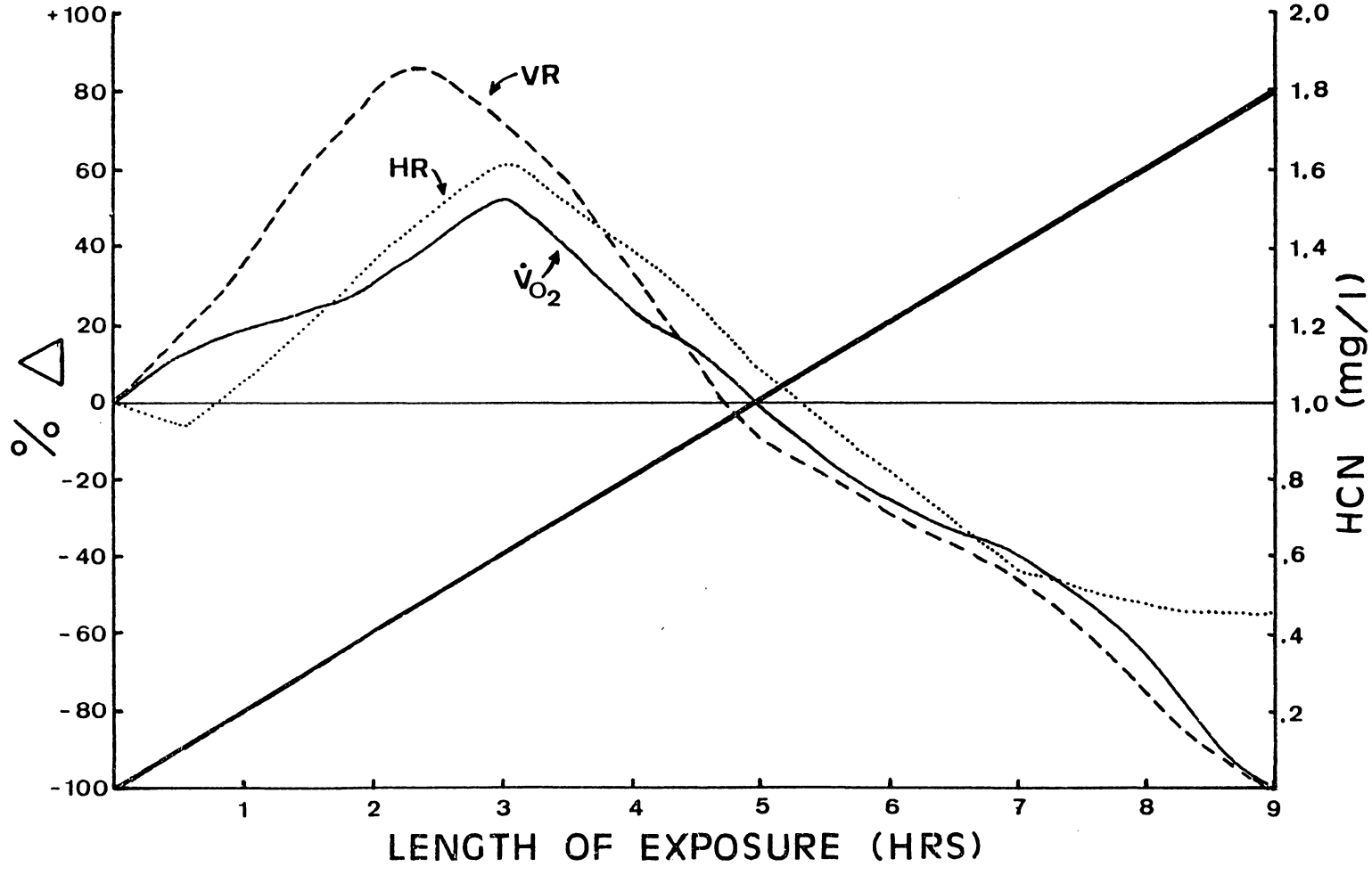

+100
80
60
40
20
0
-20
-40
-60
-80
-100
% Δ
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
HCN (mg/l)
$\dot{V}_{O_2}$
VR
HR
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

Figure 11. Relationship between heart rate (HR), ventilation rate (VR) and oxygen consumption (V_{O_2}) of brown bullhead catfish exposed to a linearly increasing cyanide concentration


+100
80
60
40
20
0
-20
-40
-60
-80
-100
% Δ
VR
HR
$\dot{V}_{O_2}$
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)

(Fig. 10) and (Fig. 11) show the relationship between changes in heart rate, ventilatory rate and oxygen consumption of the rainbow trout and brown bullhead catfish respectively.

EXPERIMENT 3 - EXPOSURE TO A CONSTANT CYANIDE CONCENTRATION

Due to equipment malfunction, only heart rate and ventilatory rates were obtained. The pattern for heart and ventilatory rates in the 4 trout used in this experiment during the initial period of increasing cyanide concentration were similar to those seen in experiment 2. Holding the cyanide concentration at a constant level (.07 mg/1) resulted in a -35% heart rate which continued throughout the exposure period (Fig. 12). The ventilation rate during this period was +30% and also remained constant.

Holding the cyanide concentration at .7 mg/1 produced a consistent heart rate in the bullheads of +60% (Fig. 13). The ventilation rate dropped from a peak of +94% at 3 hours to +52.4% over a 3 hour period and then remained at this level until the end of the experiment.

Figure 12. Changes in ventilation and heart rates of rainbow trout exposed to a linearly increasing cyanide concentration for 3 1/2 hours and then held at this level throughout the remainder of the experiment

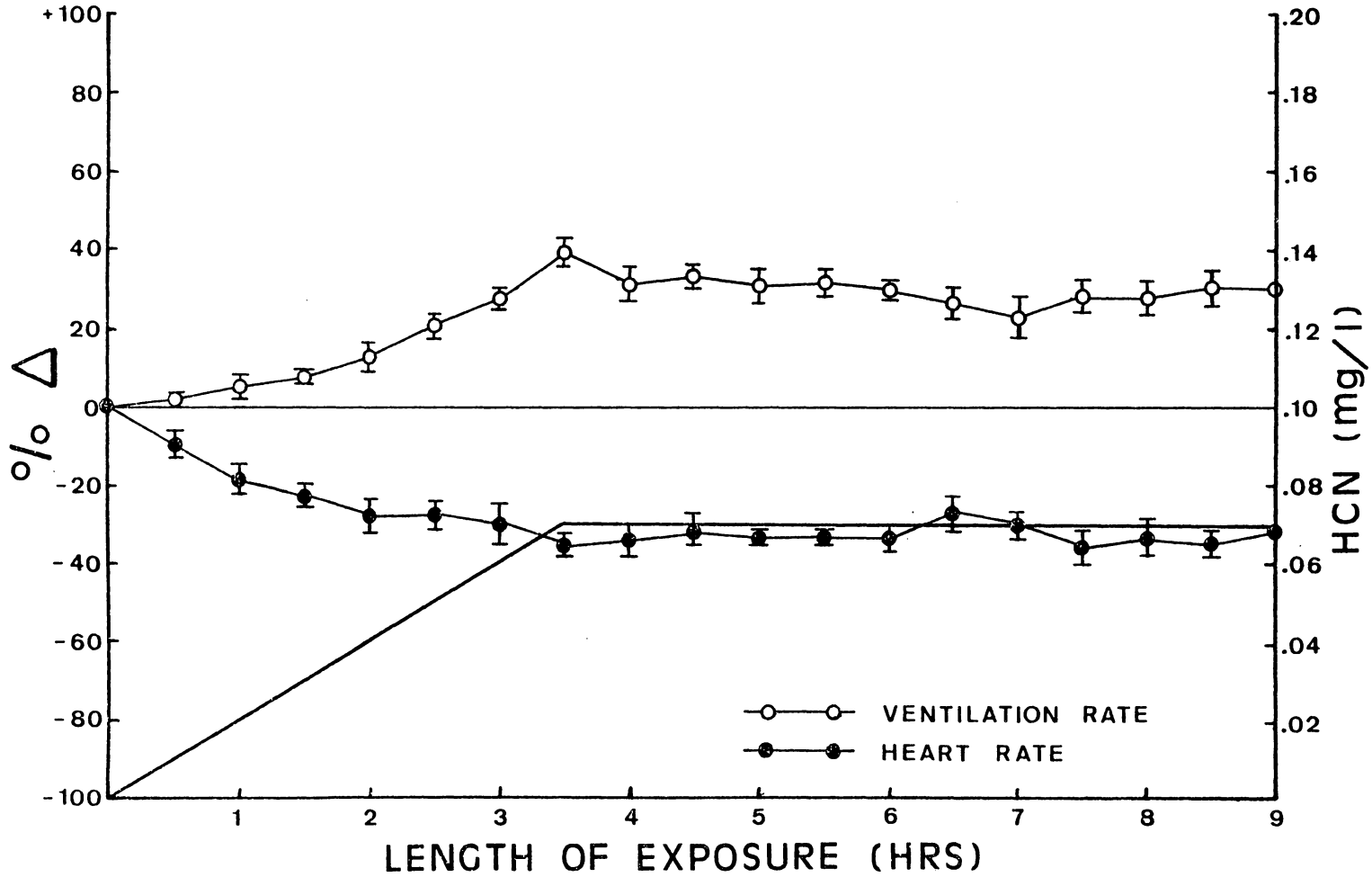
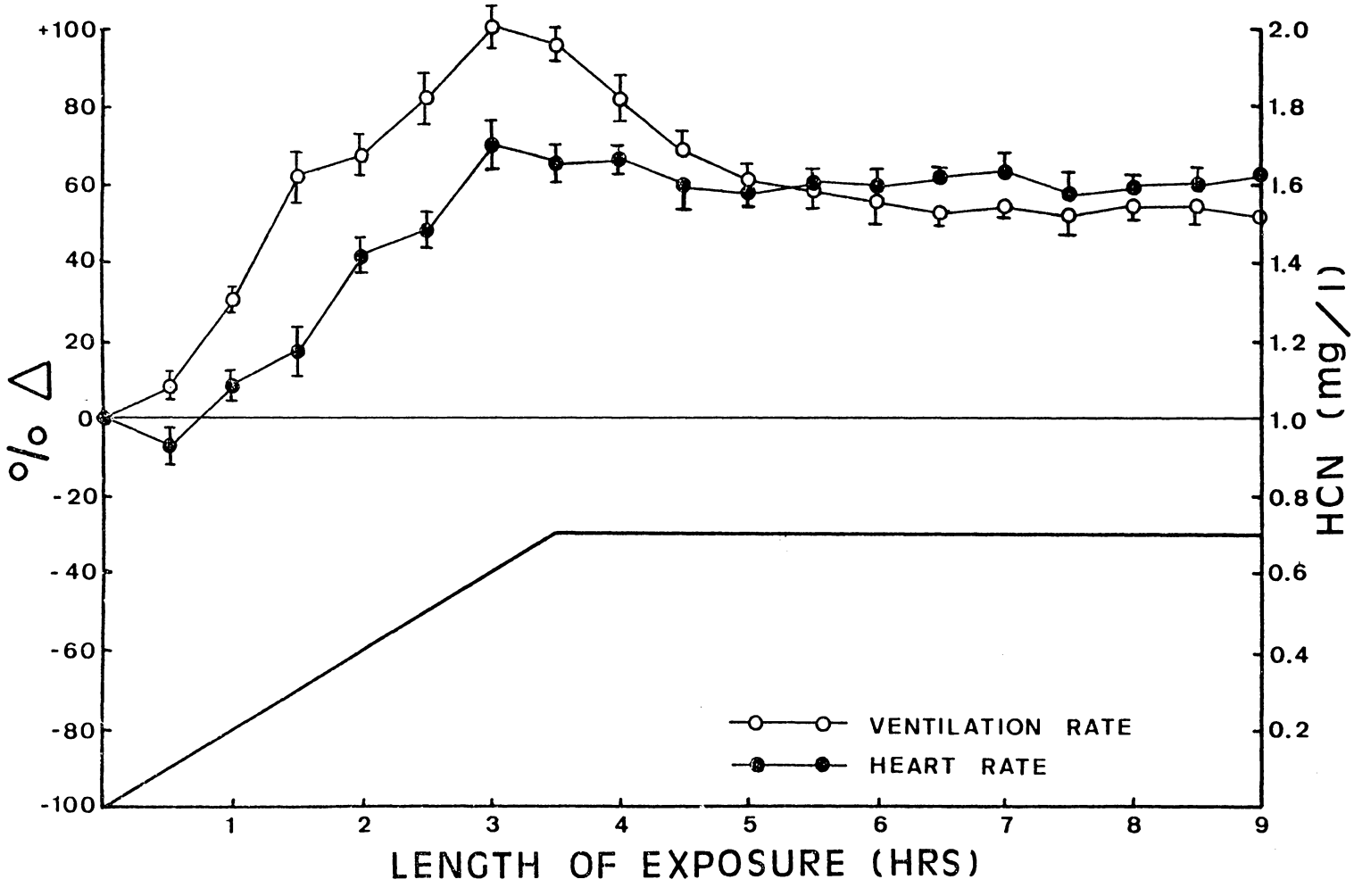

+100
80
60
40
20
0
-20
-40
-60
-80
-100
% Δ
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)
VENTILATION RATE
HEART RATE

Figure 13. Changes in ventilation and heart rates of brown bullhead catfish exposed to a linearly increasing cyanide concentration for 3 1/2 hours and then held at this level throughout the remainder of the experiment

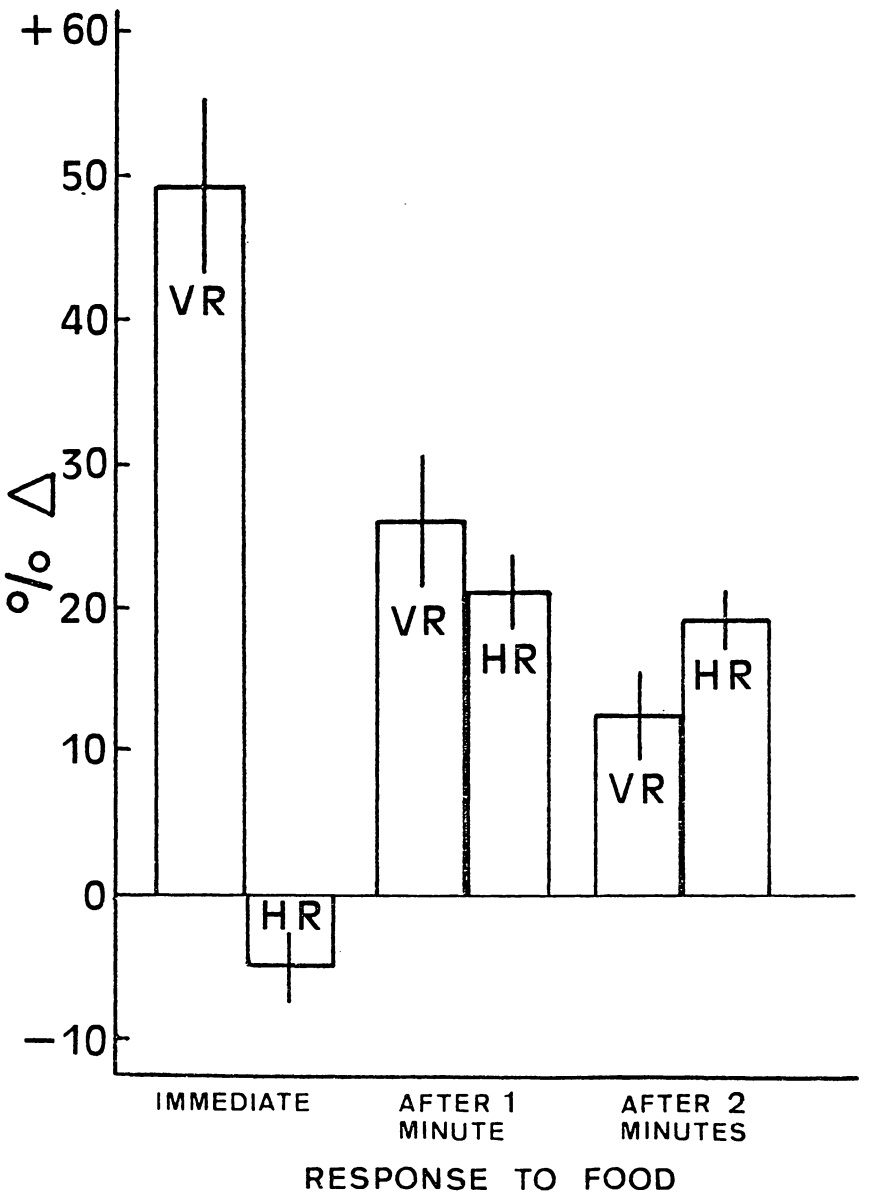

+100
80
60
40
20
0
-20
-40
-60
-80
-100
% Δ
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
HCN (mg/l)
1
2
3
4
5
6
7
8
9
LENGTH OF EXPOSURE (HRS)
VENTILATION RATE
HEART RATE

EXPERIMENT 4 - GUSTATORY STIMULATION

The purpose of this experiment was to compare the gustatory sensitivity of the brown bullhead catfish and the rainbow trout, as it was felt that some of the responses observed might be due to this form of stimulation by the cyanide. Recording of cardiac and ventilatory activity during gustatory stimulation utilized the same apparatus as above. Stimulation was achieved by injecting 5 ml of liquid food (water in which Purina Trout Chow had been soaking for 2 hours) into the inflow water line of the respirometer. The food was injected slowly to prevent a change in the water flow rate which might disturb the fish. A continuous recording was made before, during and for 2 minutes following injection of the liquid.

Injection of 5 ml liquid food into the respirometer produced an increase in ventilation and heart rate in the bullhead (Fig. 14). Exposure to identical stimulation elicited no response from the trout.

Figure 14. Changes in ventilation rate (VR) and heart rate (HR) of brown bullhead catfish induced by waterborne food stimulus


+60
50
40
30
20
10
0
−10
% Δ
VR
HR
VR
HR
VR
HR
IMMEDIATE
AFTER 1 MINUTE
AFTER 2 MINUTES
RESPONSE TO FOOD

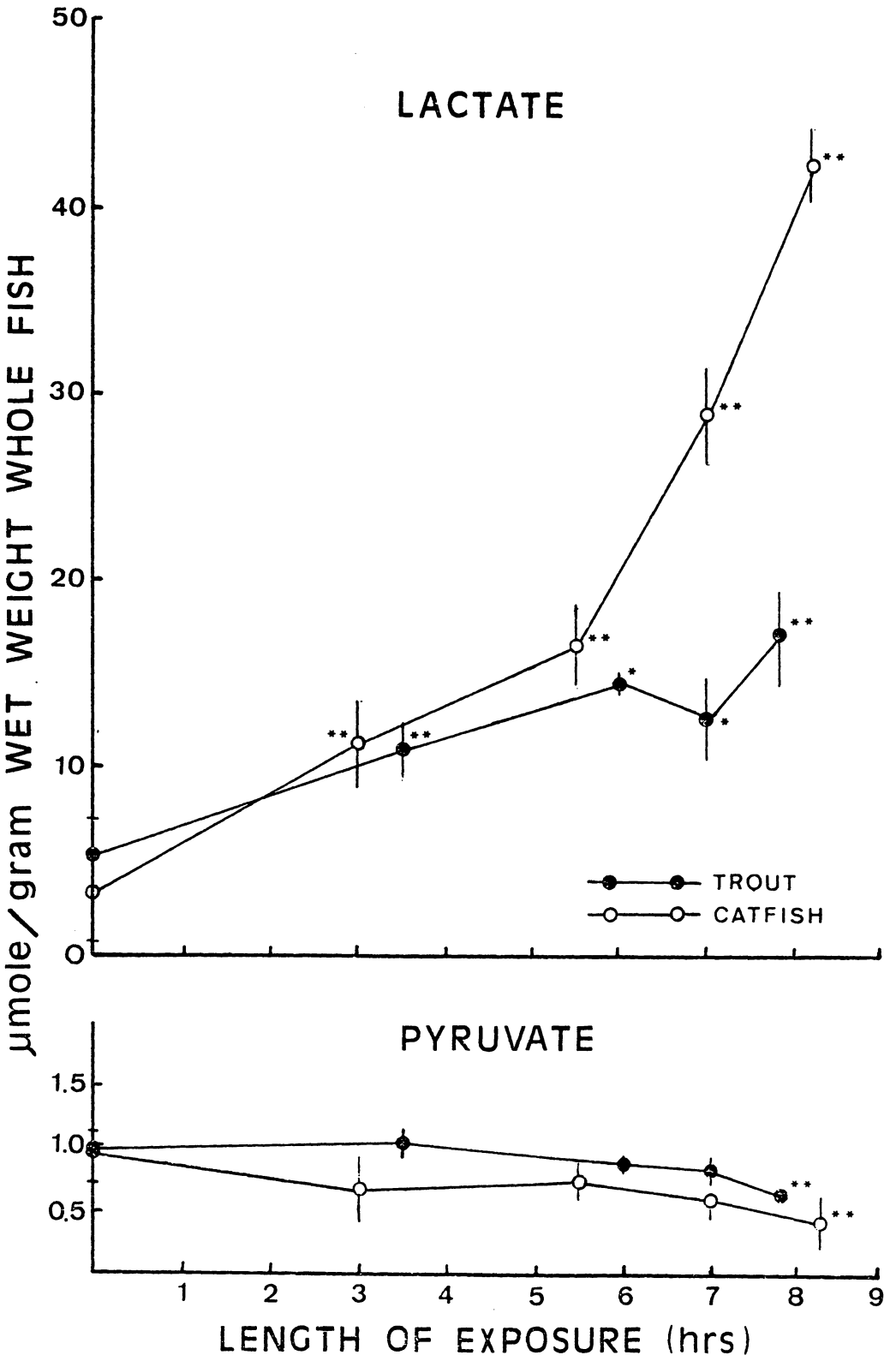
EXPERIMENT 5 - WHOLE BODY LACTATE AND PYRUVATE LEVELS

Metabolite data were treated statistically by an extension of Duncan's multiple range test to group means with unequal numbers of replications (Kramer, 1956).

Lactate levels for the fingerling trout increased significantly ($p < 0.05$) at each sampling time throughout the exposure period, reaching 3 times the control values after 7 3/4 hours (Fig. 15). Pyruvate levels showed no significant change until the last sampling period, at which time the level dropped to approximately 65% of control values.

The mean lactate level for the bullhead controls (3.31 umol/g) was somewhat less than that recorded for the trout controls (5.4 umol/g). Cyanide exposure produced a lactate level after 8 1/2 hours almost 13 times greater than control values. Pyruvate levels did not change significantly until the last sampling period (8 1/2 hours) at which time they were reduced to 40% of control values.

Figure 15. Changes in whole-body lactate and pyruvate concentrations in rainbow trout and brown bullhead catfish fingerlings exposed to a linearly increasing cyanide concentration


LACTATE
PYRUVATE
µmole/gram WET WEIGHT WHOLE FISH
LENGTH OF EXPOSURE (hrs)
TROUT
CATFISH
50
40
30
20
10
0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
9
**
*

DISCUSSION

Waterborne cyanide at lower levels induced hyperventilation and increased oxygen consumption in both the trout and bullheads. An increase in ventilation is normally employed by the fish to bring more water over the gills in response to decreases in the oxygen content of the arterial blood (Smith and Jones, 1981). The resulting increased water flow, coupled with changes in gill vascularization and decreased resistance, help maintain the blood oxygen levels during environmental hypoxia or muscular exercise. Hyperventilation is achieved via an increased ventilation rate and/or stroke volume; the relative change in the two variables depends on the species. Regardless of which mode is used predominately, hyperventilation exerts a toll due to the increase in oxygen consumption from heightened ventilatory muscle use. The tradeoff between increased oxygen consumption and the benefits derived from greater oxygen diffusion into the blood can be beneficial when environmental hypoxia is the problem. However, in cyanide poisoning the oxygen content of the blood is not altered so increased ventilation has no benefit and merely leads to an elevation in energy demand.

An increase in ventilation in mammals and fish is a general characteristic of cyanide poisoning and has been recognized for over 50 years. Gettler and St. George (1934) stated that hyperventilation was an initial response following cyanide inhalation in man, and Brinley (1927) observed an increase in rate and greater flaring of the opercula in the chub when exposed to lethal levels of cyanide. Hyperventilation in mammals is attributed to stimulation of extracranial, carotid and aortic chemoreceptor mechanisms by cyanide (Levine, 1975). In anesthesized trout and carp perfused with aerated water, injection of 10 ug/kg NaCN into the ventral aorta resulted in hyperventilation, leading Echlancher and Dejours (1975) to conclude that vascular chemoreceptor stimulation by cyanide was also present in fish, although the site(s) of these receptors are unknown.

The hyperventilation seen in the trout and bullheads in this study agree with an early investigation by Jones (1947) in which threespine sticklebacks were placed in 1 ppm NaCN for 30 minutes. During the first 5 minutes there was a 75% increase in ventilation rate followed by a rapid decline to 50% below normal levels at which time the fish were placed in fresh water and complete recovery occurred. Working with largemouth bass at 3 different (sublethal) cyanide concentrations, Morgan and Kuhn (1974) reported dose-dependent in-

creases in ventilation rate over the first 24 hours of exposure followed by a return to slightly above normal levels during the next 6 days. According to the authors, the reason for this acclimation was due, "either to the efficiency of the detoxification enzyme system, or to the activity of cyanide as a respiratory depressant, or both." (p. 76).

In the trout, the increase in ventilation during the hours immediately preceeding respiratory arrest could be due to a decrease in blood pH from a buildup of lactic acid which causes an increase in ventilation (Randall, 1982). However, based on the whole-body lactate levels for the bullheads, a lactic acidosis should have occurred in this species, yet no increase was seen. Since whole-body, rather than blood lactate levels were obtained, the possibility exists that the lactate flux from the muscles to the blood was delayed because of peripheral vasoconstriction in the bullhead, thus preventing acidosis. If acidosis was present, because of the comparatively massive cyanide concentration used with the bullheads, the continued decline in ventilation was perhaps due to a direct inhibitory effect of cyanide on the medullary respiratory control center and/or respiratory musculature, preventing any increase in ventilation regardless of the amount or nature of the stimulus.

Cyanide exposure produced somewhat similar changes in ventilation in both species, however the changes in heart rate were strikingly different. The trout developed an immediate and progressive bradycardia throughout the exposure period, whereas the bullheads exhibited an initial bradycardia lasting less than 30 minutes followed by tachycardia. These divergent results perhaps can be explained by looking at some of the extrinsic factors controlling heart rate in fish.

The teleost heart receives innervation from the parasympathetic branch of the autonomic nervous system via cardiac branches of the vagus (X cranial) nerve (Randall, 1967). Activity in the vagus causes release of acetylcholine which acts on the heart, via muscarinic acetylcholine receptors, to decrease the heart rate. The afferent arm of this reflex is from receptors which are believed to respond to changes in arterial Po_2 and are located on the first gill arch in the region of the efferent vessel (Daxboeck and Holeton, 1978). Although cyanide does not affect the gas composition of the blood or the amount of oxygen in the environment, studies in mammals indicate that it can directly stimulate the chemoreceptors sensitive to changes in arterial Po_2 (Jacobs et al., 1971), and thus initiate an hypoxia response, even though the arterial Po_2 is normal.

Atropine reduced the degree of bradycardia during early stages of exposure in the trout, suggesting that the bradycardia was due to vagal inhibition. The diminishing effect of atropine as the exposure progressed indicates that the cause of the bradycardia shifted from an increase in vagal cholinergic tone to a decrease in heart function due to myocardial anoxia (a technical misnomer, since oxygen was available but could not be utilized) and decreased pacemaker effectiveness due to cyanide's ability to affect cell membranes thereby altering their ability to conduct impulses (Schoepfle, 1963).

The greatly elevated T wave seen in the ECGs of both species during cyanide exposure demonstrate the inhibitory effect of that poison. Large T waves are characteristic of hyperkalemia (elevation of serum potassium) resulting from tissue anoxia (Smith and Kampine, 1984). Increased potassium lowers the resting potential of the cardiac cells causing a decrease in the rate and force of contraction. Severe hyperkalemia can stop the hearts of lower vertebrates in diastole (Prosser and Brown, 1961) and this could possibly account for the missed beats seen in the two trout prior to cardiac arrest. Increased T waves have also been recorded in trout exposed to environmental hypoxia (Bahr, 1973) and to chlorine, which prevents oxygen diffusion through the

gills by producing a buildup of mucus on the gill surface (Bass and Heath, 1977). Thus, T wave changes are indicative of internal tissue hypoxia regardless of how that hypoxia is induced.

Cardioacceleration in fish can result from a decrease in vagal cholinergic tone or by direct adrenergic nerve action via the vagosympathetic nerve trunk (Gannon and Burnstock, 1969), the latter being relatively more important at high temperatures (Wood et al., 1979). Catecholamines (CA) such as adrenaline and noradrenaline, released from the chromaffin tissue into the blood, produce a positive chronotropic effect by increasing self-excitation rates of pacemaker fibers (Cobb and Santer, 1973), and increase ventral and dorsal aortic blood pressures. Various types of harsh stress (i.e. handling, anoxia, exhaustive swimming) induce an increase in plasma CA, as do non-specific nociceptive reflexes such as electrical or chemical stimulation of the skin, gills, fins and stomach. The extent and swiftness of CA release depends on the species studied and temperature. Thus, the increase in heart rate seen in the bullheads during exposure to food and cyanide could be due to direct adrenergic stimulation of the heart or by circulating CA, although no direct evidence for this (measurement of plasma CA) was obtained. The decreasing heart rate fol-

lowing tachycardia is probably due to myocardial anoxia as in the trout since atropine had no effect and the ECG pattern was characteristic for this condition.

The greater magnitude of the response in cardiac and ventilatory activity by the bullheads is probably due to greater gustatory sensitivity of this species as described by Atema (1971) and confirmed in this study by injecting food soaked water into the chamber, coupled with the high cyanide concentration necessary to produce death in 8 hours. Cyanide has a bitter taste and odor and could easily elicit a classical stress (adrenergic) response from stimulation of gustatory receptors. This sympathetic response, because of its intensity, could override the parasympathetic response produced by stimulation of oxygen receptors by cyanide.

In further considering the differences in response between the two species, ecological differences may be critical. The trout lives in well-aerated water and is considered a hypoxia sensitive species and thus could have a higher set point for control of cardiac rate and respiration when exposed to low oxygen levels. This is in contrast to the bullheads which inhabit warm, sluggish water with fluctuating oxygen levels. Also, the trout live in clear water enabling prey detection by vision rather than gustation or olfaction; its sense of taste (defined here as the number of

tastebuds on the external skin surface) is less sensitive than the bullhead.

Bradycardia in the trout was initiated when the cyanide level in the aquarium was approximately .005 ppm. If the chemoreceptors responsible for changes in the heart rate in response to oxygen levels are internal, rather than external, bradycardia elicited by cyanide stimulation had to occur at very low concentration since it is improbable that the cyanide concentration in the blood could be the same as in the water. If gustatory stimulation did occur and it did produce an adrenergic stress response, the response may not have been great enough to override the already established parasympathetic influence on the heart, or it could have occurred at a time in which the heart was unable to significantly increase its rhythm due to myocardial anoxia. However, it is interesting to note that the heart rate did increase slightly during the last hours of the experiment. This could merely signify the total loss of extrinsic control and represent the intrinsic heart rate (although slower due to myocardial tissue anoxia).

An examination of the oxygen consumption and lactate data from the trout and bullheads provide insight into the mechanisms responsible for a tenfold difference in cyanide concentration required to produce death.

Heightened oxygen consumption in the trout represents the increase in aerobic respiration to meet the additional metabolic demand placed on the fish by the increase in ventilatory and motor activity. Although the percentage of the oxygen consumption attributable to individual muscle groups depends on the type and degree of muscle activity, oxygen consumption measurements in fish subjected to various forms of stress all show a correlation between increased muscular activity (whether respiratory, somatic, or both) and increased oxygen consumption. The oxygen consumption of the trout did not fall below pre-exposure levels until 30 minutes to 1 hour before ventilatory arrest (Fig. 10), and lactate levels did not increase appreciably during this time period (Fig. 15). This implies that the cyanide concentration and rate of delivery used in this experiment did not produce significant inhibition of aerobic respiration until at least 6 hours into the run. However, once inhibition was established (probably due to the cyanide detoxification system being overwhelmed by the accumulation of cyanide) the trout were only able to survive for a very short period of time. This is probably due to the inability to effectively augment the rapidly declining aerobic respiration with anaerobic respiration, as indicated by an increase in whole-body lactate levels at 7 3/4 hours of only 53% greater than those

obtained when oxygen consumption was at its peak (3 1/2 hours). Thus, depressed aerobic respiration (due to cytochrome oxidase inhibition), and insufficient anaerobic respiration probably led to rapid failure of the medullary respiratory control center and/or respiratory musculature resulting in sudden ventilatory arrest.

The bullheads were able to tolerate a tenfold higher cyanide concentration than the trout, but they did this by different methods. Whereas the trout were able to prevent a significant decrease in aerobic respiration for an extended period of time, the bullheads experienced a significant degree of aerobic respiratory inhibition during the first hours of the exposure period, as shown by lactate levels recorded during peak oxygen consumption that were 3 1/2 times greater than controls. This implies that even though aerobic respiration had increased, this increase was insufficient to satisfy the metabolic demand from heightened ventilatory and heart rates, and augmentation by anaerobic respiration was required. Cyanide does not affect the oxygen content of the blood and it is doubtful that circulation was impaired, thus, anaerobiosis was in response to inhibition of cytochrome oxidase in the tissues rather than a deficit in oxygen delivery. Since it appears that at least partial metabolic inhibition by cyanide occurred within the first 3 hours of

exposure, the bullheads had to contend with this inhibition an average of 5 - 6 additional hours. This was accomplished by effective augmentation by anaerobiosis, possibly aided by an adaptive lowering of overall metabolic demand.

It was previously suggested that the decrease in heart rate was due to the direct effect of cyanide, however an adaptive slowing of the heart in the bullhead to lower the metabolic demand cannot be entirely ruled out. Atropine injection did not reduce the bradycardia (suggesting that it was not due to vagal inhibition), but the results obtained were highly variable, possibly due to the route of injection selected. Injection of atropine directly into the pericardial cavity necessitates that the catheter is positioned so that the atropine washes over the heart in order for it to be absorbed by the myocardium. Movement of the catheter within the cavity could cause inconsistencies in the amount of atropine absorbed, thus altering the results. Post-mortem examination of each bullhead used in the atropine study revealed that the catheter was in the pericardial cavity but the exact position of the catheter in relation to the heart could not be precisely determined.

The decrease in ventilation rate of the bullheads, which dropped below pre-exposure levels after approximately 4 hours of exposure, could also be, in part, an adaptive response to

cyanide. A decline in ventilation rate would effectively lower the metabolic demand and has been reported to be an adaptation to environmental hypoxia in this species (Marvin and Heath, 1968). The periods of apnea seen in the bullhead could be a measure to further reduce the metabolic demand. Cutaneous respiration in the black bullhead catfish, a closely related species, represents about 30% of the total oxygen uptake while the oxygen consumption of the skin has been estimated at 20% of the total oxygen requirement (Nonnotte, 1984). Thus, the role of the skin as an oxygen exchanger for the benefit of other organs could be maximized by the bullhead during cyanide exposure while concomitantly reducing the metabolic demand of the respiratory musculature by stopping gill ventilation for short periods of time.

The reduction in metabolic demand from reduced heart and ventilatory rates, coupled with a decrease in skeletal muscle tone would certainly allow prolonged survival with greatly reduced aerobic respiration and anaerobic respiratory augmentation.

Anaerobiosis in trout and bullhead is by glycolysis, with lactate being the primary endproduct (Burton and Spehar, 1971). Measurement of lactate from various tissues (blood, muscle, liver) has often been used to evaluate anaerobic respiration during exercise and environmental hypoxia, how-

ever, due to the dynamic and compartmental nature of lactate production, transportation and catabolism, it has been impossible to quantify accurately the metabolic contribution of anaerobiosis to total energy utilization. Additionally, interspecific comparisons of lactate levels from tissue samples may lead to false conclusions about the extent of anaerobiosis, due to differences in the rate in which lactate moves from one compartment to the next. For example, blood lactate levels could vary between species undergoing identical rates of lactate production simply due to differences in the rate in which lactate was released from the muscles in which it was produced.

Measurement of whole-body lactate levels overcomes this problem and allows a direct comparison of anaerobiosis between the two species regardless of any differences in lactate flux between tissues. Lactate levels from control trout (5.41 umol/g) were higher than bullhead controls (3.31 umol/g) and probably reflect the greater intrinsic activity level of the trout. Similar differences in control lactate levels in muscle tissue between these two species have been reported by Burton and Spehar (1971).

The whole-body lactate level of the trout recorded during peak oxygen consumption (3 1/2 hours of exposure) was only twice that of control values and a significant increase

did not occur until the last sampling period (7 3/4 hours), which was only three times the control values. The lactate level for the bullhead after 3 hours of exposure was almost 3 1/2 times the control lactate value, and increased significantly at each consecutive sampling time, reaching a level approximately 13 times the control after 8 1/2 hours of exposure.

A comparison of whole-body lactate levels as an indicator of anaerobic capacity between these two species during the period before significant cyanide inhibition occurred is complicated by the fact that there is no way to determine what portion of the metabolic demand was met by aerobic respiration, hence the amount of anaerobiosis needed for augmentation cannot be established. It is possible to estimate the percentage contribution of anaerobic and aerobic energy sources to total energy utilized, however this required oxygen consumption measurements as well as whole-body lactate measurements. In this study the rates of oxygen consumption of the trout and bullhead fingerlings were not obtained, and using the oxygen consumption data from the adults is problematic for two reasons: (1) the oxygen consumption per unit weight is greater for smaller than for larger fish (Beamish, 1964), and (2) the activity levels of the fingerling and the adults were not the same. The adults were confined in the

respirometer which limited movement, but the fingerlings were contained in a cylinder of sufficient size to permit swimming. Because of these factors, attempting to partition the aerobic and anaerobic contribution to total energy utilization of the fingerlings would not yield meaningful data.

A comparison of anaerobiosis between the two species can be made when considering the lactate levels attained during the last sampling period, a time of maximal cyanide inhibition. During this time period a far greater increase in lactate was recorded in the bullheads, possibly due to greater amounts of available fuel for glycolysis. Glycogen, the primary fuel for glycolysis, has been measured in various species of fish, and while the levels vary between tissue types the relative total body amounts are not strikingly different among species (Love, 1970). Recently, however, DiAngelo (personal communication), measured glycogen levels in the brains of brown bullhead catfish which were four times greater than those obtained from the brains of rainbow trout, enabling the bullheads to survive total anoxia for comparatively longer periods of time.

The small size of the fish used in this portion of the study may be a factor in the relative amounts of fuel for glycolysis in these two species. Both species were deprived of food for 24 hours prior to experimentation. The high ac-

tivity level observed in the rainbow trout, compared to the sluggish behavior of the bullheads, could have resulted in fuel depletion during the acclimation and test period which would have limited the amount available for glycolysis.

Based on the preceeding discussion, it is suggested that increases in whole-body lactate in fish offer an indication that anaerobic respiration is occurring, however, due to the dynamic nature of glycolysis, quantitative information on the rate of anaerobiosis cannot be obtained.

In conclusion, the hyperventilation, bradycardia and increased motor activity seen in the rainbow trout exposed to an increasing cyanide concentration are similar to the responses seen during exposure to environmental hypoxia. During hypoxia, these cardiac and ventilatory responses are effected by central and peripheral chemoreceptors sensitive to decreases in arterial oxygen content and/or Po_2. Cyanide is able to stimulate these chemoreceptors and thus elicit the typical hypoxia response. Hyperventilation, coupled with heightened motor activity increased the metabolic demand necessitating a higher rate of oxygen consumption. As aerobic respiratory inhibition by cyanide progressed, there was a shift from aerobic to anaerobic respiration, producing a rise in whole-body lactate levels. Death was by respiratory arrest, probably the result of anoxia in the medullary respir-

atory control center caused by insufficient anaerobic augmentation to meet energy demands.

When viewed over the time-course of the cyanide exposure, it appears that cyanide was able to elicit two completely different responses in the bullhead catfish - an initial stimulation followed by suppression. An adrenergic response, characterized by hyperventilation and tachycardia, occurred during the first two hours of exposure. It is hypothesized that these responses were the result of stimulation of external chemoreceptors (gustatory and/or olfactory) by cyanide. Support for this hypothesis was obtained by exposing both species to a waterborne food stimulus while recording cardiac and ventilatory performance. The external food stimulus elicited no response in the trout, however the identical stimulus produced tachycardia and hyperventilation in the bullheads. The sensitivity of the bullheads to external chemical stimuli reflects their gustatory, rather than visual, manner of food detection.

Cardiac and ventilatory rates in the bullheads peaked after approximately 2 1/2 hours, followed by linear decreases in both parameters which continued through the remainder of the exposure period. These decreasing rates may have been the direct result of cyanide inhibition in ventilatory and

cardiac tissues, however an adaptive slowing of these functions to reduce metabolic demand cannot be ruled out.

Increasing bradycardia and progressive slowing of ventilatory and motor activity to reduce metabolic demand during gradual environmental hypoxia have been reported in this species, and are initiated by stimulation of central and peripheral chemoreceptors sensitive to changes in arterial oxygen content. If cyanide did stimulate these internal chemoreceptors, an hypoxia response characteristic for this species, could have resulted. Looking at the data from the cyanide-exposed bullheads a linear reduction in ventilatory and cardiac rates with a concomitant lowering of oxygen consumption did occur. The only difference between the cyanide-induced cardiac and ventilatory responses seen here and those induced by hypoxia is the relative difference in cardiac and ventilatory rates when the response began. In hypoxia, the "starting points" were those rates recorded during normoxia, but with the cyanide-induced response, the "starting points" were values above pre-exposure levels resulting from the adrenergic response to the external chemoreceptor stimulation by cyanide. Since the rates were initially high, a gradual reduction would still result in rates above pre-exposure levels with a concomitant increased oxygen consumption for several hours.

The shift from the excitatory response to the inhibitory response could be the result of decreased external stimulation by cyanide (gustatory and/or olfactory desensitization?), being replaced by greater stimulation from the internal chemoreceptors. The relative sensitivities of the receptors could relate to the bullhead's habitat - high external chemoreceptor sensitivity because of the manner of food detection and chemical communication in murky water, and lower internal chemoreceptor sensitivity adapted to living in water with low, fluctuating oxygen content.

Aerobic respiratory inhibition by cyanide did occur and was augmented by anaerobiosis, as indicated by an increase in whole-body lactate levels. This aerobic inhibition and insufficient anaerobic augmentation produced tissue anoxia and finally, death. An adaptive lowering of the metabolic demand may have prolonged, but could not prevent, the ultimate fate of the bullhead when exposed to a massive cyanide concentration.

SUMMARY

1. Adult rainbow trout and brown bullhead catfish were surgically fitted with electrocardiographic (ECG) leads; catheters inserted into the buccal and opercular cavities; and held in flow-through respirometers. Following an acclimation period, the fish were subjected to an increasing cyanide concentration selected to produce death in 8 hours for each species. ECG, oxygen consumption, and pressure changes associated with ventilatory activity were recorded at 15 minute intervals until the fish died - as indicated by cessation of ventilatory movements. Data were calculated as percent change from pre-exposure values in individual fish for each measured parameter.

2. Ventilation rate and amplitude were measured in trout exposed to an increasing cyanide concentration of 0.02 ppm HCN/hr. Hyperventilation was evident throughout the exposure period, although the rate and ventilatory amplitude fluctuated, and was variable between fish. In all trout the last hour of cyanide exposure was characterized by rapid, shallow respirations followed by respiratory arrest.

3. Cyanide exposure produced immediate and progressive bradycardia (slowing of the heart rate) in the trout. Atropine, delivered through a catheter entering the pericardial cavity, reduced the bradycardia when injected at hourly intervals during the first four hours of exposure but had no effect when injected after this time period. This suggests that the bradycardia was initially due to increased vagal tone, but later due to the direct effect of cyanide on the heart tissue. Cardiac arrest occurred in 2 of 7 fish tested.

4. The cardiac and ventilatory responses of the trout during cyanide exposure are similar to those seen in trout subjected to environmental hypoxia. Hyperventilation and bradycardia during cyanide exposure were attributed to cyanide stimulation of internal chemoreceptors which normally respond to changes in arterial oxygen content and elicit an hypoxia response.

5. The hyperventilation and increased motor activity of the trout during cyanide exposure resulted in an increased metabolic demand which produced a higher oxygen consumption rate for a majority of the exposure period. This suggests that significant aerobic respiratory inhibition by cyanide did not occur until the last few hours of the experiment, but once inhibition did occur the trout rapidly succumbed.

6. The increasing cyanide concentration required to produce an 8 hour survival time for the bullheads was 0.2 ppm HCN/hr. Hyperventilation occurred, as in the trout, but peaked within 3 hours and then ventilatory rate and amplitude continually dropped until cessation. Periods of apnea were observed during the latter stages of exposure.

7. Cyanide exposure in the bullheads produced bradycardia lasting less than 30 minutes and was followed by tachycardia, which peaked after 3 hours. The heart rate then dropped to the pre-exposure level followed by bradycardia which persisted until the end of the experiment. The hearts continued to beat after ventilation had stopped. Bradycardia during the first 30 minutes of exposure was reduced by pericardial atropine injection, however atropine injection during the later period of bradycardia had no effect.

8. In an experiment designed to record changes in ventilatory and cardiac rates of fish exposed to a waterborne food stimulus, it was shown that in bullheads, stimulation of external chemoreceptors by food produced tachycardia and hyperventilation. This did not occur in the trout.

9. In bullheads, the rate of oxygen consumption paralleled the changes in cardiac and ventilatory rates. It was suggested that an adaptive lowering of the ventilatory and cardiac rates, in addition to the direct effect of cyanide,

may have reduced the metabolic demand and lowered the oxygen consumption.

10. The shift from aerobic to anaerobic respiration in both species was evaluated by measuring whole-body lactate and pyruvate levels of fingerlings exposed to cyanide. These data show that anaerobiosis was used to augment a declining aerobic respiration in both species, but based on comparatively higher lactate levels in the bullhead fingerlings, anaerobiosis by the trout was far less, and unable to effectively augment the aerobic respiration to the same extent as in the bullheads.

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