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TORRANS, EUGENE LESLIE

ACUTE TOXICITY OF HYDROGEN-SULFIDE TO FISH DURING HARVESTING OPERATIONS ON COMMERCIAL CATFISH FARMS: CAUSE, PREVENTION AND CURE

The University of Oklahoma PH.D. 1980

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ACUTE TOXICITY OF HYDROGEN SULFIDE TO FISH DURING HARVESTING OPERATIONS ON COMMERCIAL CATFISH FARMS: CAUSE, PREVENTION AND CURE.

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

EUGENE LESLIE TORRANS

Norman, Oklahoma

1980

ACUTE TOXICITY OF HYDROGEN SULFIDE TO FISH DURING HARVESTING OPERATIONS ON COMMERCIAL CATFISH FARMS: CAUSE, PREVENTION AND CURE.

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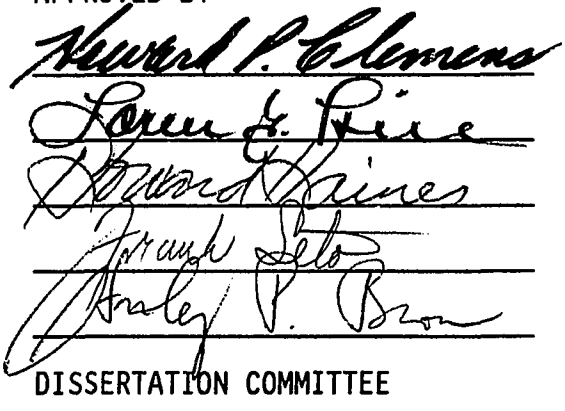

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ACKNOWLEDGMENTS

I would like to express my deep gratitude to Dr. Howard P. Clemens, under whose guidance this study was done, for convincing me that "good enough" is rarely good enough, but merely a foundation on which to build.

I am especially grateful to my wife Susan, for her advice, support, understanding and encouragement during some very difficult times.

I wish to thank the members of my committee, Professors Howard Clemens, Howard Haines, Harley Brown, Frank Seto and Loren Hill for their numerous helpful suggestions at all stages of this work, and Dr. Walter Dillard, in whose lab a part of the work was done.

I owe a special debt of gratitude to Kevin Wright, the owner of Sooner Fish Farm, for allowing me to use his facilities, and for all of his advice and cooperation. I am also endebted to Sheila Hand for her help in the preparation of the manuscript.

I express my sincere appreciation to members of the faculty, staff and students for extending various assistance and courtesies during the period of my study at the University of Oklahoma.

I wish to thank the University Research Council for providing funds for the purchase of fish, and the University of Oklahoma Biological Station for providing the computer time.

PREFACE

This dissertation is presented as two separate chapters. Chapter I will be submitted to Limnology and Oceanography, and Chapter II will be submitted to Comparative Biochemistry and Physiology.

TABLE OF CONTENTS

	Page
ABSTRACT	vi
LIST OF TABLES	viii
LIST OF ILLUSTRATIONS	x
Chapter I	1
ABSTRACT	2
INTRODUCTION	4
METHODS	7
RESULTS	14
DISCUSSION	21
LITERATURE CITED	31
TABLES	35
FIGURES	41
APPENDICES	53
Chapter II	71
ABSTRACT	72
INTRODUCTION	74
METHODS	77
RESULTS	83
DISCUSSION	89
LITERATURE CITED	102
TABLES	107
FIGURES	115

ABSTRACT

Hydrogen sulfide dissolved in the interstitial water of the sediment of commercial catfish culture ponds in Oklahoma was measured from February, 1978, through November, 1979.

The maximum concentration of sulfide in the sediment was less than 1.2 $mg \cdot liter^{-1}$ from December through February, and was as high as 18.1 $mg \cdot liter^{-1}$ in the warmer weather. The maximum sulfide concentration usually occurred from 5.7 to 12.8 cm below the surface of the sediment from December through February, and within the top two centimeters of the sediment from July through September.

Unconsumed fish food apparently resulted in the high sulfide concentrations seen near the feeding area in May or June and September of both years. Dissolved sulfide concentrations decreased near the feeding area in July and August, when the water temperatures were over 28°C and the fish consumed nearly all of the food given. The direct effect of unconsumed food on dissolved sulfide was limited to the vicinity of the feeding area.

The highest dissolved sulfide concentrations found at shoreline areas 80 meters from the feeding area occurred in September, and apparently resulted from the die-offs and deposition of phytoplankton. At those areas, the highest sulfide concentrations were at the location that was sheltered from the wind, and where the deposition of suspended matter was the greatest.

Hydrogen sulfide competitively inhibited cytochrome oxidase. The enzyme was inhibited *in vitro* 18% by 10^{-7} M H_2S, 64% by 10^{-6} M, and 100% by 10^{-4} M.

A measureable reduction in cytochrome oxidase activity was seen after exposure of fish to 0.1 mg/l H_2S at 10°C for only five minutes. Exposure of channel catfish to 0.5 mg/l H_2S at 20°C resulted in hyperpnea, followed by apnea, and finally respiratory arrest. When exposed to 0.1 mg/l H_2S at 20°C for 30 minutes, the cytochrome oxidase activity of the brain was inhibited 40% and that of the gill was inhibited 74%, while blood lactate rose from 11.6 to 38.1 mg/100 ml.

Sulfide-inhibited cytochrome oxidase recovered rapidly *in vivo*. Brain cytochrome oxidase rose from a 50% inhibition to control levels after six hours at 10°C. The recovery rate was similar in both the brain and gill.

Mortalities of fish resulting from exposure to hydrogen sulfide on commercial fish farms can be avoided by; 1) reducing dissolved sulfide concentrations in the sediment through pond design, improved feeding practices, and the use of lime in the ponds, 2) landing fish in areas of the ponds with lower sulfide concentrations, 3) treating the water with potassium permanganate during harvesting to oxidize the sulfide in the water, 4) reducing the physical exertion of the fish after sub-lethal exposure to H_2S, and 5) allowing for short-term recovery before transportation.

LIST OF TABLES

TABLE Page

Chapter I.

1. Coefficient of variation of the methylene blue spectrophotometric test for hydrogen sulfide..... 35
2. Average monthly wind speed and direction..... 36
3. Effect of wind direction on maximum dissolved sulfide concentrations in sediment profiles..... 37
4. Effect of wind direction on total filterable suspended matter..... 38
5. Effect of draining and drying the pond bottom on the maximum dissolved sulfide concentration in the sediment, Pond 5..... 39
6. Effect of draining and drying the pond bottom on the maximum dissolved sulfide concentration in the sediment, Pond 6..... 40

Chapter II

1. Cytochrome oxidase activity in four tissues of three fish species.....107
2. August and March cytochrome oxidase activity in tissues of channel catfish fingerlings (10-20 cm total length) and food fish (40-47 cm total length).....108
3. Effect of pH on hydrogen sulfide inhibition of cytochrome oxidase *in vitro*..... 109
4. Cytochrome oxidase activity in eight tissues of fathead minnows (5 to 7 cm total length), before and after lethal exposure to hydrogen sulfide.....110

5. Cytochrome oxidase activity in six tissues of fingerling channel catfish (10-15 cm total length), before and after lethal exposure to hydrogen sulfide....111
6. Effect of temperature on uptake of hydrogon sulfide.....112
7. Changes in blood lactate, and brain and gill cytochrome oxidase activity in 10 to 15 cm channel catfish, during exposure to 0.1 mg/l un-ionized sulfide.....113
8. Blood parameters of three species of fish sampled in August and March.....114

LIST OF ILLUSTRATIONS

FIGURE Page

Chapter I

1. Diffusion-chamber sampler used in study..... 41
2. Equilibration of diffusion-chamber sampler with hydrogen sulfide in aqueous solution..... 42
3. Equilibration of diffusion-chamber sampler with hydrogen sulfide in the bottom sediment of Pond 2..... 43
4. Map of study area, showing inlets, drains, sample locations and feeding areas..... 44
5. Primary sediment sample locations in Pond 2..... 45
6. Pond bottom water temperature (monthly mean, standard error of the mean, and range) 46
7. Average daily feed and total monthly feed given to Pond 2..... 47
8. Four typical dissolved sulfide profiles in the sediment of a commercial culture pond..... 48
9. Maximum dissolved sulfide concentrations in the sediment at; a) the feeding areas of Pond 2 and other ponds, and b) locations 15 meters from the north and south shores of Pond 2..... 49
10. Depth in the sediment of the maximum dissolved sulfide concentrations at; a) the feeding area of Pond 2, and b) locations 15 meters from the north and south shores of Pond 2..... 50

11. Chlorophyll α and sulfate in Pond 2..... 51

12. Maximum sulfide concentrations in sediment profiles at two shoreline locations in Pond 2..... 52

Chapter II

1. Optimum pH for cytochrome oxidase activity..... 115

2. Optimum buffer molarity for cytochrome oxidase activity..... 116

3. Effect of tissue dilution on cytochrome oxidase activity..... 117

4. Response of opercular muscle (O.M.) and heart (H) to hydrogen sulfide..... 118

5. Lineweaver-Burke plot of control and sulfide-inhibited cytochrome oxidase..... 119

6. Effect of sulfide concentration on cytochrome oxidase activity *in vitro*..... 120

7. Effect of exposure time at 10°C on *in vivo* cytochrome oxidase activity of 10-15 cm channel catfish brain and gill, at four different un-ionized sulfide concentrations..... 121

8. Effect of exposure time on *in vivo* cytochrome oxidase activity of 10-15 cm channel catfish brain and gill, in groups of fish with four different methemoglobin concentrations..... 122

9. *In vivo* recovery of cytochrome oxidase activity in 10-15 cm channel catfish brain and gill..... 123

CHAPTER I

FACTORS AFFECTING THE PRODUCTION OF HYDROGEN SULFIDE IN THE BOTTOM SEDIMENTS OF COMMERCIAL CATFISH CULTURE PONDS

By Eugene Leslie Torrans

Department of Zoology, University of Oklahoma, Norman, Oklahoma, 73019

ABSTRACT

Hydrogen sulfide dissolved in the interstitial water of the sediment of commercial catfish culture ponds in Oklahoma was measured from February, 1978, through November, 1979.

The maximum concentration of sulfide in the sediment was less than 1.2 $mg \cdot liter^{-1}$ from December through February, and was as high as 18.1 $mg \cdot liter^{-1}$ in the warmer weather. The maximum sulfide concentration usually occurred from 5.7 to 12.8 cm below the surface of the sediment from December through February, and within the top two centimeters of the sediment from July through September.

Unconsumed fish food apparently resulted in the high sulfide concentrations seen near the feeding area in May or June and September of both years. Dissolved sulfide concentrations decreased near the feeding area in July and August, when the water temperatures were over 28°C and the fish consumed nearly all of the food given. The direct effect of unconsumed food on dissolved sulfide was limited to the vicinity of the feeding area.

In 1979, chlorophyll α rose to a high of 794 mg/m^3 in July and then decreased through November. The highest dissolved sulfide concentrations found at shoreline areas 80 meters from the feeding area occurred in September, and apparently resulted from the die-offs

and deposition of phytoplankton. At those areas, the highest sulfide concentrations were at the location that was sheltered from the wind, and where the deposition of suspended matter was the greatest.

The exposure of fish to hydrogen sulfide during harvesting operations can be minimized; 1) by reducing dissolved sulfide concentrations in the sediment through pond design, improved feeding practices, and the use of lime, and 2) by landing fish in areas of the pond with lower sulfide concentrations, reducing the exposure time of the fish to the sulfide, or treating the ponds with potassium permanganate during harvesting to oxidize the sulfide in the water.

Short title: SULFIDE IN CULTURE PONDS.

INTRODUCTION

Private warmwater fish culture in the United States has grown from a few small minnow farmers in the 1920's to a multi-million-dollar-a-year industry. In 1969, over 27,000 hectares of ponds were under intensive culture, primarily in the southern United States (Meyer, et al., 1973). The channel catfish (Ictalurus punctatus) is the primary culture species and the high productivity now attained in static ponds is dependent on large energy subsidies in the form of pelleted feed. Feeding rates of 40 to 140 kg/ha·day during the growing season result in fish production of 2,000 to 10,000 kg/ha·year.

The deposition of fish feces and phytoplankton, over an extended period of time, results in the build-up of soft sediment on the pond bottom. Fish farmers have known for a long time that disturbing this sediment during a harvesting operation and exposing the fish to a sediment-water mixture, or rolling fish in the sediment in shallow water, can result in harm to the fish. This harm may range from immediate death, or mortality during transportation, to long-term deleterious effects on feeding and growth. As a result of this problem, many fish farmers are reluctant to harvest fish during the warmest summer months. This results in an interruption of cash flow at a time when the fish food, labor and pumping bills are the greatest.

Several points indicate that the problems experienced by farmers while harvesting fish in warm weather may be caused by hydrogen sulfide dissolved in the interstitial water of the sediment, which is released to the upper water when the sediment is disturbed.

It has been shown by several researchers that hydrogen sulfide is very toxic to fish (Bonn and Follis, 1966; Broderius and Smith, 1976; Smith et al., 1976). Hydrogen sulfide inhibits cytochrome oxidase activity in fish tissues, and a measurable reduction in cytochrome oxidase activity can occur after only a five minute exposure to 0.1 mg·liter^{-1} un-ionized sulfide at 10°C (Chapter II). Since the toxicity of hydrogen sulfide to fish increases logarithmically with temperature (Adelman and Smith, 1972), the presence of a few hundreths of a milligram per liter in the water during harvesting operations at 20 to 30°C may harm the fish.

Although scientific studies of the hydrogen sulfide in commercial fish culture ponds have not been done, the sulfur cycle has been studied extensively in other aquatic ecosystems. Wheatland (1954) demonstrated that the bacterial production of hydrogen sulfide is inhibited by even traces of oxygen, and that sulfide is oxidized by oxygen. Thus, in warm-water commercial fish culture ponds, which are usually homeothermic, the presence of sulfide in the water is unlikely, except for short time periods when the sediment is disturbed.

The soft sediment of commercial culture ponds is usually black and malodorous below the brown surface layer. Doyle (1968) attributed the similar dark color of lake sediments to the precipitation of hydrogen sulfide as FeS. Sulfate-reducing bacteria are

most abundant within the top two centimeters of lake sediment, and bacterial numbers increase considerably during the summer (Cappenberg, 1974). This is the period during which fish farmers have the greatest problems when harvesting fish.

Although Stuiver (1967) indicated that the sedimentation of biological material had a minor impact on the sulfur cycle in the sediment of a fresh-water lake, the study of Nriagu (1968), showed that nearly 45% of the sulfide in the sediment came from the mineralization of organic matter. The imput of organic matter into commercial fish culture ponds far exceeds that of natural ecosystems and has a large impact on the dissolved sulfide in the bottom sediment.

This study examined the occurrence and distribution of hydrogen sulfide dissolved in the interstitial water of the sediment in commercial culture ponds. Management practices are proposed that may help to avoid damage to fish caused by hydrogen sulfide during harvesting operations.

METHODS

Area of Study

This study was conducted at Sooner Fish Farm, located six miles due south of Norman, Oklahoma. The primary culture species at this commercial farm is the channel catfish (Ictalurus punctatus), however, bigmouth buffalo (Ictiobus cyprinellus), fathead minnows (Pimephales promelas), and green sunfish (Lepomis cyanellus) are also raised.

The farm consists of eight rectangular ponds with average surface areas of 1.6 hectares (175 X 91 meters). The long axes of the ponds are aligned in a north-south direction, and the ponds have individual water inlets and drains. The ponds average 0.6 meters deep, with water depth gradients of 0.25 meters (at the south end) to 1.0 meter deep (at the north end). As a result of the deposition of fish feces and phytoplankton over several years, some areas of the pond bottoms had up to 0.6 meters of soft sediment with an average water content of 53.5 ± 5% (n=6). At other areas of the ponds, particularly wind-swept shores, there was very little sediment.

Total Dissolved Sulfide

Spectrophotometric Determination

Total dissolved sulfide was measured spectrophotometrically using the methylene blue technique (APHA, 1975). The quantities of reagents were proportionally reduced to allow for testing of a 3 ml sample volume. Measurements were made in 1 cm^2 glass cuvettes at 625 nm with a Beckman D/U spectrophotometer.

In five trials with ten replicates in each, at sulfide concentrations ranging from 1.12 to 4.25 $mg \cdot liter^{-1}$, the coefficients of variation ranged from 2.0 to 5.8% and averaged 3.9% (Table 1).

Diffusion-Chamber Sampler

Construction. To sample the total sulfide dissolved in the interstitial water of the pond bottom sediments, a diffusion-chamber sampler was constructed of three layers of plexiglass, with sample chambers bound by dialysis membrane on two sides (Fig. 1). The sample chambers were 2.5 cm in diameter, 6.4 mm thick, and had a volume of 3.1 ml. The vertical center-to-center distance was 1.9 cm.

Assembly. Dialyzer tubing with a wall thickness of 0.0035" was used as the diffusion membrane (Arthur H. Thomas Company, Philadelphia 5, Pa., Cat. No. 4465-A2). The tubing was cut to size and soaked in glass distilled water prior to assembly of the sampler. The plexiglass contact areas were coated with a thin layer of Vaseline to prevent leakage. The chambers were filled with glass

distilled water and the layers joined with flat-head nylon screws. The sampler was attached to a 1.5 meter nylon cord with a floating buoy to mark the location of the sediment sampler in the pond.

Sampling procedure. Assembled samplers were pushed into the soft sediment of the pond bottom, leaving at least one chamber entirely above the sediment-water interface, and allowed to equilibrate with the dissolved sulfide in the sediment. From March through November, the samplers were allowed to equilibrate for from two to five days. It was determined that equilibration with an aqueous sulfide solution was 99.9% complete within five hours at 20°C (Fig. 2) and, in a series of seven profiles measured in the bottom sediment of Pond 2 from July 18-20, 1978, the greatest concentration of dissolved sulfide was reached after eight hours of equilibration (Fig. 3). In the colder months, from December through February, the samplers were allowed to equilibrate for from three to ten days.

After equilibration, the samplers were removed from the sediment and the location of the sediment-water interface noted. The samplers were rinsed briefly with pond water to remove the sediment adhering to the surface of the membrane. The samplers were held a few degrees above horizontal and samples removed from each chamber with a plastic-tipped volumetric pipette. The samples were placed in glass vials, fixed immediately with zinc acetate and sodium hydroxide, and analyzed within 24 hours. It took less than two minutes to remove a sampler from the sediment and fix the contents of all chambers.

Locations Sampled

From February, 1978, through November, 1979, a total of 480 dissolved sulfide profiles were determined in the sediment at 32 locations in six different ponds (Fig. 4). Of the total, 309 profiles were determined at three locations in Pond 2; 91 at the feeding area, 106 at a location 15 meters from the center of the north levee, and 112 at a location 15 meters from the center of the south levee (Fig. 5).

Climatological Factors

Water temperature

Pond bottom water temperatures were recorded in Pond 7 with a Tempscribe, 7-day recording thermometer, from March, 1978 through November, 1979 (Fig. 6). Measurements taken with a mercury thermometer showed that at any point in time, the variations within and among ponds were normally less than 1°C. The water temporarily stratified on hot, still days in July and August but destratified at night when the air temperature dropped or winds came up.

Wind

Values for wind speed and direction were obtained for Will Rogers World Airport (25 miles NNW of the study area) from the National Oceanic and Atmospheric Administration, Environmental Data and Information Service, Asheville, N.C. The vector direction was

used for the daily wind direction. The monthly wind direction was assigned by rating each month on the basis of: average vector direction for the month, the number of days that the daily vector direction was from the north or south, and the number of days the fastest wind for the day was from the north or south. From December, 1978, through March, 1979, the prevailing winds were from the north, and were very strong, averaging from 13.0 to 14.8 m.p.h. for the month (Table 2). The rest of the year the prevailing winds were from the south, or were variable in direction, and were not as strong.

Chemical/Biological Factors

Food

The fish were fed commercially prepared, sinking pellets, at a single location in each pond. As a result of continued feeding in the same area, the soft sediment was swept away by the fish, leaving a hard bottom. Immediately outside this hard area, unconsumed food accumulated.

The fish were fed a maximum of once a day by scattering the food within the swept-out area. From December, 1978, through February, 1979 (Fig. 7), when the mean monthly water temperatures were less than 6°C, the fish were fed only once. During this period the fish remain in their home areas and do not travel to the feeding area (Randolph and Clemens, 1976b).

As the mean monthly water temperature rose from 11°C in March to 25°C in June of both years, the fish became more active and came to the feeding area on a regular basis, and the farmer attempted to

keep pace with the activity of the fish and fed accordingly. During this period, the number of feeding days per month were increased, and the daily feeding rate was increased to a maximum of 42 kg/ha·day.

Experience has shown that the feeding rate needs to be limited to 42 kg/ha·day on this farm to maintain the water quality. Thus, in July and August of both years, when the mean monthly water temperatures were over 28°C, the ponds were limited to this amount, even though the fish could have consumed more.

From September through November of both years, when the mean monthly water temperatures began to decrease, the farmer again attempted to feed according to the demands of the fish, and gradually reduced both the feeding rate and frequency.

Chlorophyll α

Pond water samples were filtered through Whatman GF/C filters and chlorophyll α was determined spectrophotometrically with a Beckman D/U spectrophotometer after grinding the filtered samples and acetone extraction (APHA, 1975).

Water samples were taken from March through November, 1979, at three locations in Pond 2 (the feeding area, and 15 meters from north and south shores) on days that dissolved sulfide profiles in the sediment were determined.

Filterable Suspended Matter

Water samples were filtered through pre-weighed Whatman number 5 filters and the total filterable suspended matter was

measured by weighing the filtered samples after oven-drying at 105°C for one hour. Samples were taken from September, 1978 through February, 1979, at two locations (15 meters from the north and south shores) in each of two ponds (Ponds 2 and 3) on the same days that dissolved sulfide profiles in the sediment were determined.

Sulfate

Dissolved sulfate in the water of Pond 2 was determined from May through November, 1979, using the turbidimetric method (APHA, 1975). Measurements were made on filtered water samples in 1 cm^2 glass cuvettes with a Beckman D/U spectrophotometer at 420 nm.

Statistical Analysis

The standard procedures outlined in SAS User's Guide (1979) and Sokal and Rohlf (1969) were used for all statistical analyses.

RESULTS

Dissolved hydrogen sulfide was normally undetectable above the sediment-water interface, increased with increasing depth in the sediment to a maximum concentration, and decreased below that. The maximum sulfide concentration normally occurred within the top 12.8 cm of the sediment. Four typical dissolved sulfide profiles in the sediment, measured at a location 15 meters from the south shore of Pond 2 in different months, are shown in Fig. 8.

The amount of sulfide released to the water when the surface layer of the sediment is disturbed, such as during a harvesting operation, is of importance to the fish farmer. It was determined in this study that the average dissolved sulfide concentration in the top five centimeters of the sediment was directly related to the maximum dissolved sulfide concentration in the sediment, regardless of the time of the year. These two variables correlated at the $P<0.0001$ level during the study ($n=447$, $r=0.94$). Since the maximum concentration of sulfide was directly related to the average dissolved sulfide in the top five centimeters of sediment, and reflected a single measurement rather than an average of several determinations, it is used throughout the study to reflect the amount of dissolved sulfide in the sediment which may be released to the upper water during harvesting, and is reported hereafter as the dissolved sulfide concentration.

Factors Affecting Dissolved Sulfide in the Sediment

Several parameters were related to the concentration of dissolved sulfide in the sediment of commercial catfish culture ponds. Two of these, the water temperature and the wind, were climatological conditions not under the control of the fish farmer. Several other parameters affecting the sulfide concentrations were directly or indirectly controlled by the farmer, for example, the imput of fish food, the dense phytoplankton blooms, and the drying and excavating of the pond bottoms.

Water temperature. The dissolved sulfide concentration in the sediment correlated significantly with the water temperature over the course of the study ($P<0.0001$, $r=0.28$, $n=406$). From December through February, when the water temperatures averaged less than 6°C each month, the dissolved sulfide concentrations never exceeded 1.2 $mg \cdot liter^{-1}$ at three locations in Pond 2, and averaged less than 0.4 $mg \cdot liter^{-1}$ at each location (Fig. 9). In July and August, when the water temperatures averaged over 28°C, concentrations as high as 18.1 $mg \cdot liter^{-1}$ were found. Higher sulfide concentrations were expected in the warmer weather, since the metabolism of most organisms increases at higher temperatures. However, at a given point in time, the water temperatures were relatively uniform, but the maximum dissolved sulfide concentration ranged from 0.8 to 12.2 $mg \cdot liter^{-1}$ in the same pond (north and south shore locations in Pond 2 respectively, on September 8, 1978) and from 1.2 to 18.1 $mg \cdot liter^{-1}$ in different ponds (Ponds 3 and 5, respectively, on July 6, 1978).

The water temperature correlated significantly at all three locations in Pond 2 with the depth in the sediment at which the maximum dissolved sulfide was found, and there were no statistical differences with respect to this measurement between the three locations (T-test). The maximum dissolved sulfide concentration averaged from 5.7 to 12.8 cm below the surface of the sediment from December through February, and was normally within the top two centimeters of the sediment from July through September (Fig. 10).

Fish food. The feeding rate (kg/ha·day) did not correlate with the concentration of dissolved sulfide in the sediment near the feeding area of Pond 2 ($P<0.98$, $r=0.0034$, $n=84$) during the course of the study. In fact, the dissolved sulfide concentration decreased in the sediment near the feeding area of Pond 2 during July and August (Fig. 9) when feeding rates were high.

There was, however, a relationship between the dissolved sulfide in the sediment near the feeding area and the presence of unconsumed fish food. The dissolved sulfide concentrations were highest near the feeding area of Pond 2 during May or June, and September of both years (Fig. 9). At these times the water temperature was changing rapidly and unconsumed fish food was frequently seen in and around the feeding area. Other ponds showed a similar trend of high sulfide concentrations near the feeding area in May and June (Fig. 9).

During July and August, when both feeding rates and water temperatures were high, unconsumed fish food was seldom seen, and the dissolved sulfide concentrations decreased in the sediment near the feeding area.

The direct effect of unconsumed fish food is apparently restricted to the vicinity of the feeding area. In May and June, when dissolved sulfide concentrations in the sediment were high near the feeding area, the dissolved sulfide concentrations remained low at locations 80 meters from the feeding area (Fig. 9).

Phytoplankton. As a result of the large imput of nutrients (fish food) chlorophyll α increased in 1979 from a low of 58 mg/m^3 in April, to a high of 794 mg/m^3 in July (Fig. 11). The dissolved sulfide increased gradually at the shoreline areas during this period to July averages of 1.2 and 1.7 mg·liter^{-1}, at the north and south shore locations, respectively (Fig. 9).

Chlorophyll α decreased from the July, 1979, high to a November low of 4 mg/m^3. During this period, the dissolved sulfide increased sharply at the shoreline areas and the highest concentrations of the year at these areas were seen in September (3.4 and 5.8 mg·liter^{-1} averages at the north and south shores, respectively, as seen in Fig. 9).

Wind direction. The direction of the wind had a significant effect on the dissolved sulfide concentration at the north and south shoreline locations of Pond 2. The locations were such that with a north wind, the north shore location was in calm water; and with a south wind, the south shore location was in calm water. As shown in Table 3, during months with a northerly prevailing wind (December, 1978, through March, 1979) the dissolved sulfide concentration was 0.4 mg·liter^{-1} greater at the north shore location ($P<0.004$, T-test, $n=15$). In fact, from December through February dissolved sulfide was

not found at the windswept south shore location. When there were southerly prevailing winds for the month (August through October, 1978, and April through November, 1979) the dissolved sulfide concentration averaged 1.2 mg·liter^{-1} greater at the south shore location ($P<0.0007$, T-test, $n=83$).

Large differences in dissolved sulfide concentrations were also seen between individual measurements at the two shoreline locations (Fig. 12) and were related to the wind direction on the day of sampling. When the samples were pooled based on whether the location was exposed to or sheltered from the wind on the day of sampling, the dissolved sulfide was 0.9 mg·liter^{-1} greater at the sheltered shore ($P<0.001$, T-test, $n=102$). However, the depth in the sediment at which the maximum dissolved sulfide concentration was found at the two shorelines was not affected by the daily wind direction.

The wind direction also affected the total filterable suspended matter at opposite ends of the ponds. In both Ponds 2 and 3 there was significantly less suspended matter at locations 15 meters from the leeward shore as compared to the windward shore (Table 4).

Sulfate. The dissolved sulfide concentration did not correlate significantly with the sulfate dissolved in the pond water at any of the locations studied in Pond 2. Sulfate concentrations in Pond 2 are shown in Fig. 11.

Drying the pond bottom. Ponds 5 and 6 were harvested and drained in November, 1977, and the bottoms were allowed to dry out over-winter. The sediment in the center of both ponds and in the

northeast corner of Pond 5 was left undisturbed. Both ponds were filled with water on April 18, 1978, and stocked with channel catfish.

For the first two weeks after impoundment, dissolved sulfide was not found in the undisturbed bottoms of either pond (Tables 5 and 6), but within six weeks of impoundment sulfide concentrations of 16.7 and 6.0 mg·liter^{-1} were measured in Ponds 5 and 6 respectively. Initially, the maximum sulfide concentrations were deeper in the sediment than in the control pond (Figure 10), but this difference disappeared in Pond 5 after 10 weeks of impoundment.

Removal of sediment. Before impoundment in April, the bottoms around the periphery of both Ponds 5 and 6 were scraped out with a bulldozer to make repairs on the levees. When sampled in August, dissolved sulfide concentrations of 14.5 and 3.6 mg·liter^{-1} were found in these areas in Pond 5 (Table 5), and concentrations of 4.2 and 1.9 mg·liter^{-1} were found in these areas in Pond 6 (Table 6). The soft sediment was 16, 7, 6, and 10 centimeters deep, respectively, at the sites of these four measurements and probably came from several sources - the incomplete removal of sediment from the bottom with the bulldozer, erosion of the levees, redistribution of sediment from other parts of the pond, and deposition of fish feces and phytoplankton.

Fish activity. The activity of groups of channel catfish within a localized area over a period of time can result in the formation of "home areas" (Randolph and Clemens, 1976b). In these areas, the soft sediment is completely swept away, leaving a hard bottom. The fish are normally found here when they are not engaged

in feeding activities. When measured on August 14, 1979, the soft sediment immediately outside a home area had a dissolved sulfide concentration of 1.4 $mg \cdot liter^{-1}$ as compared to 0.3 $mg \cdot liter^{-1}$ in the hard bottom inside the home area.

DISCUSSION

The Bacterial Environment

Hydrogen sulfide can be produced by two different groups of bacteria, both of which require an anaerobic environment. The sulfate-reducing bacteria, primarily Desulfovibrio desulfericans, use sulfate as the final hydrogen acceptor in the oxidation of short-chain carbon compounds, and produce hydrogen sulfide, as well as CO_2 and acetate (Fenchel and Riedl, 1970; Cappenberg, 1974). When sulfate is absent, sulfite, thiosulfate and tetrathionate can be used as the terminal electron acceptor during respiration (Postgate, 1979). Hydrogen sulfide can also be produced by the anaerobic decomposition of sulfur-containing protein by several genera of putrefying bacteria (Cole, 1975).

Although the sulfate-reducing bacteria can occur nearly everywhere, growth and metabolism is optimum under conditions of low redox potential and pH, and in the absence of oxygen (Hutchinson, 1957; Postgate, 1979). In aquatic systems, these conditions are normally found only in the bottom sediments. If reducing conditions exist, the sediment usually appears black, due to the formation of FeS. The surface of the sediment is oxidized (unless the hypolimnion

is depleted of oxygen) and brown, due to the precipitation of ferric hydroxide (Gorham, 1958).

In this study, the maximum dissolved sulfide concentrations averaged from 5.7 to 12.8 cm deep in the sediment from December through February. Low water temperatures, cessation of feeding, and strong winds resulted in an oxidized (brown) layer up to five to ten centimeters deep. The south shores of the ponds were subjected to wave action from the strong northerly winter winds, and the oxidized layer there extended down to the hard bottom. Dissolved sulfide was not found at this area from December through February.

As the water warmed up and feeding rates increased, the chemical requirements for bacterial activity apparently became more favorable near the surface of the sediment. From July through September, the maximum dissolved sulfide concentrations were normally found within the top two centimeters of the sediment. Cappenberg (1974), in a study of the bottom deposits of a fresh water lake, found the greatest populations of sulfate-reducing bacteria within the top two centimeters of the sediment during the late summer. The ponds in the present study did not permanently stratify during the summer, and the sediment had an oxidized (brown) surface film at least a few millimeters thick at all times.

In this study, the maximum sulfide concentration occurred at a similar depth in the sediment at all locations within a pond, and that depth correlated highly significantly with the water temperature. This suggests that the optimum conditions for bacterial activity are fairly uniform within a pond, and are related, at least indirectly, to the temperature.

Bacterial Production of Sulfide

In studies of aquatic systems with low sulfate concentrations and a relatively small input of organic matter, sulfide production is proportional to the sulfate concentration (Hutchinson, 1957; Wetzel, 1975). Nriagu (1968), however, determined that 45% of the sulfide produced in a Wisconsin Lake was derived from the mineralization of organic matter. In the commercial catfish culture ponds in this study, the deposition of organic matter appears to be the major factor controlling sulfide production. The daily food input of 42 kg/ha·day can affect the production of sulfide in the sediment both directly and indirectly.

Direct effect of fish food. The metabolism of fish is directly related to the water temperature. Andrews and Stickney (1972) demonstrated that the percent weight gain of channel catfish increased linearly between the temperatures of 18° and 30°C, when the fish were acclimated to a constant temperature regime. However, acclimation to a constant temperature does not occur in culture ponds, and large variations in feeding behavior are seen on a day-to-day basis. Randolph and Clemens (1976a) found that individual channel catfish in large culture ponds fed at a demand feeder only three out of five days when the water temperature was below 22°C, and did not feed at all in March and April at times when the temperature was below average for the previous ten days.

Early and late in the growing season, when the water temperature is either generally rising or falling, farmers try to feed as

much as the fish will consume, without wasting fish food. This is very difficult to do during periods of changing water temperature, especially when the daily food ration is provided all at once, as was done in this study, rather than with a demand feeder. In this study, unconsumed fish food was frequently seen in and around the feeding area early and late in the growing season, and apparently resulted in the high dissolved sulfide concentrations found in the sediment near the feeding area.

Estimating the daily food ration is much more difficult early than late in the growing season for two reasons. First, after being "off feed" all winter, the fish must be trained to come to the feeding area. Second, there is a greater incidence of parasites and disease on commercial fish farms in the spring (Meyer, 1970) and this negatively affects the feeding behavior of the fish. Both of these factors may help to account for the fact that in both years, dissolved sulfide concentrations at the feeding area were slightly higher in May or June than in September.

The daily fish food ration was increased through the early part of the growing season to 42 kg/ha·day and held at this level through the warmer weather to prevent oxygen depletions. In July and August, when the water temperature averaged over 28°C, there was intense competition for the food, very little of it was wasted, and the dissolved sulfide concentrations decreased in the sediment near the feeding area.

The quality of the fish food may also affect the amount wasted, and subsequently, the dissolved sulfide in the sediment near

the feeding area. Fish food with a poor water stability and/or a large amount of "fines" (particles too small to be picked up by a fish) would probably result in an increased sulfide concentration.

Effect of phytoplankton decomposition. Unconsumed fish food apparently directly affects the dissolved sulfide concentration only in the vicinity of the feeding area. Dissolved sulfide concentrations early in the growing season (May or June) were high at the feeding area, while concentrations were very low at a distance of 80 meters from the feeding area. However, the large input of food eventually resulted in tremendous phytoplankton blooms in July, 1979, which declined erratically through November. When the phytoplankton blooms began to decline, dissolved sulfide concentrations increased at the shoreline areas 80 meters from the feeding area, and the sulfide concentrations there were greatest in September. The difference in dissolved sulfide concentrations between the two shoreline locations were related to the wind direction, which allowed for increased deposition of suspended matter in the quiet water in the lee of the levees.

The sulfur contained in the phytoplankton can account for a large portion of the sulfide produced at the shoreline areas in September. The 794 mg/m^3 chlorophyll α concentration measured in July, 1979, represents 80-160 g/m^3 of phytoplankton, dry weight (APHA, 1976). This plankton biomass would contain 0.5 to 1.0 gram $sulfur/m^3$, based on a 0.6% sulfur content (Schuette, 1918). If even half of the phytoplankton in a 30-cm water column settled out and all of the sulfur were converted to hydrogen sulfide in the top two

centimeters of sediment (where the greatest concentrations were found), a dissolved sulfide concentration of 7 to 14 mg·liter^{-1} would be found in the interstitial water. This is within the range of concentrations actually found at this time of the year (Fig. 12). This calculation, however, fails to take several factors into account; 1) phytoplankton populations are seldom that high, 2) total die-offs of phytoplankton occur only occasionally with a more gradual turnover of phytoplankton being more common, and 3) dissolved sulfide is lost from the sediment both by diffusion into the pond water and through precipitation as FeS in the sediment.

The decomposing phytoplankton must, therefore, be providing organic substrate for the bacterial reduction of sulfate by Desulfovibrio spp., as well as contributing to the formation of hydrogen sulfide directly through putrefacation of the protein they contain. The relative importance of these two processes cannot be determined on the basis of this data, but the distinction is not important from the standpoint of the commercial fish farmer.

Proposed Management Practices

Hydrogen sulfide can probably never be totally eliminated from the sediment of culture ponds, however, the accumulation of high concentrations of hydrogen sulfide can be minimized through pond construction, reducing wasted fish food, and liming the pond bottoms. The exposure of fish to the sulfide that is present can be reduced by taking several precautions during harvesting.

Pond construction. The large, rectangular ponds used for the culture of fish should be constructed with the long axis in line with the prevailing winds whenever practical. This will do two things. First, with a longer surface exposed to the wind there will be more turbulent mixing of the water, and more of the organic matter in the pond will be decomposed aerobically by bacteria in the water column. In addition, the length of shoreline sheltered from the wind on any given day will be reduced, and a larger shoreline suitable for landing fish will be available. The tall weeds often found along the levees should be moved as necessary to increase the effect of wind action on the ponds.

Reduce wasted food. The use of demand feeders would probably result in a higher feeding efficiency (less wasted food) and lower sulfide concentrations in the immediate area. Particular attention should be paid to feeding practices early and late in the growing season when the fish metabolism is changing rapidly, and to the quality of the food (stability in water and amount of "fines" which are not consumed by the fish). Farmers using a floating pellet would probably find lower dissolved sulfide concentrations near the feeding area, but floating pellets are much more expensive and not recommended for this reason.

Where practical, fish should be fed at an area of the pond that is not normally used for landing fish.

Liming. Bonn and Follis (1978) described the problem of sulfide poisoning of channel catfish in the acid lakes of northeast Texas. In addition to sulfide being more toxic at a low pH (Broderius

and Smith, 1976), the precipitation of hydrogen sulfide as FeS is dependent on pH. At an elevated pH, the equilibrium is strongly shifted in favor of FeS, and little free sulfide would be found as long as any unbound iron is present (Hutchinson, 1957). Many farmers now use lime, either as agricultural limestone or hydrated lime, and this practice is strongly recommended for the management of dissolved sulfide in the sediment, especially in areas with acid soil and water.

Drying and excavating the pond bottoms. Farmers should not take ponds out of production to dry or excavate the pond bottoms to reduce the dissolved sulfide in the sediment. It was determined in this study that draining and drying the ponds, and even removing the sediment with a bulldozer, produced no long-term reduction of dissolved sulfide in the pond bottoms. Within three months of draining and drying and/or excavating the pond bottom, the dissolved sulfide concentrations in the sediment were similar to those in ponds that had been under water continuously. Removal of the sediment with a bulldozer likewise produced no long-range reduction in dissolved sulfide. However, excavating the deep sediment (up to 0.6 meters deep in this study) found in very old culture ponds increases the water volume available to the fish, and the elimination of the deep home areas may increase the seining efficiency.

Harvesting practices. Even with the proper management, harmful concentrations of hydrogen sulfide may be found in the sediment during the warmer weather. However, harvesting can proceed uninterrupted year-round if certain procedures are followed.

In the cooler months of the year, from November through March, fish can be landed at any area in the pond if the water is deep enough to conduct the operation. During this period, dissolved sulfide concentrations are usually low at all areas of the pond, and in cool water (less than 10°C) fish can tolerate much more sulfide than in warmer water (Chapter II).

In the warmest months, from July through September, fish can be safely harvested but the best landing area should be selected based on the condition of the pond bottom, the water depth and the location in the pond.

If the pond bottom is hard as a result of wave action, or if the soft sediment is brown, that location is suitable. Black sediment indicates a reducing environment and the probability of sulfide in the sediment.

Deeper water is preferable when landing fish in warm weather. When the fish are crowded together in a net in deeper water, less sediment will be disturbed and the sulfide released to the water will be diluted to a greater degree.

Two areas in the pond should be avoided unless there is a compelling reason to land the fish there - the feeding area and the shoreline sheltered from the wind. Both of these areas usually have high concentrations of sulfide during this period.

If the fish cannot be landed at an ideal area (windswept shore, hard bottom and deep water) the exposure of the fish to sulfide can be minimized in two ways. First, since the exposure of fish to even 0.1 mg·liter^{-1} H_2S can cause harm to the fish within five

minutes (Chapter II), the fish should be moved to deeper water or to near the water inlet as soon as possible. If this is impractical, a current of fresh water can be directed to the fish in the net with a Crisafulli pump. Secondly, the hydrogen sulfide released to the water can be oxidized with the application of 6 $mg \cdot liter^{-1}$ potassium permanganate (Mathis et al., 1962). This may be especially useful when ponds are partially drained for a complete harvest in warm weather.

Holding fish before transportation. If black sediment is disturbed during harvesting at any time of the year, and the exposure of the fish to sulfide is suspected, the fish should not be immediately transported. It has been shown that fish exposed to sulfide have reduced cytochrome oxidase activity, and an increase in blood lactic acid (Chapter II). However, if the fish are held in fresh water (under the inlet) for several hours, and preferably overnight, the enzyme activity will return to normal levels and the fish may be transported safely.

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Table 1. Coefficient of variation of the methylene blue spectrophotometric test for hydrogen sulfide.

	Trial				
	1	2	3	4	5
n	10	10	10	10	9
Mean total sulfide ($mg \cdot liter^{-1}$)	3.25	2.88	4.25	1.52	1.12
S	0.087	0.166	0.188	0.068	0.023
Coefficient of variation (%)	2.7	5.8	4.4	4.4	2.0

Table 2. Average monthly wind speed and direction.

Year	Month	Average Wind Speed (mph)	Wind Direction: Monthly vector direction (degr./10)	Wind Direction: Number of days the fastest daily wind was from the North	Wind Direction: Number of days the fastest daily wind was from the South	Wind Direction: Number of days the daily vector was from the North	Wind Direction: Number of days the daily vector was from the South	Score N	Score S	Monthly Average direction
1978	Aug.	10.4	16	10	20	7	24	0	3	South
	Sep.	10.3	16	9	20	6	24	0	3	South
	Oct.	11.1	16	11	18	10	21	0	3	South
	Nov.	11.5	2	15	15	14	16	1	1	Tie
	Dec.	13.9	31	17	14	13	18	2	1	North
1979	Jan.	13.6	34	19	11	18	11	3	0	North
	Feb.	13.0	2	15	13	15	13	3	0	North
	Mar.	14.8	10	16	13	14	13	2	1	North
	Apr.	12.4	12	12	15	13	15	0	3	South
	May	10.2	15	10	20	11	19	0	3	South
	June	9.7	15	6	23	5	24	0	3	South
	July	7.4	14	9	20	10	20	0	3	South
	Aug.	8.2	17	10	21	5	26	0	3	South
	Sep.	6.9	11	11	13	11	16	0	3	South
	Oct.	13.6	16	12	18	10	20	0	3	South
	Nov.	12.0	24	0	28	10	21	0	3	South

Table 3. Effect of wind direction on maximum dissolved sulfide concentrations in sediment profiles. T-test of mean difference in the maximum dissolved sulfide concentration ($mg \cdot liter^{-1}$), north minus south shore samples (Pond 2).

Monthly Wind Direction	n	Mean Difference in Maximum Sulfide ($mg \cdot liter^{-1}$)	S.E.	T	P>|T|
South	83	-1.2	0.33	-3.54	0.0007
North	15	0.4	0.11	3.38	0.004

Table 4. Effect of wind direction on total filterable suspended matter. Mean difference in suspended matter ($mg \cdot liter^{-1}$) between the leeward and windward shores for each of two ponds, based on the average wind direction for the sampling day.

Pond	n	Mean Difference in suspended matter ($mg \cdot liter^{-1}$)	S.E.	T	P> |T|
2	39	-30.8	11.4	-2.69	0.01
3	16	-20.7	8.3	-2.48	0.02

Table 5. Effect of draining and drying the pond bottom on the maximum dissolved sulfide concentration in the sediment, Pond 5. All samples through August 2 were taken from areas in which the sediment was undisturbed. The samples on August 17 were taken from areas in which the sediment had been scraped out with a bulldozer prior to impoundment.

Date	Maximum Dissolved Sulfide Concentration ($mg \cdot liter^{-1}$)	Depth in Sediment (cm)	Comments
Nov. 19, 1977			Pond drained
Apr. 14, 1978			Pond filled
Apr. 18, 1978			Pond stocked
Apr. 26	0.0	0.0	
May 3	0.0	0.0	
May 28	16.7	-3.8	
June 6	5.2	-10.0	
June 20	6.6	-9.5	
July 6	14.5 18.1	-2.0 -4.0	
Aug. 2	0.8	-1.9	
Aug. 17	14.5 3.6	-1.9 0.0	

Table 6. Effect of draining and drying the pond bottom on the maximum dissolved sulfide concentration in the sediment, Pond 6. All samples through June 6 were taken from areas in which the sediment was undisturbed. The samples on August 23 were taken from areas in which the sediment had been scraped out with a bulldozer prior to impoundment.

Date	Maximum Dissolved Sulfide Concentration ($mg \cdot liter^{-1}$)	Depth in Sediment (cm)	Comments
Nov. 19, 1977			Pond drained
Apr. 18, 1978			Pond filled Pond stocked
May 3	0.0	0.0	
June 6	6.0	-13.3	
Aug. 23	4.2 1.9	-1.9 -3.8	

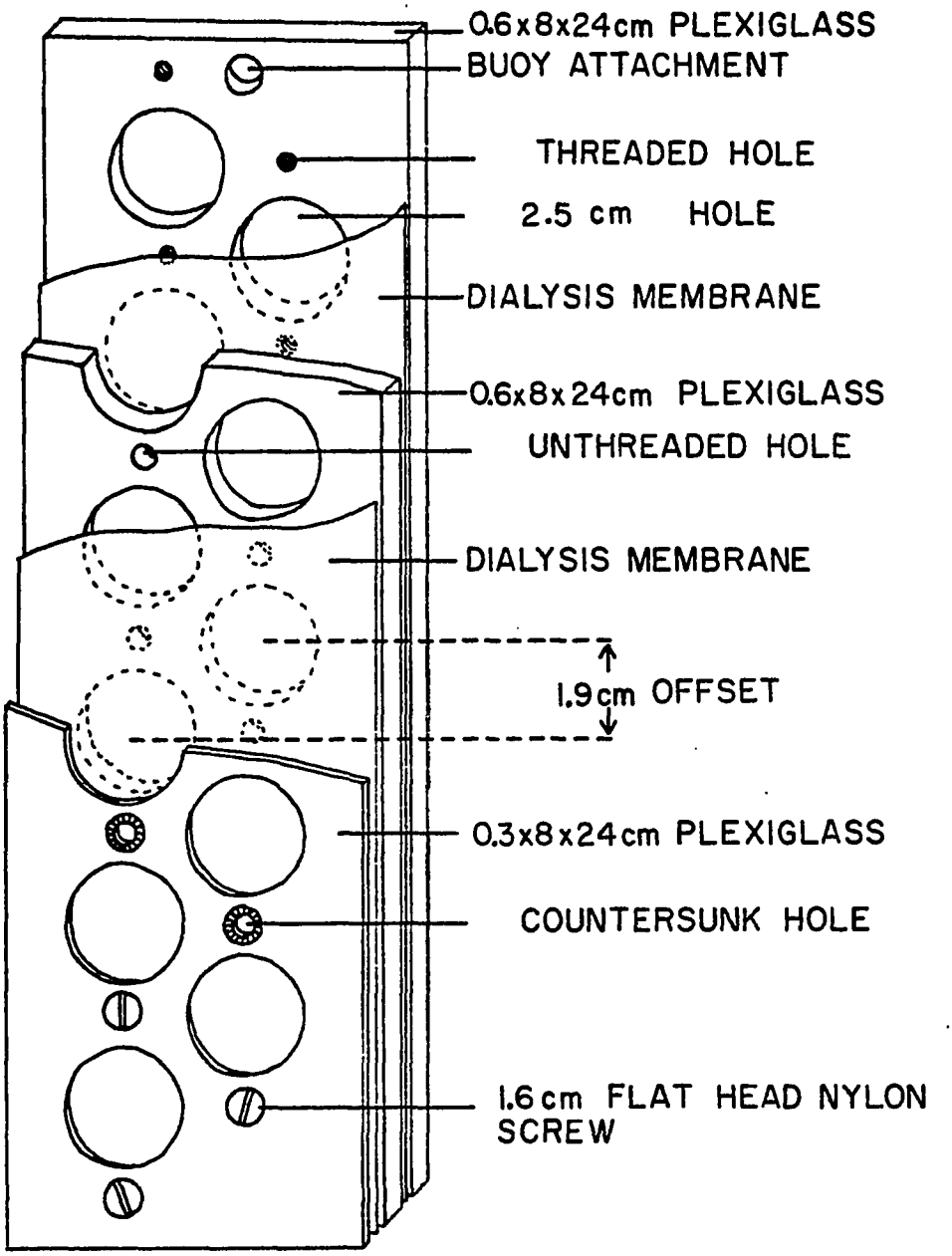


Fig. 1. Diffusion-chamber sampler used in study.

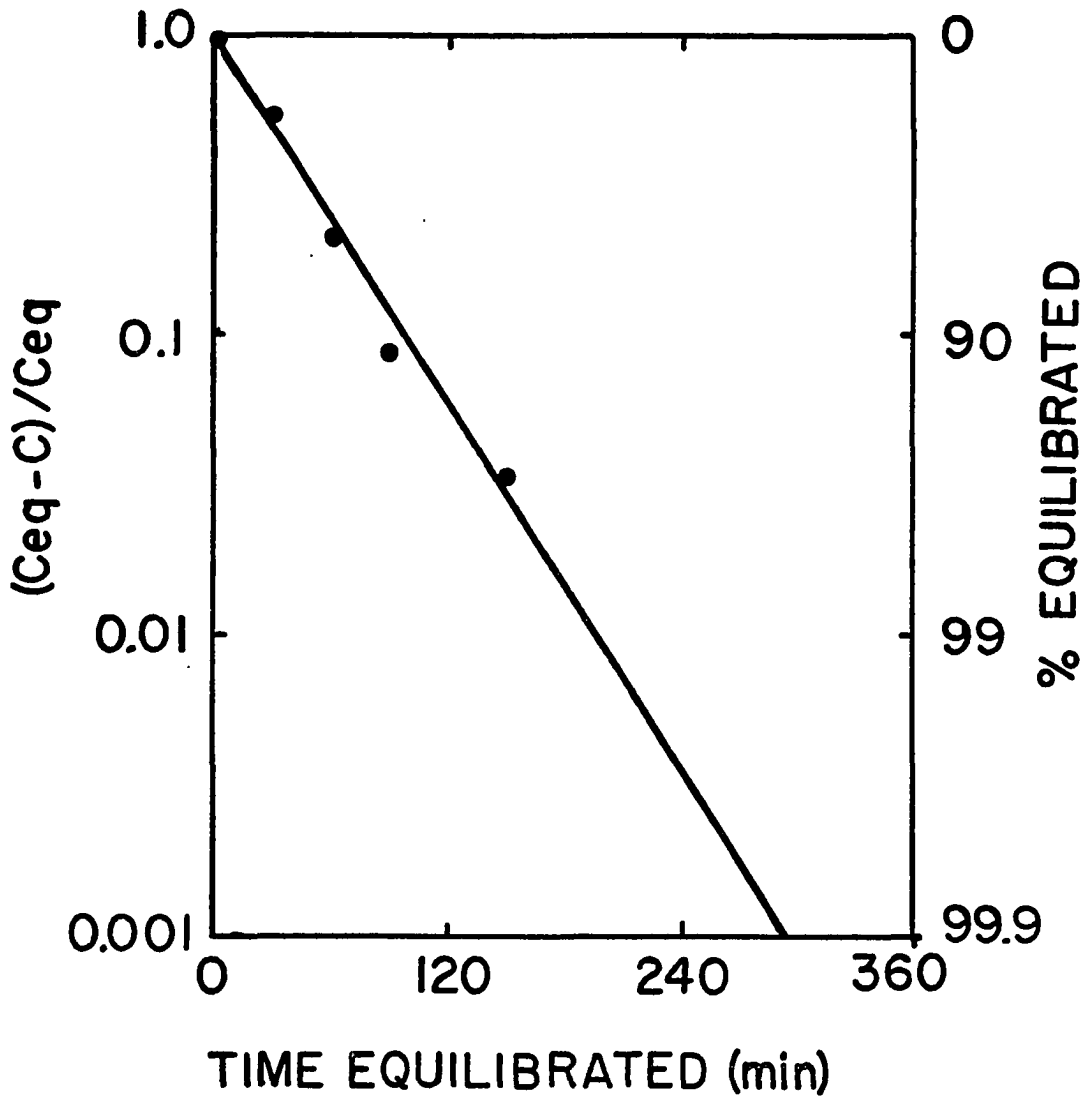


Fig. 2. Equilibration of diffusion-chamber sampler with hydrogen sulfide in aqueous solution.

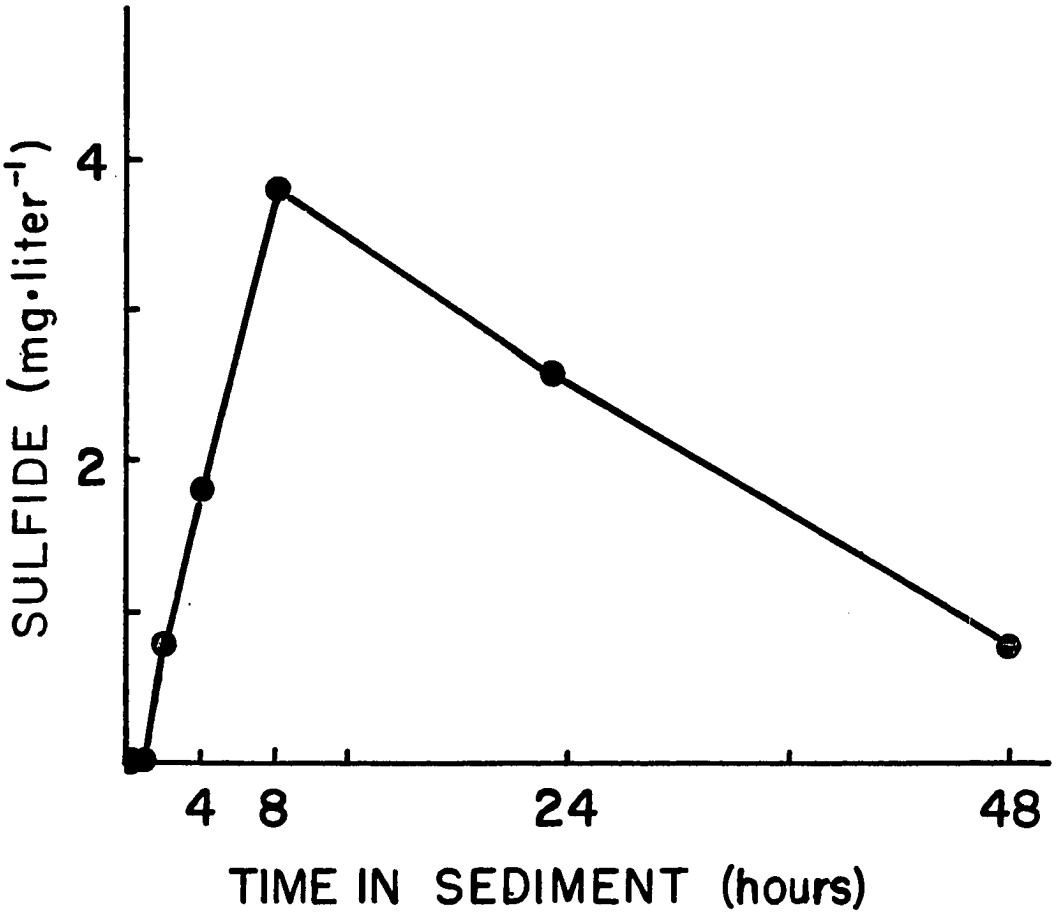


Fig. 3. Equilibration of diffusion-chamber sampler with hydrogen sulfide in the bottom sediment of Pond 2. The maximum dissolved sulfide concentration in the sediment is shown.

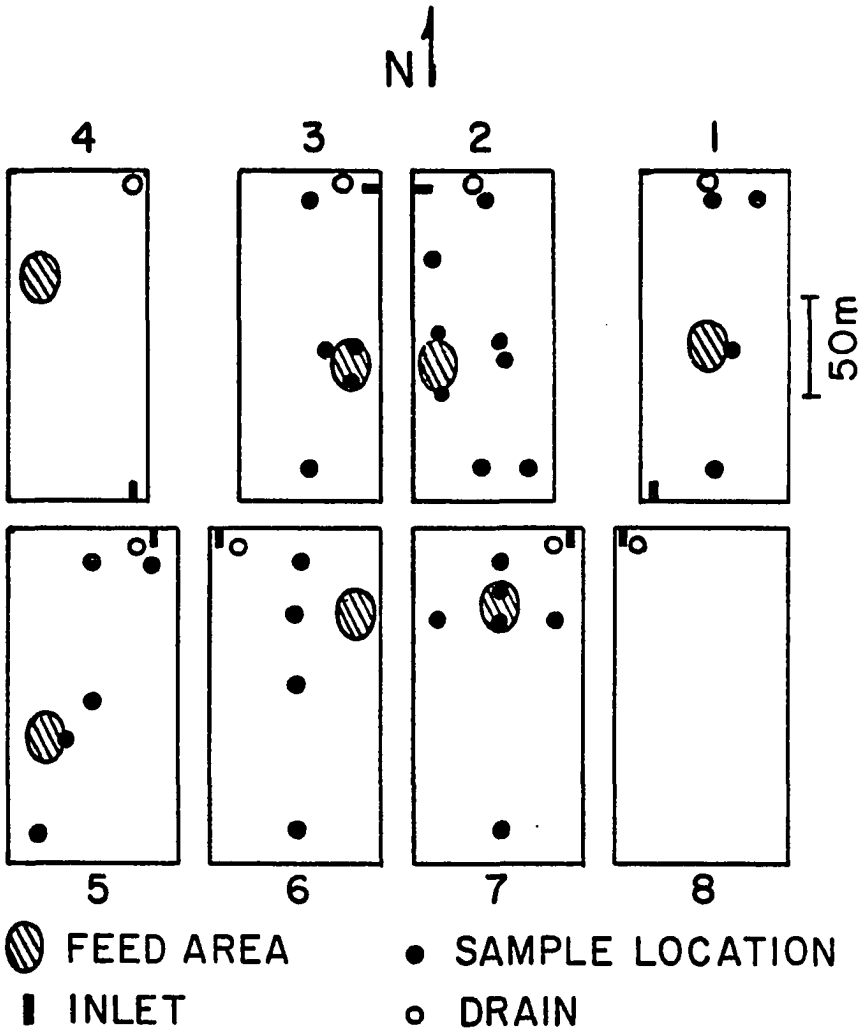


Fig. 4. Map of study area, showing inlets, drains, sample locations and feeding areas.

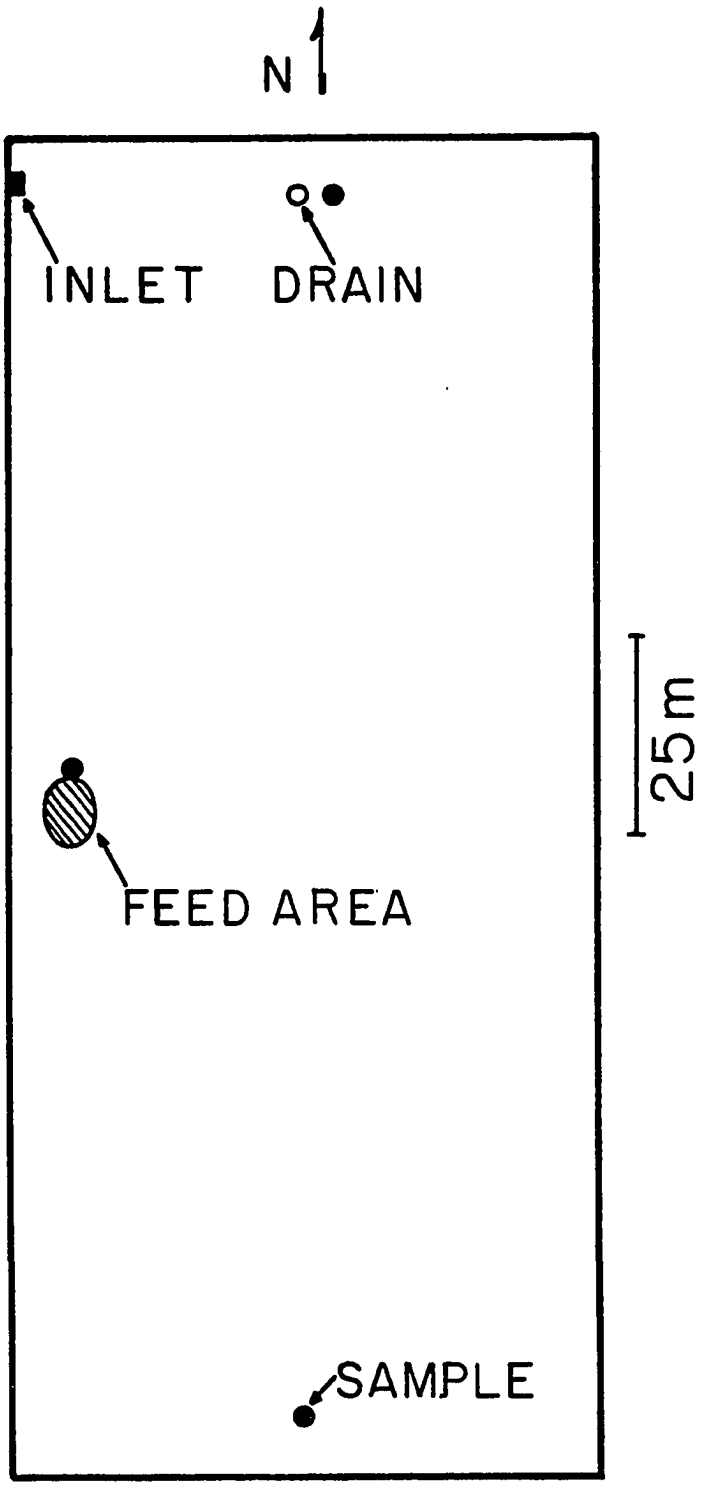


Fig. 5. Primary sediment sample locations in Pond 2.

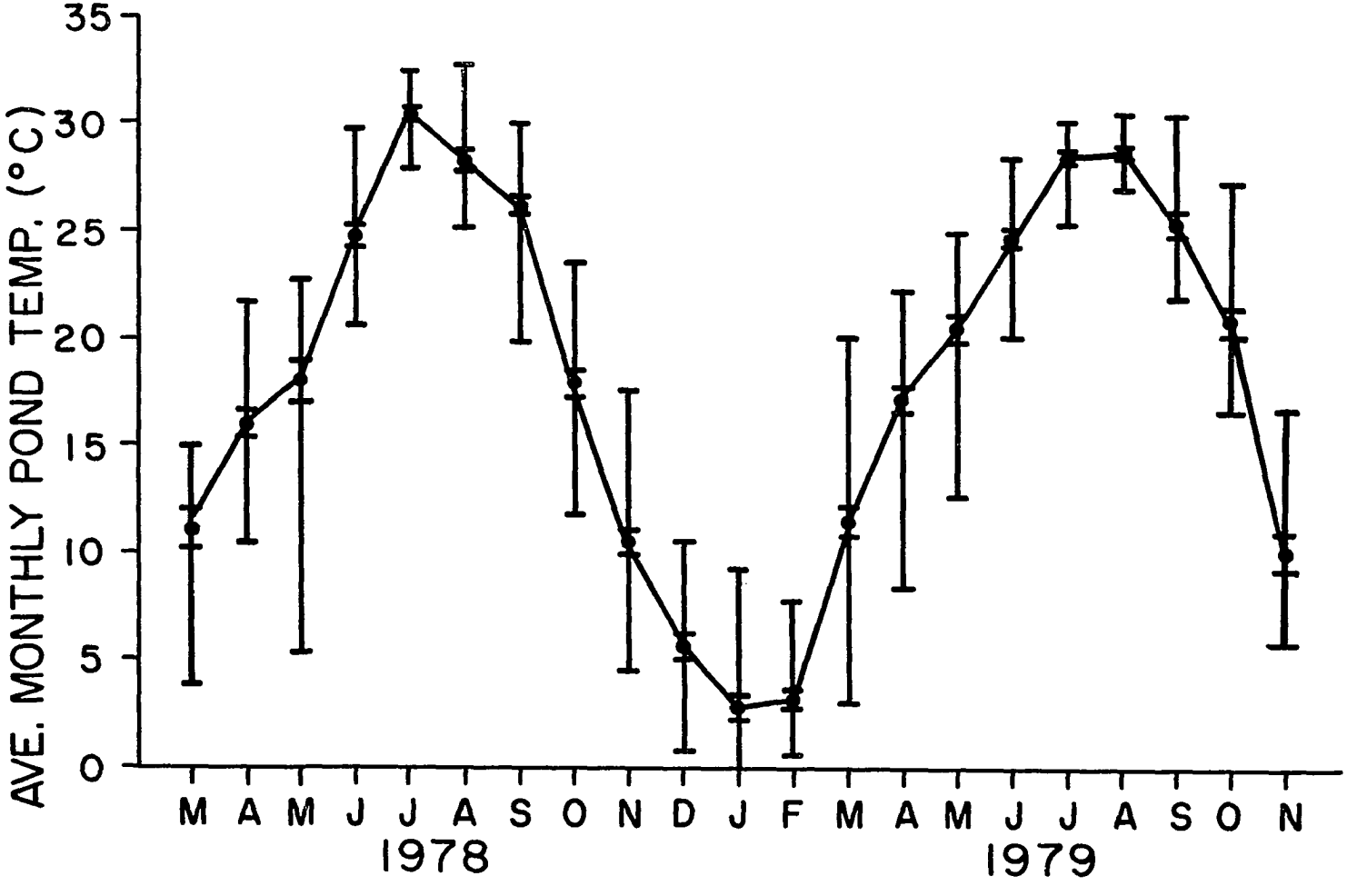


Fig. 6. Pond bottom water temperature (monthly mean, standard error of the mean, and range).

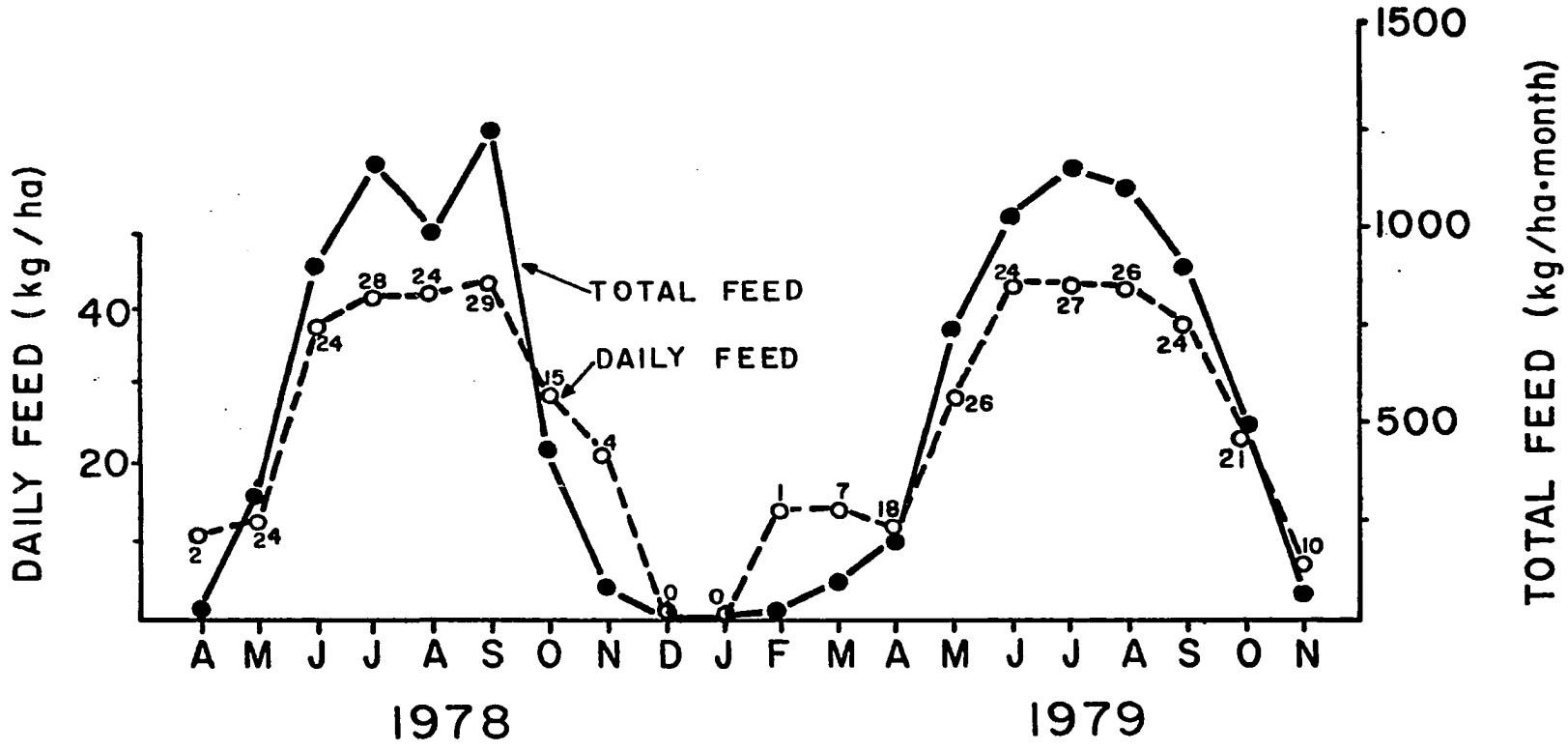


Fig. 7. Average daily feed and total monthly feed given to Pond 2. Numbers are days fed during the month; daily feed is average of days fed during the month.

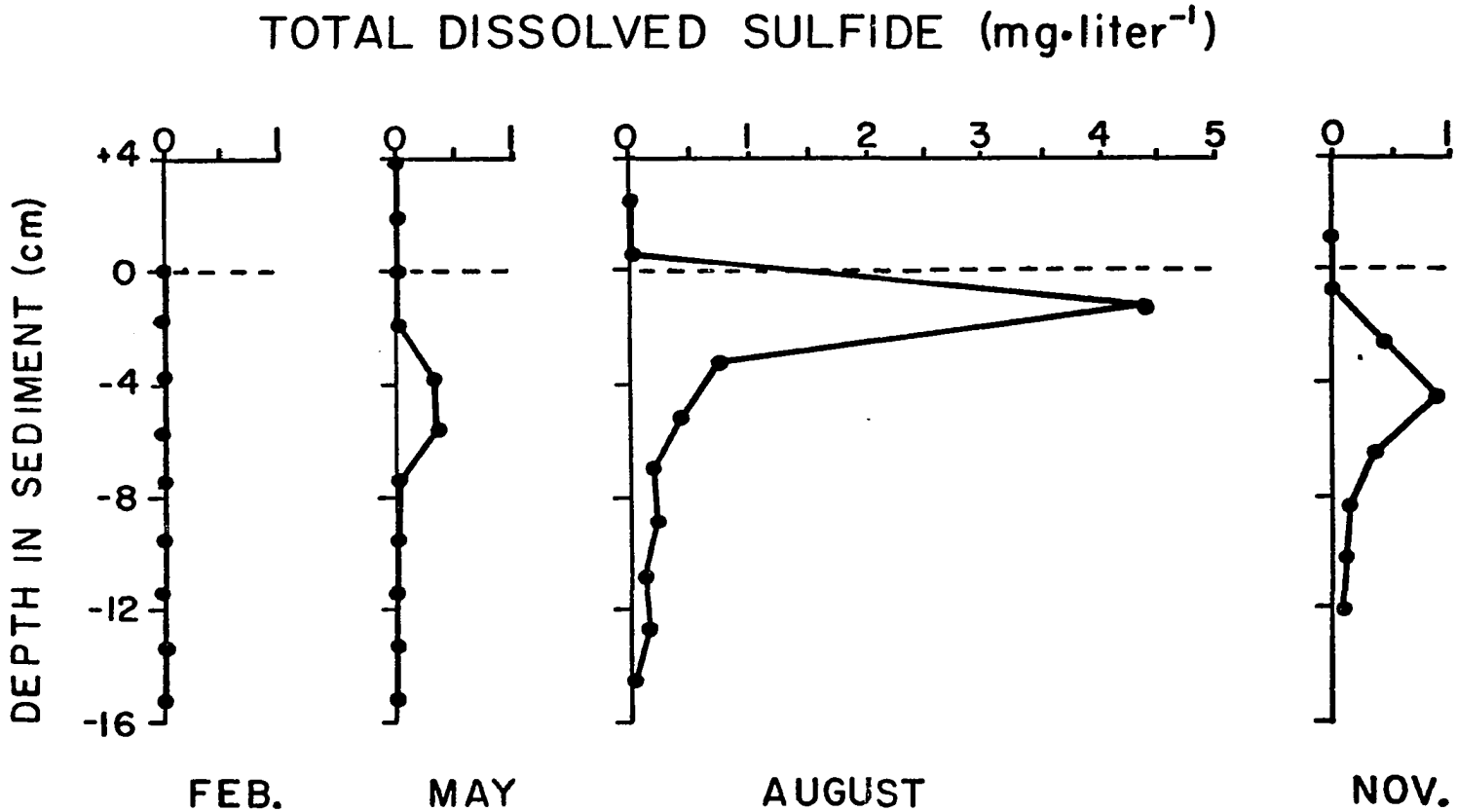


Fig. 8. Four typical dissolved sulfide profiles in the sediment of a commercial culture pond. The profiles were determined at the south shore location (15 meters from the levee) in Pond 2 in February, May, August and November, 1979.

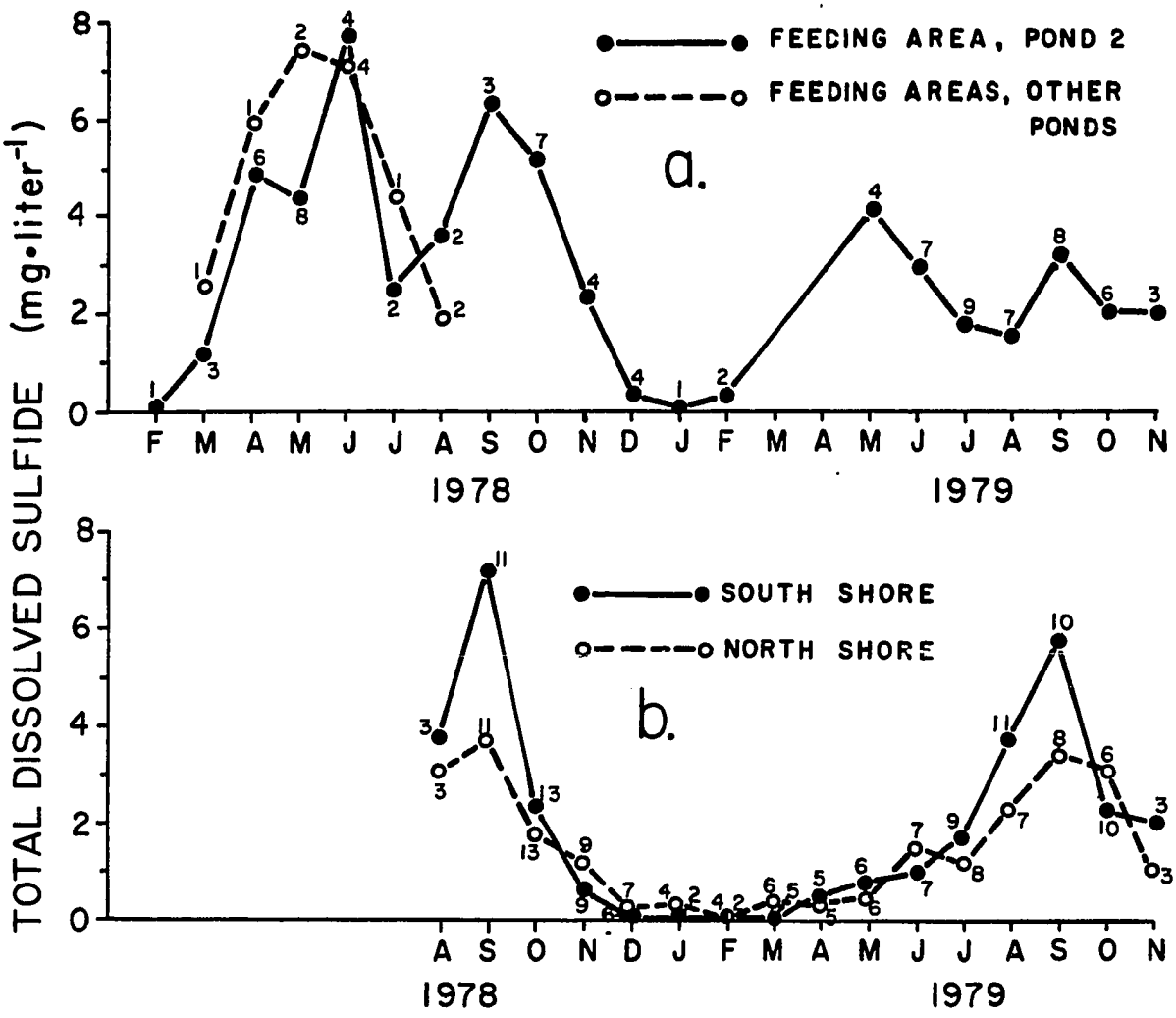


Fig. 9 Maximum dissolved sulfide concentrations in the sediment at; a) the feeding areas of Pond 2 and other ponds, and b) locations 15 meters from the north and south shores of Pond 2. Numbers are sample sizes.

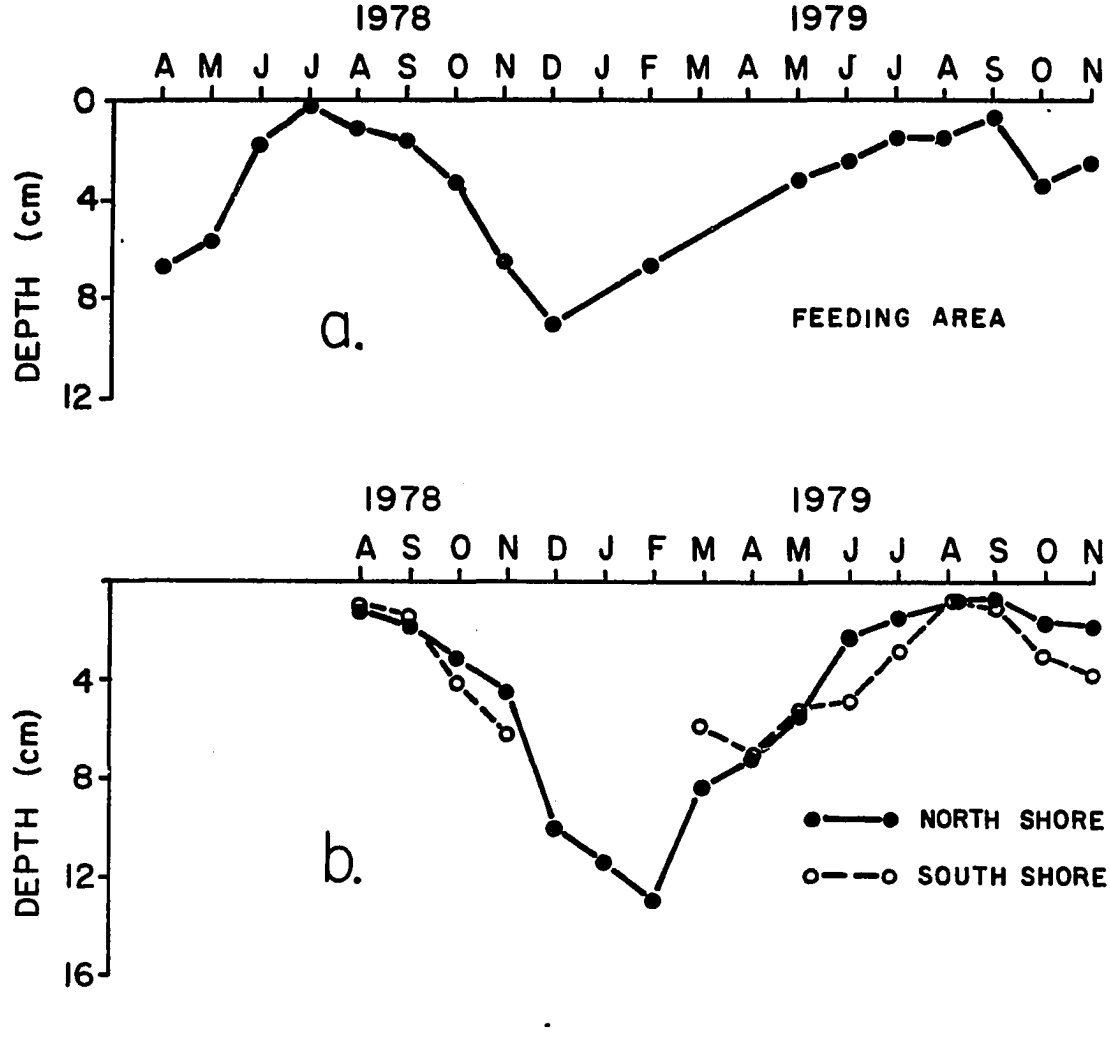


Fig. 10. Depth in the sediment of the maximum dissolved sulfide concentrations at; a) the feeding area of pond 2, and b) locations 15 meters from the north and south shores of Pond 2.

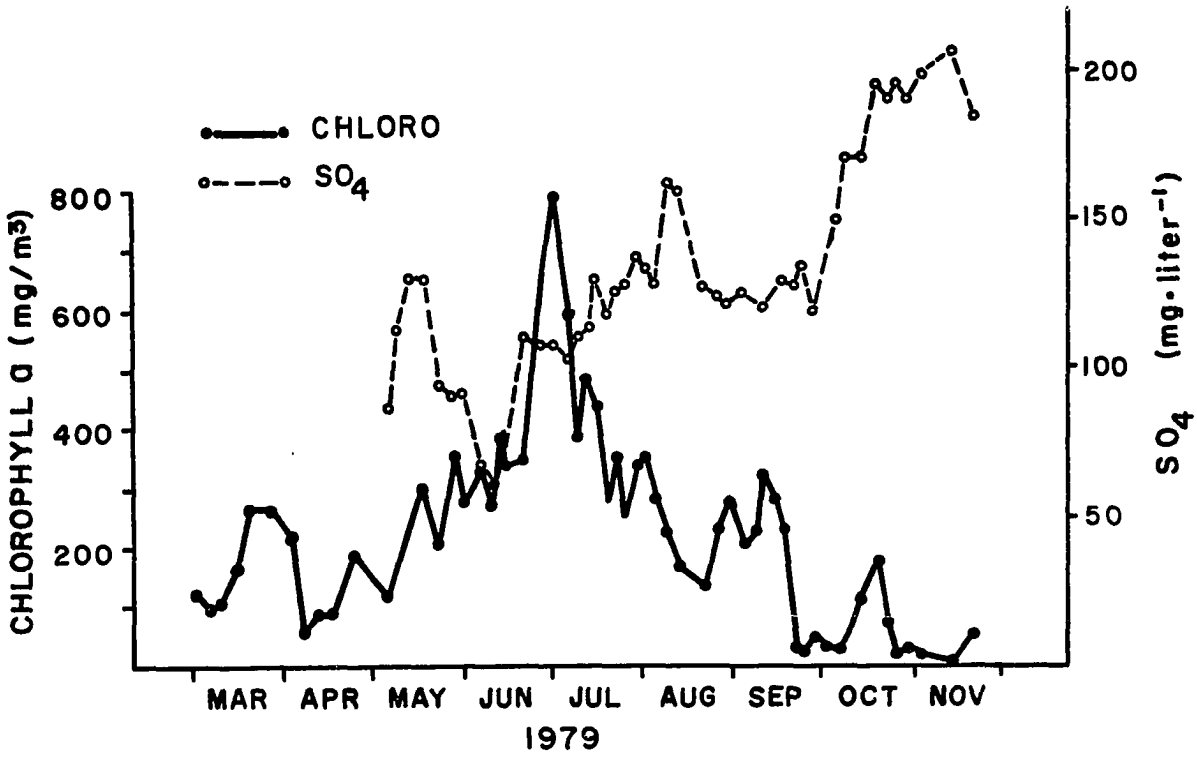


Fig. 11. Chlorophyll α and sulfate in Pond 2. Each point represents the mean of determinations at the feeding area, north and south shore sample locations.

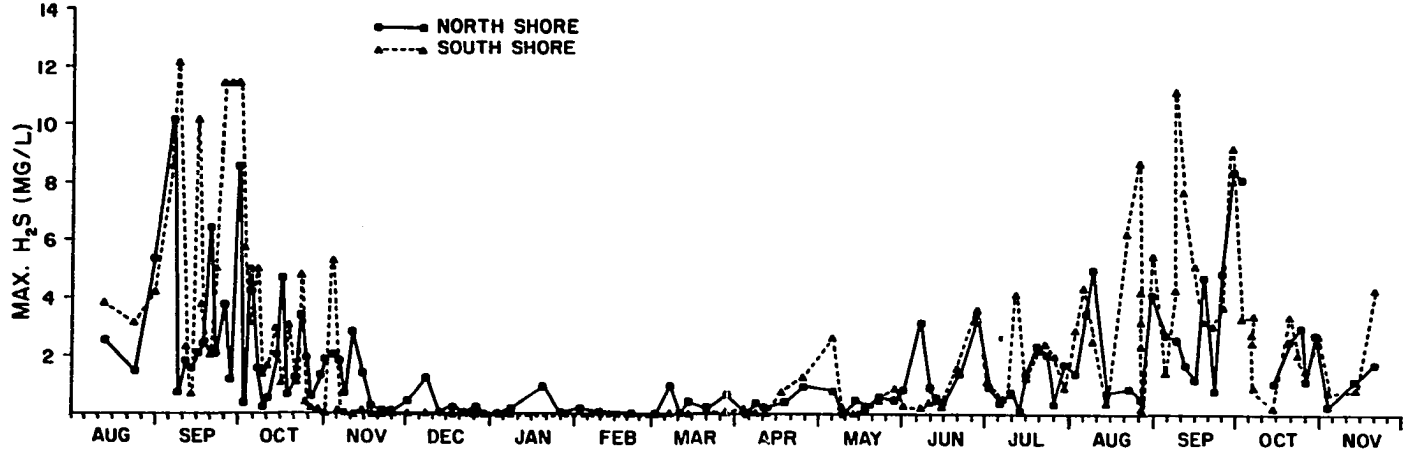


Fig. 12. Maximum dissolved sulfide concentration in sediment profiles at two shoreline locations in Pond 2. Individual measurements are shown.

Appendix A. Locations at which dissolved sulfide profiles were determined.

Number	Location
21	15 meters from center of north shore
22	15 meters from west shore at feeding area
23	15 meters from center of south shore
24	15 meters from west shore, 15 meters south, of feeding area
25	30 meters from north levee, 15 meters from west levee
26	15 meters from southeast corner
27	center of pond, soft mud outside home area
28	center of pond, inside home area
11	15 meters from southeast corner
12	center feed area
13	15 meters from center of south shore
14	15 meters from center of north shore
31	15 meters from center of north shore
32	15 meters from center of south shore
33	15 meters from east shore, near feeder
34	15 meters from shore, 15 meters south of feeder
35	30 meters from east shore, 15 meters west of feeder
51	15 meters from northeast corner, in unexcavated sediment
52	30 meters from west shore at feeding area, excavated area
53	center of pond, in unexcavated sediment

Appendix A Continued

54	15 meters from center of north shore, excavated area
55	15 meters from southwest corner, excavated area
62	15 meters from center of north shore, excavated area
63	15 meters from center of south shore, excavated area
64	center, in unexcavated sediment
71	15 meters from shore, at feeding area on west bank, fall 1978
72	center of pond, feeding area in spring-summer 1978
73	15 meters from east bank
74	45 meters south of center of north shore, near feeding area
75	15 meters from center of north shore
76	15 meters from center of south shore

Appendix B. Raw data, complete sulfide records.

SEQ#	DATE	LOC	MAX H2S	AVE H2S	AVE SID	AVE 10 MIN	MAX DEPTH	WAT DEPTH	MUD DEPTH	TIME
1	15FEB1976	24	0.00	0.00	0.00	0.00	•	•	•	•
2	08MAR1976	25	0.60	0.60	0.40	0.10	•	•	•	•
4	14MAR1978	24	0.00	0.00	0.00	0.00	•	•	•	•
3	14MAR1978	25	3.00	1.55	1.25	0.02	•	•	•	•
5	28MAR1978	71	1.50	0.46	0.64	0.27	-3.4	8	9	•
6	28MAR1978	72	2.80	1.30	0.88	0.50	-1.8	9	9	•
7	28MAR1978	73	1.10	0.60	0.45	0.30	-3.8	8	8	•
9	11APR1978	22	4.80	0.90	1.20	0.20	-11.4	4	4	•
10	11APR1978	24	5.60	3.72	3.61	2.97	-3.2	2	2	•
8	11APR1978	25	4.20	0.65	1.74	0.30	-11.4	4	4	•
12	24APR1978	22	8.40	0.40	5.40	0.73	-8.9	9	9	•
13	24APR1978	24	5.40	3.40	0.86	0.47	-3.2	2	2	•
11	24APR1978	25	1.40	1.40	1.00	0.80	-1.9	9	9	•
14	24APR1978	33	6.00	0.30	0.67	1.50	-5.8	8	8	•
15	26APR1978	51	0.00	0.00	0.00	0.00	22	33	33	36
22	03MAY1978	11	2.51	1.72	1.82	1.67	-10.2	33	33	30
20	03MAY1978	22	2.66	0.47	1.10	1.78	-14.5	33	33	16
21	03MAY1978	24	2.00	0.34	1.75	0.46	-8.6	30	30	18
19	03MAY1978	33	16.00	11.41	3.66	0.54	-2.5	30	30	30
16	03MAY1978	51	0.00	0.00	0.00	0.00	•	15	30	15
17	03MAY1978	64	0.00	0.00	0.00	0.00	•	36	15	26
18	03MAY1978	74	4.80	2.76	1.48	1.20	-1.9	36	26	17
28	11MAY1978	22	1.70	1.22	1.22	1.02	-7.6	36	17	23
29	11MAY1978	24	2.60	1.78	1.20	0.85	-1.9	36	23	19
27	11MAY1978	25	0.80	0.25	0.52	0.45	-8.8	43	19	31
23	11MAY1978	33	2.50	1.53	0.65	0.76	0.0	31	31	31
24	11MAY1978	33	1.00	0.75	0.47	0.50	0.0	31	31	30
25	11MAY1978	34	1.00	•	0.88	0.71	-8.0	30	30	20
26	11MAY1978	35	0.70	0.37	0.35	0.58	-12.9	41	20	22
33	28MAY1978	22	4.70	3.38	0.59	0.62	-1.9	40	22	25
34	28MAY1978	24	15.40	11.70	1.14	0.00	-1.9	40	25	14
32	28MAY1978	25	5.80	5.17	1.33	0.69	-1.9	57	14	36
30	28MAY1978	33	15.40	13.08	1.48	0.27	-3.2	51	36	36
31	28MAY1978	33	13.00	12.85	0.48	0.32	-3.2	51	36	20
35	28MAY1978	51	16.70	8.90	5.08	5.12	-2.9	25	20	22
36	28MAY1978	74	12.20	10.25	1.33	0.00	-2.9	70	22	22
38	06JUN1978	24	9.37	9.37	2.44	0.00	-2.9	45	22	20
37	06JUN1978	25	5.94	5.57	0.00	0.00	-1.9	48	20	18
42	06JUN1978	51	5.20	1.83	4.56	2.75	-10.2	56	18	15
43	06JUN1978	52	1.50	1.09	•	•	-3.8	54	15	16
41	06JUN1978	64	6.90	2.62	2.25	0.69	-13.5	55	16	20
40	06JUN1978	72	12.40	7.15	3.04	0.00	-3.8	63	20	25
39	06JUN1978	74	9.20	7.86	3.02	0.50	-3.8	62	25	30
44	20JUN1978	12	1.38	0.94	0.56	0.15	-0.6	60	30	22
46	20JUN1978	24	10.80	7.39	1.34	0.53	-1.9	45	22	42
45	20JUN1978	25	4.80	3.71	0.77	0.50	-1.9	45	42	33
47	20JUN1978	33	2.60	2.08	0.48	0.38	-1.9	45	33	33
48	20JUN1978	51	6.60	2.48	5.83	2.18	-9.5	45	33	24
49	20JUN1978	72	12.20	6.85	6.51	3.06	-2.9	45	24	•

Appendix B Continued

SEQ SULF	DATE	LOC	MAXES	AVE 5	AVE 10	AVE 15	MAXDEPTH	WATDEPTH	MUDDEPTH	TIME
50	20JUN1978	74	10.60	8.18	0.00	0.00	-1.0	43	23	•
51	06JUL1978	12	6.25	2.54	0.30	0.25	0.0	•	•	•
53	06JUL1978	24	3.00	1.41	0.28	0.38	0.0	48	18	•
52	06JUL1978	25	2.12	1.13	0.62	1.04	0.0	•	•	•
54	06JUL1978	33	1.25	1.12	0.67	0.62	1.9	57	23	•
55	06JUL1978	51	14.50	9.58	7.92	5.11	-2.0	40	38	•
56	06JUL1978	53	18.10	10.20	9.00	13.48	-4.6	50	19	•
57	06JUL1978	72	5.62	3.71	0.87	•	-1.9	47	8	•
59	02AUG1978	13	2.06	2.06	0.54	0.41	-1.3	40	28	•
58	02AUG1978	26	2.19	1.78	0.71	1.28	-1.3	30	35	•
60	02AUG1978	32	0.44	0.36	0.25	0.25	0.0	40	30	•
62	02AUG1978	51	0.81	0.44	0.25	0.12	-1.9	60	32	•
64	02AUG1978	73	0.62	0.46	0.42	0.56	1.0	59	40	•
63	02AUG1978	75	0.94	0.78	0.41	0.16	-1.0	61	30	•
66	12AUG1978	12	1.22	1.08	0.34	0.40	0.0	59	24	11:45
67	12AUG1978	13	1.50	0.68	0.48	0.30	-1.9	46	28	12:00
65	12AUG1978	14	1.19	0.76	0.43	0.33	-1.3	68	40	11:30
68	12AUG1978	21	2.56	1.71	0.28	0.15	-2.0	53	57	11:30
69	12AUG1978	23	3.88	1.71	0.81	0.49	-2.0	35	25	11:00
70	12AUG1978	75	2.06	1.45	0.43	0.39	-1.3	59	22	11:00
71	12AUG1978	76	4.88	2.36	1.23	•	-1.9	46	16	11:00
72	17AUG1978	31	5.00	2.22	0.65	0.32	-1.9	66	20	8:15
74	17AUG1978	32	2.69	2.25	0.75	0.52	-1.6	40	38	8:35
73	17AUG1978	33	2.62	1.28	0.36	0.24	0.0	70	25	8:25
76	17AUG1978	53	14.50	7.59	0.27	0.16	-1.9	•	16	8:55
75	17AUG1978	54	3.56	1.87	•	•	0.0	80	7	8:55
77	17AUG1978	75	5.75	2.81	0.44	0.32	0.0	62	20	9:10
78	17AUG1978	76	11.40	5.74	2.22	1.02	0.0	40	19	9:15
79	23AUG1978	21	1.50	0.84	0.47	0.38	-1.9	54	30	7:45
80	23AUG1978	22	2.75	2.75	0.94	0.62	-2.2	50	19	8:00
81	23AUG1978	23	3.12	2.29	0.21	0.22	0.0	30	20	8:00
82	23AUG1978	62	4.25	2.37	•	•	-1.9	66	6	8:05
83	23AUG1978	63	1.88	1.66	0.75	•	-3.8	46	19	8:35
84	23AUG1978	75	6.50	4.03	0.72	0.56	-3.8	56	27	8:20
85	23AUG1978	76	3.12	1.31	1.32	1.23	-4.0	38	20	8:25
87	30AUG1978	13	1.31	1.10	0.35	0.26	-1.3	54	30	8:45
86	30AUG1978	14	0.75	0.40	0.20	0.40	-1.3	74	22	8:30
88	30AUG1978	21	5.37	3.31	0.42	0.38	0.0	45	60	8:55
89	30AUG1978	22	4.50	2.73	0.64	0.81	0.0	38	12	9:00
90	30AUG1978	23	4.25	2.90	0.80	0.62	-1.3	24	24	9:10
91	30AUG1978	75	5.00	3.03	0.41	0.46	-1.0	58	24	9:20
92	30AUG1978	76	1.88	1.67	1.10	1.14	0.0	36	24	9:30
96	06SEP1978	21	10.20	4.88	0.92	0.86	0.0	50	70	7:15
97	06SEP1978	23	8.60	6.42	1.44	0.58	-1.3	30	30	7:15
98	08SEP1978	21	0.75	0.32	0.38	0.54	-6.4	58	67	17:00
99	08SEP1978	23	12.24	5.89	1.19	0.44	-1.5	34	30	17:00
100	11SEP1978	21	1.88	1.32	0.22	0.12	-2.0	55	60	13:30
101	11SEP1978	23	2.35	1.66	0.50	0.16	0.0	35	25	13:30
102	13SEP1978	21	1.56	1.47	0.50	0.40	-3.2	50	60	8:05

Appendix B Continued

SEQUENCE	DATE	LOC	MAX H2S	AVE H2S	AVE TD	AVE TDS	MAX DEPTH	WATER DEPTH	MUD DEPTH	TIME
103	13SEP1978	22	3.06	3.06	0.69	•	-1.3	42	16	6:20
104	13SEP1978	23	0.62	0.39	0.17	0.06	0.0	30	25	8:20
105	13SEP1978	31	0.94	0.77	0.34	0.39	0.0	62	29	8:15
106	13SEP1978	32	5.00	3.33	0.71	0.28	-1.9	40	38	8:45
107	15SEP1978	21	2.12	1.31	0.88	•	-2.0	50	60	17:15
108	15SEP1978	23	10.20	6.30	0.44	0.12	-2.0	25	30	17:00
109	17SEP1978	21	2.44	1.96	0.55	0.32	-1.9	45	60	10:30
110	17SEP1978	22	1.50	1.13	0.32	0.36	-1.9	46	17	9:45
111	17SEP1978	23	3.88	3.13	1.09	0.50	-1.3	29	33	9:45
112	17SEP1978	31	1.38	1.07	0.35	0.09	-1.9	55	23	10:00
113	17SEP1978	32	0.50	0.23	0.31	•	-1.9	43	30	9:30
114	20SEP1978	21	6.50	3.29	0.41	0.42	0.0	44	60	8:15
115	20SEP1978	23	2.00	0.87	0.22	0.17	-2.0	25	30	8:30
116	22SEP1978	21	2.06	1.37	0.25	0.20	-1.9	55	60	18:00
117	22SEP1978	23	5.00	3.11	0.84	0.32	-1.9	25	30	18:00
118	22SEP1978	31	0.50	0.47	0.33	0.26	-1.3	58	20	18:15
119	22SEP1978	32	4.38	2.65	0.65	0.50	-1.9	42	35	18:25
120	25SEP1978	21	3.75	1.42	0.18	0.11	0.0	55	60	17:15
121	25SEP1978	23	11.40	10.80	2.44	0.28	0.0	30	25	17:30
122	27SEP1978	21	1.19	0.58	0.10	0.02	0.0	58	52	14:30
123	27SEP1978	22	14.50	9.44	4.65	0.66	-1.9	49	13	14:45
124	27SEP1978	23	11.40	7.86	1.92	0.34	-1.9	35	21	14:55
125	27SEP1978	31	0.56	0.27	0.22	0.28	-1.9	62	20	14:15
126	27SEP1978	32	2.56	1.44	0.99	0.40	-1.9	44	32	15:00
127	30SEP1978	21	8.60	4.63	1.26	0.29	-2.0	50	60	17:10
128	30SEP1978	23	11.40	8.95	0.62	0.00	-2.8	30	30	17:25
129	02OCT1978	21	0.38	0.27	0.23	0.25	0.0	55	60	13:00
130	02OCT1978	22	8.12	8.12	1.00	0.39	-1.3	42	16	12:45
131	02OCT1978	23	5.75	5.10	1.58	0.26	-1.3	30	25	14:00
132	02OCT1978	31	0.62	0.37	0.39	0.44	1.9	58	20	13:40
133	02OCT1978	32	10.60	6.66	0.83	0.56	-1.9	35	38	14:30
134	04OCT1978	21	5.00	2.33	0.53	0.47	-2.5	55	60	16:00
135	04OCT1978	23	3.12	1.67	0.44	0.22	-2.0	25	30	15:00
136	07OCT1978	21	1.60	•	•	•	•	47	70	14:40
137	07OCT1978	22	1.25	0.73	0.66	0.27	-3.6	41	13	15:15
138	07OCT1978	23	5.07	2.86	3.00	0.25	-4.7	25	30	15:30
139	07OCT1978	31	0.62	0.56	0.47	0.26	-1.9	55	27	14:55
140	07OCT1978	32	7.25	5.03	0.81	0.42	-1.3	30	40	15:05
141	09OCT1978	21	0.19	0.00	0.06	0.15	-12.0	55	65	10:15
142	09OCT1978	23	1.31	0.72	0.38	0.29	0.0	22	34	10:20
143	11OCT1978	21	0.56	0.48	0.28	0.31	-2.5	55	70	13:15
144	11OCT1978	22	11.40	7.60	2.40	0.68	-3.2	40	17	13:40
145	11OCT1978	23	1.75	1.50	0.60	0.30	-3.8	25	25	13:20
146	11OCT1978	31	0.94	0.67	0.53	0.53	-0.6	55	22	13:00
147	11OCT1978	32	8.12	4.76	1.88	0.97	-1.9	32	38	13:00
148	14OCT1978	21	2.06	1.34	0.34	0.28	-1.9	50	60	15:00
149	14OCT1978	23	3.06	1.33	0.17	0.22	-2.0	25	20	16:00
150	16OCT1978	21	4.75	2.52	0.83	0.20	-1.9	50	60	16:00
151	16OCT1978	22	10.20	8.28	3.11	0.62	-3.2	47	13	16:20

Appendix B Continued

SEQUENCE	DATE	LOC	MAXH2S	AVE05	AVE510	AVE10IE	MAXDEPTH	WATDEPTH	MUDDEPTH	TIME
152	16OCT1978	23	1.00	0.46	0.45	0.14	-3.6	26	20	16:15
153	16OCT1978	31	0.38	0.26	0.19	0.20	-1.9	55	24	16:30
154	16OCT1978	32	2.50	1.45	0.92	0.75	-1.9	35	35	16:00
155	18OCT1978	21	0.62	0.34	0.29	0.17	-5.0	50	60	14:00
156	18OCT1978	23	3.12	1.46	1.46	0.69	-4.0	30	20	14:15
157	21OCT1978	21	1.25	0.57	0.19	0.26	-1.9	50	60	10:50
158	21OCT1978	22	4.38	3.88	0.36	0.25	-1.3	44	15	10:40
159	21OCT1978	23	1.12	0.56	0.35	0.24	-3.2	28	30	10:35
160	21OCT1978	31	1.12	0.80	0.55	0.45	-3.2	55	19	10:55
161	21OCT1978	32	5.07	2.88	0.53	0.33	-1.3	37	33	10:30
162	23OCT1978	21	3.50	2.67	0.47	0.15	-2.5	50	60	17:00
163	23OCT1978	23	4.88	1.75	0.68	0.22	-4.0	26	36	17:20
164	25OCT1978	21	1.94	1.17	0.46	0.42	-1.9	59	60	11:45
165	25OCT1978	22	0.62	0.16	0.33	0.10	-5.7	21	39	11:50
166	25OCT1978	23	0.44	0.09	0.28	0.21	-7.6	25	26	12:00
167	25OCT1978	31	0.62	0.43	0.28	0.34	-1.9	49	20	11:35
168	25OCT1978	32	3.38	2.25	0.63	0.25	-1.9	30	30	12:10
169	27OCT1978	21	0.56	0.23	0.44	0.50	-4.0	50	60	14:40
170	27OCT1978	23	0.25	0.22	0.25	0.18	-8.0	25	25	14:50
171	30OCT1978	21	1.31	0.62	0.26	0.50	-1.9	47	60	12:45
172	30OCT1978	22	0.53	0.20	0.35	0.18	-3.8	40	17	12:55
173	30OCT1978	23	0.19	0.12	0.14	0.19	-8.9	25	30	13:05
174	30OCT1978	31	0.62	0.40	0.47	0.19	-3.6	44	28	12:30
175	30OCT1978	32	5.37	3.46	0.65	0.39	-1.9	22	36	13:05
176	01NOV1978	21	1.88	1.04	0.73	0.14	-3.0	45	70	10:25
177	01NOV1978	23	0.06	0.02	0.06	0.06	•	25	26	10:10
178	04NOV1978	21	2.06	1.24	0.14	0.04	-2.5	47	70	9:25
179	04NOV1978	22	6.12	4.37	1.10	0.21	-3.2	32	22	9:40
180	04NOV1978	23	3.30	2.31	0.39	0.20	-1.9	25	29	9:50
181	04NOV1978	31	2.19	1.66	0.27	0.27	-1.3	42	22	9:30
182	04NOV1978	32	1.88	0.41	0.77	0.06	-6.6	22	40	9:45
183	06NOV1978	21	1.88	1.00	0.40	0.18	-1.9	50	65	14:00
184	06NOV1978	23	0.19	0.03	0.15	0.06	-8.6	28	27	14:20
185	08NOV1978	21	0.69	0.39	0.18	0.00	-3.8	50	60	10:55
186	08NOV1978	22	2.32	0.85	1.26	0.20	-3.8	44	18	11:00
187	08NOV1978	23	0.00	0.00	0.00	0.00	•	29	30	11:25
188	08NOV1978	31	2.06	1.12	0.32	0.17	-2.5	48	28	10:45
189	08NOV1978	32	0.91	0.61	0.61	0.20	-3.8	26	38	11:10
190	11NOV1978	21	2.88	1.46	0.64	0.12	-3.0	50	60	9:55
191	11NOV1978	23	0.06	0.00	0.06	0.02	-9.0	25	30	10:05
192	15NOV1978	21	1.38	0.51	0.10	0.09	-3.8	•	•	14:15
193	15NOV1978	22	0.22	0.00	0.10	0.12	-9.5	•	•	14:20
194	15NOV1978	23	0.19	0.00	0.07	0.00	-8.9	•	•	14:35
195	15NOV1978	31	0.47	0.30	0.22	0.10	-1.9	•	•	14:10
196	15NOV1978	32	1.00	0.45	0.09	0.00	-3.8	•	•	14:30
197	18NOV1978	21	0.19	0.05	0.14	0.12	-8.0	50	60	11:15
198	18NOV1978	23	0.00	0.00	0.00	0.00	•	26	30	11:40
199	22NOV1978	21	0.09	0.01	0.07	0.03	-5.7	50	60	9:50
200	22NOV1978	22	0.69	0.30	0.48	0.28	-9.5	42	15	10:15

Appendix B Continued

SEQUENCE	DATE	LOC	MAX 2S	AVG E	AVG 5 TC	AVG 15	MAX DEPTH	WATER DEPTH	MUD DEPTH	TIME
01	22NOV1978	23	0.00	0.00	0.00	0.00	.	25	30	10:00
02	22NOV1978	31	0.16	0.07	0.12	0.02	-5.7	42	23	9:40
03	22NOV1978	32	0.22	0.10	0.13	0.04	-5.7	30	30	10:10
04	25NOV1978	21	0.03	0.01	0.02	0.01	-8.0	50	60	12:30
05	25NOV1978	23	0.03	0.02	0.02	0.00	-2.0	25	30	12:45
06	01DEC1978	21	0.38	0.07	0.15	0.00	-7.6	50	60	12:25
07	01DEC1978	22	1.16	0.33	1.09	0.37	-6.4	45	15	11:10
08	01DEC1978	23	0.00	0.00	0.00	0.00	.	25	25	11:00
09	01DEC1978	31	0.42	0.24	0.13	0.15	-1.9	50	20	11:15
10	01DEC1978	32	0.22	0.13	0.11	0.08	-4.4	25	37	12:40
11	08DEC1978	21	1.25	0.03	1.10	0.32	-6.0	.	.	15:00
12	08DEC1978	23	0.00	0.00	0.00	0.00	.	.	.	15:30
13	13DEC1978	21	0.00	0.00	0.00	0.00	.	50	60	16:40
14	13DEC1978	22	0.00	0.00	0.00	0.00	.	40	15	16:45
16	13DEC1978	31	0.22	0.10	0.14	0.10	-1.9	50	20	16:30
17	13DEC1978	32	0.14	0.02	0.07	0.00	-5.7	20	40	16:55
18	18DEC1978	21	0.17	0.00	0.02	0.10	-16.0	.	.	15:15
19	18DEC1978	23	0.00	0.00	0.00	0.00	.	.	.	15:30
20	22DEC1978	21	0.00	0.00	0.00	0.00	.	55	70	13:15
21	22DEC1978	22	0.12	0.01	0.08	0.06	-7.6	42	15	13:20
22	22DEC1978	23	0.00	0.00	0.00	0.00	.	30	25	13:30
23	22DEC1978	31	0.47	0.24	0.34	0.09	-5.7	42	24	13:00
24	22DEC1978	32	1.19	0.72	0.24	0.08	-3.8	28	32	13:05
25	26DEC1978	21	0.19	0.00	0.14	0.16	-10.0	55	70	12:15
26	26DEC1978	23	0.00	0.00	0.00	0.00	.	30	25	12:45
27	29DEC1978	21	0.00	0.00	0.00	0.00	.	55	65	13:10
28	29DEC1978	22	0.17	0.02	0.08	0.14	-13.3	42	14	13:20
29	29DEC1978	23	0.00	0.00	0.00	0.00	.	28	30	13:25
31	29DEC1978	32	0.19	0.10	0.08	0.02	-1.9	25	35	13:05
32	03JAN1979	21	0.00	0.00	0.00	0.00	.	55	.	15:00
33	08JAN1979	21	0.16	0.02	0.03	0.09	-13.3	57	.	13:00
34	08JAN1979	22	0.06	0.00	0.02	0.02	-5.7	52	13	13:20
35	08JAN1979	23	0.00	0.00	0.00	0.00	.	45	20	13:45
36	08JAN1979	31	0.25	0.11	0.11	0.03	-4.8	58	.	14:10
37	08JAN1979	32	0.12	0.03	0.07	0.02	-5.7	36	40	13:30
38	20JAN1979	21	0.94	0.05	0.94	0.79	-9.0	75	60	15:00
39	20JAN1979	23	0.00	0.00	0.00	0.00	.	51	30	15:40
40	26JAN1979	21	0.00	0.00	0.00	0.00	.	72	.	14:50
41	02FEB1979	21	0.16	0.03	0.04	0.06	-18.0	75	.	13:20
42	02FEB1979	22	0.42	0.06	0.15	0.08	-7.6	70	.	13:45
43	02FEB1979	23	0.00	0.00	0.00	0.00	.	55	.	13:40
44	02FEB1979	31	0.06	0.04	0.03	0.01	-2.8	.	.	13:30
45	02FEB1979	32	0.06	0.01	0.04	0.02	-5.7	55	.	13:55
46	09FEB1979	21	0.06	0.00	0.04	0.00	-7.6	.	.	14:20
47	21FEB1979	21	0.00	0.00	0.00	0.00	.	.	.	8:10
48	21FEB1979	22	0.25	0.00	0.08	0.00	-5.7	.	.	8:10
49	21FEB1979	23	0.00	0.00	0.00	0.00	.	.	.	8:30
50	21FEB1979	31	0.00	0.00	0.00	0.00	.	.	.	7:45
51	02MAR1979	21	0.00	0.00	0.00	0.00	.	60	50	9:00

Appendix B Continued

SEQUENCE	DATE	LOC	MAXH2S	AVE2E	AVE10	AVE15	MAXDEPTH	WATDEPTH	MUDDEPTH	TIME
252	02MAR1979	32	0.16	0.09	0.02	0.06	-11.0	45	45	9:40
253	07MAR1979	21	0.57	0.02	0.54	0.23	-8.2	50	50	8:45
254	07MAR1979	23	0.00	0.00	0.00	0.00	•	55	55	9:00
255	07MAR1979	31	0.00	0.00	0.00	0.00	•	45	20	8:30
256	07MAR1979	32	0.68	0.00	0.07	0.02	-5.0	25	35	9:10
257	11MAR1979	21	0.00	0.00	0.00	0.00	•	45	45	10:30
258	11MAR1979	23	0.00	0.00	0.00	0.00	•	20	35	10:15
259	11MAR1979	32	0.12	0.02	0.08	0.09	-10.0	15	35	10:00
260	14MAR1979	21	0.44	0.06	0.24	0.03	-7.6	40	50	9:10
261	14MAR1979	23	0.00	0.00	0.00	0.00	•	20	30	9:00
262	14MAR1979	31	0.22	0.03	0.15	0.14	-5.7	40	20	9:20
263	14MAR1979	32	0.00	0.00	0.00	0.00	•	20	35	8:50
264	21MAR1979	21	0.16	0.06	0.10	0.00	-8.2	44	50	8:55
265	21MAR1979	23	0.11	0.10	0.04	0.00	-5.7	22	36	9:10
266	21MAR1979	32	0.36	0.29	0.21	0.03	-4.0	15	35	9:00
267	28MAR1979	21	0.64	0.07	0.47	0.16	-9.5	40	50	9:40
268	28MAR1979	23	0.00	0.00	0.00	0.00	•	20	25	9:20
269	28MAR1979	31	0.88	0.22	0.56	•	-5.7	40	20	9:50
270	28MAR1979	32	3.60	1.10	0.60	0.00	-4.0	20	35	9:00
271	04APR1979	21	0.00	0.00	0.00	0.00	•	35	50	9:40
272	04APR1979	23	0.23	0.04	0.09	0.00	-7.6	15	25	9:15
273	04APR1979	32	0.83	0.35	0.38	0.12	-2.0	15	35	9:05
274	08APR1979	21	0.39	0.00	0.21	0.30	-8.2	45	50	17:20
275	08APR1979	23	0.00	0.00	0.00	0.00	•	30	30	17:15
276	11APR1979	21	0.24	0.06	0.16	0.18	-8.9	50	50	9:25
277	11APR1979	23	0.00	0.00	0.00	0.00	•	30	23	9:45
278	11APR1979	31	1.30	0.17	0.84	0.34	-5.7	50	20	9:30
279	11APR1979	32	1.79	0.94	0.94	•	-3.0	30	38	9:55
280	18APR1979	21	0.44	0.19	0.13	0.38	-11.4	45	50	9:15
281	18APR1979	23	0.83	0.38	0.52	0.00	-9.5	30	25	9:30
282	18APR1979	32	0.94	0.58	0.44	0.46	-1.0	25	40	9:40
283	25APR1979	21	1.00	0.33	0.03	0.04	0.0	40	60	9:00
284	25APR1979	23	1.31	1.28	0.08	0.00	-3.8	25	25	9:20
285	25APR1979	31	1.25	0.85	0.07	0.12	-1.0	50	20	9:05
286	25APR1979	32	0.46	0.00	0.27	0.30	-5.1	30	35	9:35
287	06MAY1979	21	0.84	0.50	0.04	0.14	-2.5	50	45	14:20
288	06MAY1979	23	2.73	1.34	1.16	0.00	-7.8	30	20	14:35
289	06MAY1979	33	2.75	1.18	0.36	0.22	0.0	30	40	14:45
290	09MAY1979	21	0.00	0.00	0.00	0.00	•	45	55	8:40
291	09MAY1979	23	0.27	0.20	0.19	0.21	-11.0	25	25	8:30
292	09MAY1979	31	0.64	0.37	0.39	0.33	-3.8	55	20	9:00
293	09MAY1979	32	1.11	0.70	0.87	0.52	-2.0	30	38	8:20
294	14MAY1979	21	0.52	0.00	0.14	0.46	-10.1	40	50	10:00
295	14MAY1979	22	9.87	4.19	3.64	0.48	-4.0	40	20	10:10
296	14MAY1979	23	0.00	0.00	0.00	0.00	•	25	25	10:20
297	18MAY1979	21	0.26	0.21	0.20	•	0.0	40	50	8:55
298	18MAY1979	22	0.75	0.44	0.57	0.25	-4.0	30	20	8:55
299	18MAY1979	23	0.30	0.10	0.10	0.00	-3.8	22	28	8:30
300	18MAY1979	31	0.72	0.24	0.00	0.00	-1.9	55	18	9:05

Appendix B Continued

SEQUENCE	DATE	LOC	MAX2S	AVG 5	AVE 10	AVE 15	MAX DEPTH	WATER DEPTH	MUD DEPTH	TIME
1	23MAY1979	21	0.64	0.24	0.07	0.00	-1.9	40	50	15:00
2	23MAY1979	22	4.58	1.65	0.26	0.29	-4.6	42	20	15:05
3	23MAY1979	23	0.51	0.32	0.00	0.00	-1.6	30	25	15:25
4	29MAY1979	21	0.51	0.24	0.14	0.44	-13.3	40	50	13:10
5	29MAY1979	22	1.40	1.13	0.45	0.44	-1.7	45	15	13:00
6	29MAY1979	23	0.95	0.44	0.52	0.34	-5.7	30	20	12:40
7	29MAY1979	32	0.76	0.46	0.48	0.32	-4.4	35	35	12:30
8	01JUN1979	21	0.87	0.45	0.22	0.40	-1.9	45	50	15:00
9	01JUN1979	22	7.30	3.30	1.42	0.86	-4.0	45	15	14:45
10	01JUN1979	23	0.38	0.31	0.23	0.23	-0.6	30	20	14:40
11	07JUN1979	21	3.20	1.63	0.03	0.00	-1.9	40	50	11:30
12	07JUN1979	22	2.90	1.49	0.24	0.16	-2.5	45	20	11:55
13	07JUN1979	23	0.22	0.08	0.07	0.13	-0.6	30	25	12:00
14	07JUN1979	32	0.25	0.16	0.25	0.07	-0.8	48	35	12:10
15	10JUN1979	21	0.96	0.89	0.35	0.40	-2.6	•	•	14:40
16	10JUN1979	22	4.90	2.25	0.74	0.36	-2.0	•	•	15:00
17	10JUN1979	23	0.41	0.37	0.18	0.12	-1.6	•	•	15:05
19	13JUN1979	21	0.51	0.46	0.18	0.28	-1.6	45	55	10:55
C	13JUN1979	22	1.70	1.15	1.10	0.66	-3.0	49	27	10:40
2	13JUN1979	23	0.58	0.25	0.41	0.26	-7.6	35	25	10:25
1	13JUN1979	27	4.40	2.16	0.68	0.70	-3.8	45	25	10:35
3	15JUN1979	21	0.44	0.41	0.17	0.17	-3.2	50	25	10:40
4	15JUN1979	22	1.45	0.66	1.25	0.58	-7.0	50	17	9:55
5	15JUN1979	23	0.36	0.23	0.25	0.15	-4.4	35	25	10:00
6	21JUN1979	21	1.45	0.73	0.22	0.11	-3.8	45	25	10:00
7	21JUN1979	22	2.71	1.20	0.50	0.49	0.0	47	9	9:55
8	21JUN1979	23	1.62	0.69	0.97	0.66	-7.6	33	9	9:30
9	21JUN1979	27	2.60	2.33	0.72	0.50	-0.6	45	20	9:40
C	28JUN1979	21	3.30	1.45	0.56	0.65	-0.6	45	50	14:30
1	28JUN1979	22	0.62	0.23	0.42	0.39	0.0	45	20	15:55
2	28JUN1979	23	3.63	1.69	•	•	-1.6	35	20	13:45
3	02JUL1979	21	0.94	0.43	0.44	•	-1.9	45	55	9:25
4	02JUL1979	22	2.62	2.44	0.69	0.55	-2.9	45	20	9:35
6	02JUL1979	23	1.11	0.85	0.49	0.74	-1.9	32	20	9:45
5	02JUL1979	27	5.35	2.25	2.02	0.90	-3.8	50	35	9:40
8	07JUL1979	22	1.76	1.35	0.66	0.65	-2.4	52	25	14:50
7	07JUL1979	23	0.44	0.19	0.28	0.44	-11.4	38	25	15:10
40	10JUL1979	21	0.91	0.40	0.09	0.00	-1.9	45	55	11:15
41	10JUL1979	22	3.68	1.67	0.62	0.22	-2.6	58	25	11:00
42	10JUL1979	23	0.90	0.20	0.56	0.30	-5.7	32	25	10:45
43	10JUL1979	27	3.63	3.10	0.55	0.46	-3.2	60	30	10:55
45	13JUL1979	21	0.10	0.07	0.07	0.05	-1.9	48	55	15:55
46	13JUL1979	22	0.64	0.56	0.46	0.33	-2.6	52	18	16:00
47	13JUL1979	23	4.20	1.69	0.29	0.24	-0.6	32	30	14:20
48	16JUL1979	21	1.43	0.81	0.62	0.28	-0.6	50	50	14:20
49	16JUL1979	22	3.20	1.54	0.47	0.46	-1.6	48	20	13:30
51	16JUL1979	23	1.24	0.85	0.25	0.16	-1.9	37	55	13:00
50	16JUL1979	27	2.62	1.58	0.74	0.48	-3.2	58	50	13:20
52	20JUL1979	21	2.43	1.37	0.44	0.42	-1.9	50	50	9:40

Appendix B Continued

SEQ	DATE	LOC	MAX H2S	AVE H2S	AVE IO	AVE IS	MAX DEPTH	WAT DEPTH	MUD DEPTH	TIME
353	20JLL1979	22	1.71	0.91	0.28	0.32	0.0	49	20	9:55
354	20JLL1979	23	2.32	1.30	0.12	0.14	0.0	34	18	10:05
355	23JLL1979	21	2.07	0.68	0.13	0.17	-1.5	48	50	14:45
356	23JLL1979	22	0.67	0.65	0.34	0.27	-4.0	47	21	14:20
357	23JLL1979	23	2.52	1.07	0.13	0.00	-1.9	36	24	13:30
358	23JLL1979	27	3.08	1.67	0.48	0.38	-1.9	52	32	14:15
359	26JLL1979	21	0.33	0.18	0.05	0.04	-1.9	49	50	10:40
360	26JLL1979	22	1.37	0.97	0.45	0.33	0.0	49	22	10:30
361	26JLL1979	23	2.00	0.50	0.25	0.27	-1.3	34	22	13:15
362	30JLL1979	21	1.74	0.85	0.03	0.12	0.0	46	50	9:15
363	30JLL1979	22	1.10	0.66	0.06	0.07	0.0	46	20	9:30
365	30JLL1979	23	0.97	0.62	0.28	0.17	-0.6	34	20	9:45
364	30JLL1979	27	1.49	0.97	0.44	0.42	-0.6	51	30	9:45
366	03ALG1979	21	1.38	0.87	0.27	0.21	0.6	45	40	10:00
367	03ALG1979	22	2.12	1.67	0.48	0.40	-1.9	43	20	9:45
368	03ALG1979	23	3.00	1.80	0.33	0.21	-1.3	29	35	9:15
369	06AUG1979	21	3.50	1.39	0.10	0.10	0.0	45	40	9:45
370	06ALG1979	22	1.48	0.95	0.18	0.32	-2.5	48	15	9:45
372	06ALG1979	23	4.40	2.57	0.27	0.08	-1.3	30	25	9:10
371	06ALG1979	27	0.62	0.43	0.16	0.05	0.0	50	25	9:20
373	09ALG1979	21	5.00	1.96	0.10	0.04	-0.6	58	40	9:45
374	09ALG1979	22	1.55	0.86	0.19	0.15	-1.9	60	18	13:55
375	09ALG1979	23	2.68	1.31	0.26	0.08	0.6	42	21	14:15
383	14ALG1979	21	0.75	0.43	0.25	0.32	0.0	50	60	14:20
384	14AUG1979	22	0.91	0.62	0.74	0.48	-7.0	58	15	14:30
387	14AUG1979	23	0.47	0.33	0.21	•	-1.3	45	20	14:05
385	14ALG1979	27	1.38	0.63	0.66	0.16	-1.9	52	20	14:35
386	14ALG1979	28	0.27	0.23	0.22	•	-5.7	90	0	14:40
395	22AUG1979	21	0.94	0.66	0.04	0.02	-1.3	60	60	14:00
396	22ALG1979	22	0.79	0.49	0.26	•	0.0	69	10	14:05
399	22ALG1979	23	6.30	3.60	0.21	0.08	-1.3	40	28	14:35
397	22ALG1979	27	0.13	0.13	0.13	•	-1.3	73	17	14:15
398	22ALG1979	28	0.86	0.55	0.00	•	0.0	100	•	14:20
400	27ALG1979	21	0.53	0.33	0.16	0.20	-1.9	63	50	13:00
401	27AUG1979	22	0.53	0.46	•	•	0.0	68	15	13:15
402	27AUG1979	23	8.80	7.44	2.14	•	-1.3	40	24	13:50
403	27ALG1979	23	2.48	2.15	0.70	•	0.6	40	24	14:00
404	27ALG1979	23	3.20	1.47	0.44	•	-1.9	40	24	14:03
405	27ALG1979	23	4.34	2.06	•	•	0.0	40	24	14:07
406	27AUG1979	23	0.17	0.13	0.16	•	0.0	40	24	14:10
407	31ALG1979	21	4.16	1.94	0.35	0.35	-1.9	65	60	12:55
408	31ALG1979	22	3.37	1.54	0.42	•	0.0	68	16	13:10
410	31ALG1979	23	5.54	3.57	0.14	0.14	-1.5	45	24	13:25
409	31ALG1979	27	1.57	1.02	0.46	•	0.0	79	10	13:20
411	05SEP1979	21	2.72	1.03	0.22	0.33	0.0	•	•	8:15
412	05SEP1979	22	1.44	0.73	0.44	0.26	1.9	63	17	8:30
413	05SEP1979	23	1.47	0.58	0.19	0.42	-1.9	42	20	9:45
414	09SEP1979	21	2.58	0.94	0.12	0.06	-2.5	60	50	14:10
415	09SEP1979	23	4.48	2.15	0.37	0.24	-1.9	42	27	14:55

Appendix B Continued

SEQ SUF	DATE	LOC	MAX 2 S	AVE 0 5	AVE 5 I 0	AVE 1 C I S	MAX DEPTH	WAT DEPTH	MUD DEPTH	TIME
416	09SEP1979	2[illegible]	8.18	3.23	0.16	•	0.0	42	27	15:00
417	09SEP1979	2[illegible]	11.22	4.00	0.29	0.46	-0.6	42	27	15:05
418	12SEP1979	21	1.73	0.71	0.11	0.16	0.0	•	•	7:50
419	12SEP1979	22	1.03	0.54	0.41	0.25	-2.0	•	•	7:30
420	12SEP1979	23	7.70	4.98	0.24	0.34	0.0	•	•	7:20
421	16SEP1979	21	1.17	0.82	0.26	0.28	0.0	55	45	14:00
422	16SEP1979	22	0.75	0.43	0.24	0.27	-2.0	•	•	14:20
424	16SEP1979	23	5.24	2.34	0.19	0.30	0.0	32	30	14:40
423	16SEP1979	27	2.36	1.03	0.52	0.26	-3.6	56	22	14:30
425	19SEP1979	21	4.78	1.95	0.16	0.15	0.0	55	45	9:15
426	19SEP1979	22	1.51	0.97	0.37	0.40	0.0	54	20	9:00
427	19SEP1979	23	3.26	0.26	0.35	0.21	-1.3	•	•	8:55
428	23SEP1979	21	0.76	0.37	0.[illegible]	0.00	1.6	55	40	10:00
429	23SEP1979	22	3.51	1.37	0.14	0.00	0.0	54	17	10:10
430	23SEP1979	22	5.06	2.06	0.16	0.09	-1.9	54	17	10:15
431	23SEP1979	23	3.04	2.19	0.09	0.09	-1.9	33	30	10:25
432	26SEP1979	21	4.92	3.56	0.27	0.09	-3.2	45	40	9:10
433	26SEP1979	22	9.10	3.68	0.44	0.25	0.0	45	20	9:25
434	26SEP1979	23	3.77	1.63	0.18	0.14	-1.9	33	20	9:40
435	30SEP1979	21	8.47	3.10	0.14	0.32	-0.6	50	40	16:15
436	30SEP1979	22	3.56	2.31	0.38	0.14	-1.0	53	16	16:20
426	30SEP1979	2[illegible]	9.30	5.28	0.48	0.44	-1.3	30	24	16:55
437	30SEP1979	27	2.43	1.28	0.40	•	0.0	53	24	16:35
439	03OCT1979	21	8.22	5.08	0.17	0.18	-0.6	45	50	18:00
440	03OCT1979	22	2.60	1.85	0.50	0.32	0.0	44	20	18:15
441	03OCT1979	23	3.38	1.21	0.52	0.24	-3.8	26	28	18:20
442	07OCT1979	23	3.43	1.49	0.57	0.20	-3.8	30	22	16:00
443	07OCT1979	23	2.71	1.95	0.40	0.28	-2.5	30	22	16:10
444	07OCT1979	23	2.56	1.09	0.35	0.23	-1.9	30	20	16:15
445	07OCT1979	23	0.96	0.57	0.42	0.32	-2.0	30	20	16:30
450	15OCT1979	21	1.09	0.46	0.06	0.08	-0.6	40	50	11:05
451	15OCT1979	22	6.76	3.57	0.24	0.19	-1.9	45	17	11:15
452	15OCT1979	23	0.24	0.07	0.16	0.22	-5.7	23	23	11:25
457	21OCT1979	21	2.50	1.18	0.19	0.20	-1.9	40	45	14:27
458	21OCT1979	22	1.67	1.00	0.29	0.31	-1.3	40	18	14:37
459	21OCT1979	23	3.41	1.74	0.20	0.20	-0.6	20	20	15:00
460	24OCT1979	21	3.00	1.22	0.30	0.14	-1.9	36	54	8:07
461	24OCT1979	22	0.58	0.36	0.36	0.34	-8.0	39	14	8:26
463	24OCT1979	23	2.05	1.05	0.38	0.15	-3.2	20	20	8:45
462	24OCT1979	27	1.56	1.54	0.31	0.24	-3.8	34	32	8:25
464	27OCT1979	21	1.14	0.89	0.20	0.19	-2.5	36	50	14:40
465	27OCT1979	22	0.82	0.32	0.71	0.34	-5.7	36	18	14:13
466	27OCT1979	23	1.43	0.72	0.16	0.02	-3.2	20	20	13:59
467	31OCT1979	21	2.75	1.65	0.14	0.14	-2.5	35	43	8:05
466	31OCT1979	22	0.30	0.22	0.16	0.21	-4.0	40	15	9:15
470	31OCT1979	23	2.73	1.43	0.14	0.11	-3.8	20	22	9:30
469	31OCT1979	27	0.46	0.40	0.11	0.07	-1.9	36	37	9:20
471	04NOV1979	21	0.20	0.11	0.17	0.14	-1.9	38	45	10:00
472	04NOV1979	22	0.40	0.27	0.30	0.33	-3.8	30	20	11:00

Appendix B Continued

SEQSULF	DATE	LOC	MAXH2S	AVE05	AVE5 T0	AVE10 T5	MAXDEPTH	WATDEPTH	MUDDEPTH	TIME
473	04NOV1979	23	0.79	0.57	0.30	0.28	-4.4	20	20	10:45
474	14NOV1979	21	1.18	0.49	0.05	0.10	-1.9	35	45	8:45
475	14NOV1979	22	1.75	0.70	0.17	0.21	-2.0	35	20	8:51
477	14NOV1979	23	0.89	0.45	0.26	0.09	-4.4	17	25	9:05
476	14NOV1979	27	0.21	0.13	0.13	0.10	-3.0	40	30	9:00
478	21NOV1979	21	1.75	0.91	0.07	0.06	-1.3	40	50	11:45
479	21NOV1979	22	4.19	1.92	0.18	0.24	-1.9	40	20	12:00
480	21NOV1979	23	4.40	4.28	0.70	0.14	-2.5	20	25	12:36

N=449

Appendix C. Depth in the sediment of the maximum concentration of dissolved sulfide in sediment profiles at the feeding area in Pond 2. Monthly averages, distribution statistics and the results of the Duncan multiple range test; months not joined by the same letter are significantly different at the P=0.05 level.

Month	Year	n	Mean (cm)	Range Min	Range Max	S.D.	S.E.	Duncan
July	1978	2	0.00	0.00	0.00	0.00	0.00	A
September	1979	8	-0.63	-2.00	1.90	1.37	0.48	A
August	1978	2	-1.10	-2.20	0.00	1.56	1.10	A B
September	1978	3	-1.50	-1.90	1.30	0.35	0.20	A B
July	1979	9	-1.56	-4.00	0.00	1.42	0.47	A B
August	1979	7	-1.57	-7.00	0.00	2.51	0.95	A B
June	1978	4	-1.75	-2.20	-1.00	0.52	0.26	A B
November	1979	3	-2.57	-3.80	-1.90	1.07	0.62	A B C
June	1979	7	-2.57	-7.00	0.00	2.44	0.92	A B C
October	1978	7	-3.16	-5.70	-1.30	1.53	0.58	A B C
May	1979	4	-3.25	-4.00	-1.00	1.50	0.75	A B C
March	1978	1	-3.40	-3.40	-3.40	0.00	0.00	A B C D
October	1979	6	-3.48	-8.00	0.00	3.00	1.22	A B C D
May	1978	8	-5.65	-14.30	-1.00	4.73	1.67	B C D
January	1979	1	-5.70	-5.70	-5.70	0.00	0.00	B C D
November	1978	4	-6.50	-9.50	-3.10	3.47	1.74	B C D
February	1979	2	-6.65	-7.60	-5.70	1.34	0.95	B C D
April	1978	6	-6.67	-11.40	-1.90	4.39	1.79	C D
December	1978	3	-9.10	-13.30	-6.40	3.69	2.13	D

Appendix D. Depth in the sediment of the maximum concentration of dissolved sulfide in sediment profiles at the north shore sample location in Pond 2. Monthly averages, distribution statistics and the results of the Duncan multiple range test; months not joined by the same letter are significantly different at the P=0.05 level.

Month	Year	n	Mean (cm)	Range Max	Range Min	S.D.	S.E.	Duncan
September	1979	8	-0.60	-3.20	1.00	1.35	0.48	A
August	1979	7	-0.73	-1.90	0.60	0.99	0.38	A
August	1978	3	-1.30	-2.00	0.00	1.13	0.65	A B
July	1979	8	-1.50	-1.90	0.00	0.76	0.27	A B
October	1979	6	-1.67	-2.50	0.60	0.87	0.35	A B
November	1979	3	-1.70	-1.90	-1.30	0.35	0.20	A B
September	1978	11	-1.76	-6.40	0.00	1.90	0.57	A B
June	1979	7	-2.27	-3.80	-0.60	1.16	0.44	A B
October	1978	12	-3.09	-12.00	0.00	3.08	0.89	A B
November	1978	9	-4.41	-8.00	-1.90	2.29	0.76	B C
May	1979	6	-5.56	-13.30	0.00	5.79	2.59	B C D
April	1979	4	-7.12	-11.40	0.00	4.94	2.47	C D E
March	1979	4	-8.37	-9.50	-7.60	0.80	0.40	D E F
December	1978	4	-9.90	-16.00	-6.00	4.39	2.19	E F
January	1979	2	-11.15	-13.30	-9.00	3.04	2.15	E F
February	1979	2	-12.80	-18.00	-7.60	7.35	5.20	F

Appendix E. Depth in the sediment of the maximum concentration of dissolved sulfide in sediment profiles at the south shore sample location in Pond 2. Monthly averages, distribution statistics and the results of the Duncan multiple range test; months not joined by the same letter are significantly different at the P=0.05 level.

Month	Year	n	Mean (cm)	Range Max	Range Min	S.D.	S.E.	Duncan
August	1979	11	-0.83	-1.90	0.60	0.94	0.28	A
September	1979	10	-1.08	-1.90	0.00	0.85	0.27	A
August	1978	3	-1.10	-2.00	0.00	1.01	0.59	A B
September	1978	11	-1.37	-2.80	0.00	0.97	0.29	A B
July	1979	9	-2.81	-11.40	0.00	3.62	1.21	A B C
October	1979	10	-3.05	-5.70	-0.60	1.39	0.44	A B C
November	1979	3	-3.77	-4.40	-2.50	1.10	0.63	A B C D
October	1978	13	-4.10	-8.90	0.00	2.67	0.74	B C D
June	1979	7	-4.79	-9.50	-0.60	3.47	1.31	B C D
May	1979	5	-5.24	-11.00	-1.90	3.49	1.56	C D
March	1979	1	-5.70	-5.70	-5.70	0.00	0.00	C D
November	1978	5	-6.08	-9.00	-1.90	3.77	1.69	D
April	1979	3	-6.97	-9.50	-3.80	2.90	1.68	D
December	1978	6	0 $mg \cdot liter^{-1}$ in all samples					
January	1979	2	0 $mg \cdot liter^{-1}$ in all samples					
February	1979	2	0 $mg \cdot liter^{-1}$ in all samples					

Appendix F. Maximum sulfide concentrations in sediment profiles at the feeding area in Pond 2. Monthly averages, distribution statistics, and the results of the Duncan multiple test; months not joined by the same letters are significantly different at the P=0.05 level.

Month	Year	n	Mean ($mg \cdot liter^{-1}$)	Range Min	Range Max	S.D.	S.E.	Duncan
June	1978	4	7.75	4.48	10.80	2.80	1.40	A
September	1978	3	6.35	1.50	14.50	7.10	4.10	A B
October	1978	7	5.21	0.53	11.40	4.67	1.77	A B
April	1978	6	4.98	1.40	8.40	2.26	0.92	A B C
May	1978	8	4.36	0.55	15.40	4.78	1.69	A B C
May	1979	4	4.15	0.75	9.87	4.16	2.08	A B C
August	1978	2	3.63	2.75	4.50	1.24	0.88	A B C
September	1979	8	3.24	0.75	9.10	2.81	0.99	B C
June	1979	7	2.95	0.62	7.30	2.21	0.84	B C
July	1978	2	2.56	2.12	3.00	0.62	0.44	B C
November	1978	4	2.34	0.22	6.12	2.68	1.34	B C
October	1979	6	2.12	0.30	6.76	2.42	0.99	B C
November	1979	3	2.11	0.40	4.19	1.92	1.11	B C
July	1979	9	1.86	0.64	3.68	1.09	0.36	B C
August	1979	7	1.54	0.53	3.37	0.97	0.37	B C
March	1978	3	1.20	0.00	3.00	1.59	0.92	B C
December	1978	4	0.36	0.00	1.16	0.54	0.27	C
February	1979	2	0.33	0.25	0.42	0.12	0.08	C
January	1979	1	0.06	0.00	0.00	0.00	0.00	C
February	1978	1	0.00	0.00	0.00	0.00	0.00	C

D11

Appendix G. Maximum sulfide concentrations in sediment profiles at the north shore sample location in Pond 2. Monthly averages, distribution statistics, and the results of the Duncan multiple test; months not joined by the same letters are significantly different at the P=0.05 level.

Month	Year	n	Mean ($mg \cdot liter^{-1}$)	Range		S.D.	S.E.	Duncan
				Min	Max			
September	1978	11	3.73	0.75	10.20	3.22	0.97	A
September	1979	8	3.39	0.76	8.47	2.56	0.90	A B
August	1978	3	3.14	1.50	5.37	2.00	1.15	A B C
October	1979	6	3.14	1.09	8.22	2.63	1.07	A B C
August	1979	7	2.32	0.53	5.00	1.84	0.70	A B C D
October	1978	13	1.82	0.19	5.00	1.62	0.45	B C D
June	1979	7	1.53	0.44	3.30	1.22	0.46	B C D
July	1979	8	1.24	0.10	2.43	0.83	0.29	C D
November	1978	9	1.23	0.03	2.88	1.02	0.34	C D
November	1979	3	1.04	0.20	1.75	0.78	0.45	C D
May	1979	6	0.46	0.00	0.84	0.29	0.12	C D
April	1979	5	0.41	0.00	1.00	0.37	0.17	C D
March	1979	6	0.37	0.00	0.97	0.39	0.16	C D
December	1978	7	0.28	0.00	1.25	0.45	0.17	D
January	1979	4	0.28	0.00	0.94	0.45	0.22	D
February	1979	3	0.07	0.00	0.16	0.08	0.05	D

Appendix H. Maximum sulfide concentrations in sediment profiles at the south shore sample location in Pond 2. Monthly averages, distribution statistics, and the results of the Duncan multiple test; months not joined by the same letters are significantly different at the P=0.05 level.

Month	Year	n	Mean ($mg \cdot liter^{-1}$)	Range Min	Range Max	S.D.	S.E.	Duncan
September	1978	11	7.19	0.62	12.24	4.46	1.35	A
September	1979	10	5.77	1.47	11.22	3.16	1.00	A
August	1979	11	3.76	0.17	8.80	2.51	0.76	B
August	1978	3	3.75	3.12	4.25	0.58	0.33	B C
October	1978	13	2.39	0.19	5.75	1.93	0.53	B C D
October	1979	10	2.29	0.24	3.43	1.10	0.35	B C D
November	1979	3	2.03	0.79	4.40	2.06	1.19	B C D
July	1979	9	1.74	0.44	4.20	1.16	0.39	B C D
June	1979	7	1.03	0.22	3.63	1.24	0.47	C D
May	1979	6	0.79	0.00	2.73	1.00	0.41	C D
November	1978	9	0.65	0.00	5.30	1.75	0.58	C D
April	1979	5	0.47	0.00	1.31	0.58	0.26	C D
March	1979	5	0.02	0.00	0.11	0.05	0.02	C D
January	1979	2	0.00	0.00	0.00	0.00	0.00	C D
February	1979	2	0.00	0.00	0.00	0.00	0.00	C D
December	1978	6	0.00	0.00	0.00	0.00	0.00	D

CHAPTER II

PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF ACUTE EXPOSURE OF FISH TO HYDROGEN SULFIDE

By Eugene Leslie Torrans

Department of Zoology, University of Oklahoma, Norman, Oklahoma, 73019

ABSTRACT

Hemoglobin and methemoglobin concentrations in the channel catfish (Ictalurus punctatus), the bigmouth buffalo (Ictiobus cyprinellus), and the green sunfish (Lepomis cyanellus) were determined in March and August. Cytochrome oxidase activities of the liver, brain, gill and epaxial muscle were also determined during March and August for the same three fish species.

Hydrogen sulfide competitively inhibited cytochrome oxidase. The enzyme was inhibited in vitro 18% by 10^{-7} M H_2S, 64% by 10^{-6} M, and 100% by 10^{-4} M.

After in vivo exposure to hydrogen sulfide, the percent inhibition of cytochrome oxidase varied with the tissue, and differiences were seen between the channel catfish and the fathead minnow (Pimephales promelas).

A measureable reduction in cytochrome oxidase activity was seen after exposure of fish to 0.1 mg/l H_2S at 10°C for only five minutes. Exposure of channel catfish to 0.5 mg/l H_2S at 20°C resulted in hyperpnea, followed by apnea, and finally respiratory arrest. When exposed to 0.1 mg/l H_2S at 20°C for 30 minutes, the cytochrome oxidase activity of the brain was inhibited 40% and that of the gill was inhibited 74%, while blood lactate rose from 11.6 to 38.1 mg/100 ml.

Sulfide-inhibited cytochrome oxidase recovered rapidly *in vivo*. Brain cytochrome oxidase rose from a 50% inhibition to control levels after six hours at 10°C. The recovery rate was similar in both the brain and gill.

Mortalities of fish resulting from exposure to hydrogen sulfide on commercial fish farms can be avoided by limiting the exposure of the fish, reducing the physical exertion of the fish after sub-lethal exposure to H_2S, the use of lime and potassium permanganate in the ponds, and allowing for short-term recovery before transportation.

Short title; Exposure of fish to sulfide.

INTRODUCTION

The commercial culture of fish in the United States has grown into a multi-million-dollar-a-year industry in the last 20 years. In 1979, over 18,000 metric tons of channel catfish (Ictalurus punctatus) were delivered to the processors alone (National Marine Fisheries Service, 1980). Nearly all of the fish produced were harvested by seining in the traditional way, and loaded into transport trucks at the pond bank for delivery elsewhere.

During a harvest operation, the black, malodorous sediment on the pond bottom is often disturbed. Fish farmers have known for a long time that exposing fish to this sediment-water mixture, especially in the warm summer months, can result in immediate death or mortalities during transport. Although farmers are not sure of the exact cause of the fish mortalities, they try to avoid harvesting fish during the warmest summer months. This results in an interruption of cash flow at a time when the fish food, labor, and pumping bills are the greatest.

Research conducted on a commercial catfish farm in Oklahoma indicates that hydrogen sulfide dissolved in the interstitial water of the pond bottom sediment may be the toxic agent (Chapter I). Dissolved sulfide concentrations as high as 18.1 mg/l in the sediment were measured in the warmer weather.

Studies of the effects of hydrogen sulfide on fish have been restricted to acute toxicity (24 to 96 hour exposure), using death as the end point; or chronic toxicity (weeks to months of exposure) which examined the secondary effects of hydrogen sulfide on reproduction, feeding and growth (Smith et al., 1976a). Investigations of primary effects of short-term exposure of fish (less than 30 minutes) to hydrogen sulfide, as would occur during harvesting, have not been made.

Much is known about the toxicology of hydrogen sulfide in mammals from the numerous physiological and biochemical studies of the last half-century. Although it was previously presumed that sulfide bound to hemoglobin and reduced the oxygen-carrying capacity of the blood, it is now known that this is not true. Sulfide does bind, however, to methemoglobin, and this appears to be a major defense mechanism against sulfide poisoning (Smith et al., 1977; Smith and Gosselin, 1964, 1966).

The primary biochemical "lesion" of hydrogen sulfide, as with cyanide, is the reversible inhibition of cytochrome oxidase (cytochrome c oxidase; cytochrome a-a_3), the terminal cytochrome in the electron transport system (Albaum et al., 1946b; Evans, 1967; Griffiths and Wharton, 1961; Smith et al., 1977; Smith and Gosselin, 1964; Stine et al., 1976). Although the inhibition of cytochrome oxidase by hydrogen sulfide has been studied in many mammals, it has not been documented in fish.

If respiration is promptly restored, recovery of mammals from acute sulfide poisoning is often possible (Evans, 1967; National

Research Council, 1979). Although measurements of recovery of cytochrome oxidase activity have not been made, most physiological functions return to normal within several hours (Stine et al., 1976).

In the present study, the direct effect of short-term exposure to hydrogen sulfide on cytochrome oxidase activity was studied in the channel catfish. The secondary physiological and metabolic effects of sulfide poisoning were also examined, as was the protective effect of induced methemoglobinemia. Improved management practices are recommended which may help the commercial fish farmer alleviate the problem of the toxicity of sulfide to fish during harvest operations.

METHODS

Maintenance of Fish

All fish used in this study were obtained from Sooner Fish Farm, Washington, Oklahoma. The fish used for the determination of normal levels of cytochrome oxidase activity and methemoglobin concentration were either sampled immediately after harvest or were held overnight in wire mesh cages in the culture pond. The fish used in the sulfide toxicity studies were held indoors in 140-liter glass aquaria equipped with floss/charcoal filters and air stones. Water was supplied from the University of Oklahoma water system and had a pH of 8.7 and a specific conductance of 554 micromhos (Clemens and Jones, 1954). Half of the water in each tank was exchanged twice weekly, and the period of temperature fluctuation resulting from water exchange was brief.

The aquaria were kept in a walk-in environmental chamber equipped with fluorescent lights that were controlled with a timer to maintain photoperiod. Fish were brought into the lab when environmental temperatures approximated desired acclimation temperatures to minimize acclimation time. The fish were held at 10°C and a 12:12 L:D cycle or 20°C and a 16:8 L:D cycle for a minimum of two weeks prior to testing. All fish were used within six weeks from the date of collection.

Fish were fed 32% protein commercial catfish pellets to satiation once daily. Excess food was removed by siphon after feeding ceased.

Recording of Muscular Activity

A channel catfish was submerged in oxygenated water in a shallow, plastic pan and held in place with a clamp on the dorsal spine. Platinum electrodes were implanted in the opercular muscle and near the heart, and muscular activities of breathing and heart rate were recorded with a Grass Model 79 EEG and Polygraph Data Recording System. When both rates stabilized, the sulfide solution was added and the recordings continued.

Assay of Cytochrome Oxidase Activity

Principle of the assay. The micro-spectrophotometric method used by Cooperstein and Lazarow (1951) for the determination of cytochrome oxidase activity (cytochrome c oxidase; cytochrome a-a_3) in rat tissues was modified for use in this study. The test is based on the fact that reduced and oxidized solutions of cytochrome c have different optical densities at 550 nm. When a tissue homogenate is added to a reduced cytochrome c solution, the decrease in optical density at 550 nm is proportional to the cytochrome oxidase activity in the tissue (the rate of enzymatic oxidation of reduced cytochrome c).

Procedure. Tissues were removed from live fish, blotted to remove excess fluids, and weighed to the nearest half milligram. Whole brains were used from fish weighing less than 100 grams.

Gill lamellae were removed from the gill arches. Where possible, tissue samples of at least 20 mg were collected. Tissues were homogenized in a glass/glass tissue grinder at a 1:50 dilution (w/v) with ice-cold, 20 mM citrate buffer, containing 1% Brij-58 to solubilize the membranes. Homogenates were kept in an ice bath and normally tested within 30 minutes of removal from the fish.

Cytochrome c solutions (Sigma C2506, type III, 95-100% pure) were prepared with a 20 mM citrate buffer in 100 ml batches. The solutions were reduced by adding 20 µl increments of a freshly prepared, 1.15 M sodium hydrosulfite solution until they were completely reduced, as determined by measuring the optical density at 550 nm. For a 16.2 µM solution of cytochrome c, 100 µl of the hydrosulfite solution was required. The reduced cytochrome c solutions were then shaken for several minutes to oxidize any excess sodium hydrosulfite.

A 10 or 20 µl aliquot of tissue homogenate was added to 3 ml of the reduced cytochrome c solution in a 1 cm square glass cuvette, mixed by inversion, and placed in the sample chamber of a Beckman D/U spectrophotometer. The instrument was blanked against a cuvette containing only citrate buffer, and $O.D._{550}$ was recorded on a Gilford 2000 recorder. The slope of the initial linear portion of the line was taken as the cytochrome oxidase activity (the rate of enzymatic oxidation of cytochrome c).

All assays were run at room temperature (25 ± 4°C), and comparative tests were run at the same time to reduce error due to temperature. All assays were run with a 10 µM or 16.2 µM substrate

concentration, at pH 5.6, in a 20 mM citrate buffer. The cytochrome c solution was such that a suitable optical density was obtained; the pH and citrate molarity were empirically-determined optima using fathead minnow (Pimephales promelas) brain homogenates (Figs. 1 and 2). Since enzyme activity was linear within the range of 0 to 0.33 µg of tissue per ml of cytochrome c (0 to 50 µl of a 1:50 tissue homogenate per 3 ml of cytochrome c) (Fig. 3), cytochrome oxidase activities were normally expressed as $\Delta O.D._{550} \cdot min^{-1}$ for a 1:7500 final tissue dilution.

Storage of tissues. Tissues that could not be tested immediately for enzyme activity were frozen on dry ice. Tissues collected during the toxicity studies were all tested within six hours of the completion of each trial. Tissues collected in the field for studies of normal levels of enzyme activity were frozen on dry ice and returned to the lab for analysis. Tissues not analyzed on the day of sampling were held overnight at -60°C and tested the following day.

Toxicity Studies

A glass aquarium containing 125 liters of tap water was equilibrated with air by overnight aeration. In the morning the temperature was adjusted to acclimation temperature, if necessary, with the addition of ice. All fish were tested at their acclimation temperature. The dissolved oxygen was determined at the start of each trial using the Winkler method (APHA, 1975) and the required amount of sodium sulfide was dissolved and added to the aquarium.

The pH of the water was adjusted to 8.0 for all tests with HCl, and the total dissolved sulfide concentration was determined spectrophotometrically (APHA, 1975).

Tissues from the control fish were sampled and the test fish were placed in the sulfide solution. A sheet of plastic the size of the aquarium was placed on the water to reduce the loss of sulfide to the atmosphere. The total dissolved sulfide concentration in the aquarium remained stable for the entire 30 minute test period.

All tests began between 0900 and 1100 CST. Sub-groups of five fish were removed from the aquarium and tissues sampled after 5, 10, 20 and 30 minutes of exposure to the sulfide solution. The pH and total dissolved sulfide concentration of the water were measured at the same time intervals. Using the pH of the water and the total dissolved sulfide concentration, the concentration of the un-ionized sulfide (molecular sulfide) was calculated (APHA, 1975).

The fish used for the recovery experiments were removed from the test tank after showing visible signs of distress, but before death, and returned to the acclimation aquarium. Enzyme activities were determined in sub-groups of five fish upon removal from the tank, and after 3, 6 and 24 hours of recovery.

Replicate enzyme determinations were made for each brain sampled. The gill tissues within sub-groups were pooled (due to time constraints) and four replicate enzyme determinations were normally made on each pooled sample.

Blood Lactate

Blood samples were collected by severing the caudal peduncle and analyzed for lactic acid using an enzymatic technique (Sigma Company, 1974). Measurements were made in quartz cuvettes at 340 nm. Standard curves were made on the day that blood lactate determinations were made.

Oxyhemoglobin and Methemoglobin

With fish weighing less than 100 gms, the caudal peduncle was severed and blood was collected in heparinized micro-hematocrit tubes. A heparinized syringe was used to withdraw blood from the heart or dorsal aorta of larger fish. The method of Dubowski (1960) was used for the determination of oxyhemoglobin and methemoglobin. All measurements were made in 1 cm square glass cuvettes with a Beckman D/U spectrophotometer.

RESULTS

Effect of H_2S on Respiration and Circulation

Acute exposure of fish to hydrogen sulfide resulted in an initial stimulation of both respiration and circulation, followed immediately by a reduction of both functions. Within one minute of exposure to a 0.5 mg/l un-ionized sulfide solution (molecular H_2S) at 20°C, the heart rate of a 20 cm channel catfish increased from a resting rate of 88 beats per minute to 128 b.p.m. The respiratory rate decreased from 140 to 128 cycles per minute during the same period, but the depth of ventilation greatly increased (Fig. 4). After the first two minutes, both the heart rate and respiration decreased. After five minutes of exposure the heart rate dropped to 60 b.p.m.; respiration decreased to 88 c.p.m. and became both shallow and irregular. After six minutes and 40 seconds of exposure, the opercular muscle went into a state of tetanus and respiration ceased entirely.

When the fish was returned to fresh water after eight minutes of exposure, the opercular muscle experienced occasional spasms, but even these ceased entirely after four minutes in the fresh water. Although the fish was moribund at this time, the heart continued to beat for over one hour (the length of the experiment) at a steadily decreasing rate (down to 23 b.p.m. after one hour in the fresh water).

Effect of Sulfide on Cytochrome Oxidase Activity

Normal levels of cytochrome oxidase activity. There were large differences in cytochrome oxidase activity among tissues in each of the three fish species tested in August (Table 1). Enzyme activity was highest in the liver and lowest in the epaxial muscle or the gill in all three species. In the channel catfish, cytochrome oxidase activity ranged from 0.017 units in the epaxial muscle of the 11.6 cm group to 0.173 units in the liver of the 42.5 cm group. Enzyme activities of the bigmouth buffalo (Ictiobus cyprinellus) ranged from 0.028 units in the gill to 0.107 units in the liver, and in the green sunfish (Lepomis cyanellus) ranged from 0.032 units in the muscle to 0.155 units in the liver.

There were also species-related differences in enzyme activity. Channel catfish of both size groups tested had a greater enzyme activity in the liver, and less enzyme activity in the epaxial muscle, than did the bigmouth buffalo. These differences may be related to the relative aerobic and anaerobic capacities of these two species.

The gill, brain and liver of both size groups all showed reduced enzyme activity in March, with the liver showing the greatest decrease. The muscle tissue of the fingerlings showed an increase in enzyme activity in March, while that of the food fish showed a slight decrease.

In vitro inhibition of cytochrome oxidase. Hydrogen sulfide was shown to be a competitive inhibitor of cytochrome oxidase in a fathead minnow brain homogenate. A 10^{-6} M sulfide concentration

increased the K_m of the enzymatic oxidation of cytochrome c from 1.45 X 10^{-5} M to 1.00 X 10^{-4} M (Fig. 5).

Hydrogen sulfide at very low concentrations inhibited cytochrome oxidase *in vitro*. The inhibition of the enzyme in a channel catfish brain preparation was 18% with 10^{-7} M H_2S, 64% with 10^{-6} M and 100% with 10^{-4} M (Fig. 6). The effect of hydrogen sulfide on a brain homogenate from the fathead minnow was similar.

Un-ionized sulfide (H_2S) appears to be the major inhibitor of cytochrome oxidase. With a pK_1 of 7.04, 98% of the sulfide is in the un-ionized form at pH 5.0 and only 14% is un-ionized at pH 7.5. When the pH of my assay system was raised from 5.0 to 7.5, with the total sulfide concentration held constant at 10^{-6} M, cytochrome oxidase activity of a channel catfish brain homogenate increased from 34.6% to 54.3% of control levels (Table 3).

In vivo inhibition of cytochrome oxidase. The effect of acute exposure to hydrogen sulfide on the inhibition of cytochrome oxidase varied with the type of tissue. At the point of respiratory arrest, the tissues of the fathead minnow showed cytochrome oxidase activities ranging from control levels in the testes, to a 55% inhibition in the kidney (Table 4). In the channel catfish the effect ranged from a 28% decrease of the brain cytochrome oxidase activity, to a 66% decrease in the heart, when the fish were sampled at the point of respiratory arrest (Table 5).

The inhibition of cytochrome oxidase in the channel catfish brain and gill was affected by the un-ionized sulfide concentration. When fish were exposed to a 0.1 mg/l H_2S at 10°C, the cytochrome

oxidase of the brain was not affected by even a 30 minute exposure (Fig. 7). The enzyme of the gill, however, was inhibited 15% after only five minutes and 39% after 30 minutes in the sulfide.

Increased inhibition of the enzyme in both tissues was seen when the fish were exposed to 0.3 mg/l H_2S at 10°C, and when exposed to 0.5 mg/l H_2S, the brain enzyme was inhibited 56% and that of the gill 48% after only five minutes of exposure (Fig. 7). This was the maximum effect seen at that concentration and it coincided with the onset of respiratory arrest, which was first seen in some fish after 4.5 minutes of exposure. The fish were left in the sulfide an additional 25 minutes but the inhibition of the enzyme did not increase beyond that seen after five minutes of exposure.

The same pattern was seen when fish were exposed to 1.1 mg/l H_2S. The enzyme activity of both tissues was inhibited 42% after five minutes of exposure and decreased only slightly after an additional 25 minutes in the sulfide (Fig. 7).

The test temperature greatly affected the action of hydrogen sulfide. Channel catfish exposed at 20°C to only 0.1 mg/l H_2S showed similar decreases in the enzyme activity of both tissues as did fish exposed at 10°C to 0.5 mg/l H_2S (Table 6).

Effect of Exposure to Sulfide on Blood Lactate

While cytochrome oxidase activity decreased in tissues of fish exposured to hydrogen sulfide, blood lactate increased. When channel catfish were exposed to a 0.1 mg/l un-ionized sulfide solution at 20°C for 30 minutes, enzyme activity decreased to 40% and

26% of control levels in the brain and gill respectively. During the same exposure blood lactate rose from 11.6 mg/100 ml to 38.1 mg/100 ml (Table 7).

The Role of Methemoglobin in Sulfide Poisoning

Normal levels of circulating methemoglobin. Mean methemoglobin concentrations in three species of fish sampled in August ranged from 0.07 gms MHb/100 ml of blood in the bigmouth buffalo to 0.36 gms MHb/100 ml in the green sunfish (1.8% and 4.9% of the total hemoglobin, respectively) (Table 8). Individual fish had concentrations as high as 0.57 gms MHb/100 ml of blood in an adult channel catfish and 0.54 gms MHb/100 ml in a green sunfish (7.2% and 7.7% of the total hemoglobin, respectively). Although higher than mammalian concentrations, these methemoglobin concentrations are similar to those reported by other authors for various fish species (Cameron, 1971; Shterman, 1970; Smith and Russo, 1975).

In March the mean methemoglobin concentrations ranged from 0.12 gms MHb/100 ml of blood in the bigmouth buffalo, to 0.60 gms MHb/100 ml in the green sunfish (1.0% to 9.2% of the total hemoglobin, respectively) with one green sunfish having 1.3 g MHb/100 ml (14% of the total hemoglobin). All three species and two size groups examined had methemoglobin concentrations in March higher than or equal to the August values.

Protective effect of methemoglobin. Channel catfish with high levels of circulating methemoglobin showed less inhibition of cytochrome oxidase when exposed to hydrogen sulfide than did fish

with low methemoglobin concentrations. Two groups of fish were independently exposed to nitrite solutions to produce elevated methemoglobin concentrations of 2.00 and 2.67 g MHb/100 ml. Two groups were untreated and had normal (low) levels of 0.14 and 0.18 g MHb/100 ml. Each group was exposed to 0.1 mg/l H_2S at 20°C and tissues were sampled as in the previous bioassay (Fig. 8). In the groups with high levels of methemoglobin, the brain cytochrome oxidase activity was reduced an average of 10% after 30 minutes of exposure, as compared to a 60% inhibition of enzyme activity in both of the low methemoglobin groups.

High levels of methemoglobin did not protect the enzyme of the gill as much as that of the brain. The gill cytochrome oxidase activity in the high methemoglobin groups was inhibited an average of 56% after 30 minutes of exposure, as compared to a 73% inhibition in the low methemoglobin groups after the same exposure.

Recovery from Sulfide Poisoning

The inhibition of cytochrome oxidase by hydrogen sulfide is reversible within a short period of time. The enzyme activity of the brain recovered from a 50% inhibition to control levels within six hours in fresh water at 10°C, and it recovered at a similar rate at both 10°C and 20°C. At 20°C the recovery of the enzyme in both the brain and the gill proceeded at the same rate.

DISCUSSION

Action of Hydrogen Sulfide

Physiological effects. In these experiments, exposure of fish to 0.5 mg/l H_2S at 20°C resulted in hyperpnea, followed immediately by apnea and finally respiratory arrest. The same response to hydrogen sulfide was demonstrated in the cat by Evans (1967). Evans showed that the hyperpnea was a result of the stimulation of receptors in the carotid body by hydrogen sulfide. Isolating the receptors by vagotomy and local anesthesia of the carotid body eliminated the hyperpnea, and only the depressant effect was seen. Likewise, reducing the sulfide concentration below the threshold level for the receptor produced only a depression of respiration. The same general response is seen with acute exposure of both rats and humans to hydrogen cyanide (Albaum et al., 1946b; Stine et al., 1976).

Biochemical effect. Cytochrome oxidase, the terminal cytochrome in the electron transport system, catalyzes the reaction shown in Equation 1 (Yonetani and Ray, 1965).

$$\text{Ferrocytochrome } \underline{c} + \tfrac{1}{4}O_2 + H^+ \xrightarrow{\text{cytochrome oxidase}} \text{ferricytochrome } \underline{c} + \tfrac{1}{2}H_2O \qquad 1)$$

In the present study, at pH 5.6 and 23°C, the K_m of cytochrome oxidase

in a fathead minnow brain homogenate was 14.5 μM. This compares favorably with a K_m of 14 μM for a beef heart preparation at pH 6.0 (Yonetani and Ray, 1965), and a K_m of 15 to 25 μM for a goldfish (carassius auratus) gill preparation assayed at pH 7.5 (Caldwell, 1969). Thus there is a great similarity in the kinetics of the reaction when tested under similar conditions, regardless of the source of the enzyme. The slightly higher values seen by Caldwell (1969) can be explained by the increase in K_m with increasing pH as was demonstrated by Yonetani and Ray (1965).

In this study, hydrogen sulfide was shown to be a competitive inhibitor of cytochrome oxidase. An 18% inhibition of cytochrome oxidase was seen when the hydrogen sulfide concentration was 10^{-7} M and a 64 % inhibition resulted from a 10^{-6} M concentration. Griffiths and Wharton (1961), using a manometric assay of beef heart cytochrome oxidase at pH 7.2 with a 50 μM substrate concentration, saw a 10% inhibition with 10^{-4} M sulfide and a 90% inhibition with 10^{-3} M. Part of this difference in results can be explained by the effects of substrate concentration and pH. Due to the nature of competitive inhibition, the higher substrate concentration used by Griffiths and Wharton would reduce the effect of the inhibitor. In addition, my studies have shown that at a pH of 7.5, a solution of hydrogen sulfide is much less inhibitory than at pH 5.0. This confirms the findings of Smith et al. (1977) that it is mainly the un-ionized molecule (H_2S) that binds to cytochrome oxidase, and may account for the reduced effect of the inhibitor in the study of Griffiths and Wharton.

There is great similarity in the action of cyanide and sulfide at the enzyme level. Albaum et al. (1946b) caused a 50% reduction in brain cytochrome oxidase in adult rats by injecting 5 mg/kg NaCN intraperitoneally. Smith et al. (1977) found that cyanide inhibited cytochrome oxidase in a beef heart preparation but that it was not as potent an inhibitor as sulfide.

Secondary metabolic effects of acute exposure. Since sulfide interferes with the ability to utilize oxygen at the enzyme level (equation 1), exposure to sulfide should result in a histotoxic anoxia, and a consequent increase in the by-products of glycolysis. In this study, exposure of fingerling channel catfish to 0.1 mg/l H_2S at 20°C for 30 minutes resulted in increasing the blood lactic acid from 11.6 mg/100 ml to 38.1 mg/100 ml. This increase in blood lactic acid was similar to that shown by Caillouet (1968) in channel catfish during five to ten minutes of anoxic anoxia (environmental anoxia). Evans (1967) noted a decreased oxygen consumption in cats during sulfide injections. Stine et al. (1976) measured a 41.2 meq/l anion gap in the blood of a human subject 30 minutes after exposure to sulfide in an industrial accident. All these facts point to a decreased reliance on oxidative phosphorylation and an increase in glycolysis after an acute exposure to hydrogen sulfide.

Many studies are available that describe the metabolic effects of the inhibition of cytochrome oxidase by cyanide. Olsen and Klein (1947) observed that an increased dosage of cyanide in the cat caused decreased oxygen consumption and increased brain lactic acid. Albaum et al. (1946b) observed decreases in rat brain glycogen and ATP, and

increases in lactic acid and ADP, after intraperitoneal injections of NaCN.

Similar metabolic changes are probably the immediate cause of most sulfide-related mortalities occurring during the harvest and transport of fish on commercial fish farms. The aerobic capacity of the fish is pushed to its limit during harvest. The fish are crowded together in a net, often in very shallow water. The fish are scooped out of the water, either with a dip net or a boom basket, and are weighed out of the water. Only then are they placed in a transport tank, and at such high densities (1 to 3 pounds/gallon of water) that they must continually work to maintain position. They often are hauled for several hours before being unloaded. Even normal fish can succumb to such physical exertion, and the loss of an entire load of fish occurs occasionally. Thus, the exposure of fish to even very low sulfide concentrations (less than 0.1 mg/l H_2S) for as little as five minutes, may reduce their aerobic capacity to the point where fatal metabolic acidosis results. It has been shown in fact that chronic exposure of the bluegill (Lepomis macrochirus) to low levels of sulfide reduced their swimming endurance significantly (Oseid and Smith, 1972).

Many other detrimental side effects of hydrogen sulfide are known. Smith et al. (1976a), in a review of sulfide toxicity studies on fish and invertebrates, discuss the negative effects of hydrogen sulfide on spawning success, sperm viability, per cent hatching, time to hatching, size to hatching, food conversion and growth. Probably all of these effects can be attributed in some way to the inhibition

of cytochrome oxidase and the subsequent reduction of aerobic respiration.

Factors Affecting The Toxicity Of Sulfide

pH. Bonn and Follis (1966) showed that increasing the pH of a sulfide solution resulted in reducing the toxicity to the channel catfish. Since hydrogen sulfide ionizes in aqueous solution, a higher pH would result in reduced concentrations of the un-ionized form (Equation 2).

$$H_2S \underset{}{\overset{}{\rightleftharpoons}} HS^- + H^+ \underset{}{\overset{}{\rightleftharpoons}} S^{2-} + H^+ \qquad 2)$$

$pK_1=7.04$ $pK_2=11.96$

Hunn and Allen (1974) demonstrated in a study on the absorption of acids and bases, that fish gills resist the passage of ionized molecules. Although some HS^- ions apparently do enter the fish (Broderius and Smith, 1976), the majority of the effects observed are related to the concentration of the un-ionized sulfide. At normal environmental pH's (6.5-9.5) the concentration of S^{2-} is negligible.

The effect of pH on sulfide toxicity is also seen at the enzyme level. Raising the pH of my assay system from 5.0 to 7.5 reduced the un-ionized sulfide concentration by 84% and decreased the inhibition of the enzyme by 20%. It is apparent that any condition that may lower the intra-mitochondrial pH, such as metabolic acidosis, will increase the toxicity of sulfide.

Temperature. The effect of sulfide is increased greatly with increasing temperature. In this study, 0.5 mg/l H_2S at 10°C was required to produce the same inhibition of enzyme activity *in vivo* as 0.1 mg/l H_2S at 20°C. This is similar to the findings of Adelman and Smith (1972) which showed a logarithmic relationship between 96-hr TL50 and temperature in the goldfish. This magnifies the problem faced by the commercial fish farmer. Much of the harvesting is done in July and August when water temperatures may average 30°C. At this time of the year, sulfide concentrations which are relatively harmless during the winter could cause significant damage to the fish within a few minutes.

Age of the fish. Smith et al. (1976b) showed that the sulfide tolerance of eggs and fry was much less than that of juvenile and adult bluegill. The 96-hr LC50 for fry was 0.0131 mg/l H_2S and for adults was 0.0448 mg/l H_2S at temperatures near 20°C. Channel catfish fingerlings and fry are also much more succeptible to sulfide than adults (Bonn and Follis, 1966). This may account for the greater incidence of sulfide poisoning with smaller channel catfish reported by Martin (1978).

Lines of Defense Against Sulfide Poisoning

Behavioral. Most organisms have a very low olfactory threshold for hydrogen sulfide (National Research Council, 1979). If given the opportunity, fish will avoid hydrogen sulfide. Magnuson and Karlen (1970), in a study of a shallow northern lake, found that northern pike (*Esox lucius*), could, through behavioral means, survive

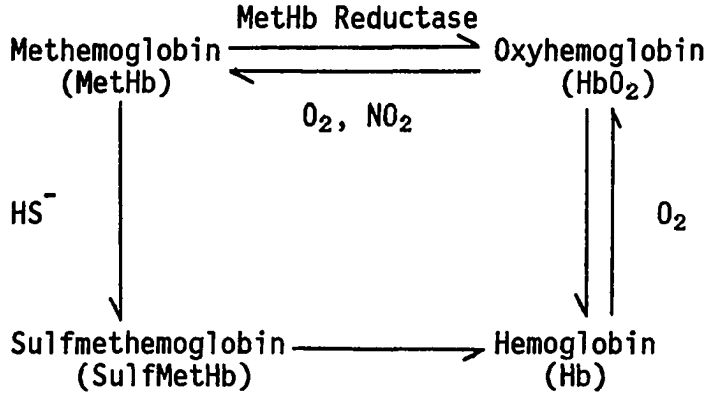
conditions of low oxygen and high hydrogen sulfide which killed other species. In a harvest operation on a commercial farm, however, fish can be forced into a harmful set of conditions from which there is no escape - at that point the other lines of defense become very important.

The gill mucus barrier. Fish can alter mucus production in response to environmental factors. Measurements made on the common carp (Cyprinus carpio) show that the mucus retards the diffusion of oxygen across the gill, both by acting as a diffusion barrier and by reducing the flow of water between the secondary lamellae (Ultsch and Gros, 1979). It is known that the irritating effect of hydrogen sulfide produces pulmonary edema during chronic exposure in human subjects (National Research Council, 1979) but the effect of sulfide on fish gill mucus production, and the protective effect of this, is not known.

Respiratory arrest. Respiratory arrest, mediated through the central nervous system, appears to be a valuable protective adaptation against acute sulfide poisoning. In this study, the uptake of sulfide ceased with the onset of respiratory arrest. This is in accord with the findings of other researchers (Evans, 1967; Stine et al., 1976), and allows an animal to survive a short-term exposure that would otherwise be fatal. Fish that show severe symptoms of sulfide poisoning during harvest can be saved if they are removed to fresh water immediately.

Methemoglobin. The binding of hydrogen sulfide by methemoglobin (ferrihemoglobin, oxidized hemoglobin) is well documented in the literature (Smith et al., 1977; Smith and Gosselin, 1964, 1966).

The compound formed by this reaction is sulfmethemoglobin, and has properties similar to cyanomethemoglobin (Albaum et al., 1946a). It is the anion (HS^-) that binds to the methemoglobin, reacting mole for mole with the ferric heme (Corydell et al., 1937). An excellent discussion of the sulfide-hemoglobin-methemoglobin relationships was presented by the National Research Council (1979) and is summarized, in part, below (Equation 3).



3)

The formation of sulfmethemoglobin apparently speeds the formation of ferrohemoglobin (Smith and Gosselin, 1964).

Methemoglobin protects cytochrome oxidase from sulfide *in vitro* (Smith et al., 1977), and has been shown to increase survival in several species *in vivo* (Smith and Gosselin, 1964). However, the direct protective effect of methemoglobin on cytochrome oxidase has not been demonstrated *in vivo*. In this study, therapeutic methemoglobinemia in the channel catfish significantly protected cytochrome oxidase against acute exposure to hydrogen sulfide, *in vivo*. The cytochrome oxidase of the brain was protected to a greater degree than was that of the gill. At very low sulfide concentrations (0.1 to 0.3 mg/l H_2S at 10°C) even normal levels of methemoglobin appeared

to protect the brain to some extent. The high levels of methemoglobin seen in March would give a great degree of protection to the fish at that time of year.

Sulfide-resistant cytochrome oxidase. Hall et al.(1971) showed that the cytochrome oxidase of two species of millipedes (Euryurus leachii and Pleuroloma flavipes) were resistant to the toxic effect of cyanide. The rates of cyanide penetration and detoxification were ruled out, as was the ability to tolerate anaerobiosis. Perhaps a similar mechanism exists for hydrogen sulfide, and if so, it would help to explain the range of sulfide tolerance found in the animal kingdom.

Resistance to hypoxia. Fish species show large differences in the ability to withstand hypoxia (Heath and Pritchard, 1965). Since hydrogen sulfide poisoning reduces aerobic respiration, and causes a buildup of lactic acid, it follows that an animal's anaerobic capacity could, to a large degree, affect its ability to survive hydrogen sulfide poisoning.

Recovery from Acute Sulfide Poisoning

Rate of recovery. The National Research Council (1979) reviewed several cases of acute sulfide poisoning in humans. In most cases, if respiration was restored immediately, recovery proceeded at a rapid rate, and most physiological functions returned to normal within a few hours. But the time course of recovery from sulfide poisoning has not been documented at the enzyme level. In this study, cytochrome oxidase in channel catfish poisoned by hydrogen

sulfide returned from a 50% inhibition to control levels within six hours in live fish. Thus, hydrogen sulfide acts as a noncumulative poison which is rapidly cleared from the body.

Role of methemoglobin in recovery. Therapeutic methemoglobinemia has long been used as a standard treatment for cyanide poisoning. The similarity in the action and effects of cyanide and sulfide suggest that a similar treatment may be of use in sulfide poisoning. Smith et al. (1977) showed that activity was restored to sulfide-inhibited cytochrome oxidase, in vitro, by the addition of methemoglobin. Stine et al. (1976) saw an improved rate of recovery in a human poison victim after treatment with amyl nitrite, a standard cyanide antidote. The treatment of poisoned fish with nitrite or methylene blue (methemoglobin-producing compounds) may help to speed recovery if the dosage rates are understood and strictly controlled, but this is not recommended for practical reasons.

Hyperbaric oxygen. Although hyperbaric oxygen is often used for the treatment of acute cyanide poisoning, the actual benefit of this procedure is in doubt (Way et al., 1972), since cyanide affects the utilization of O_2 at the tissue level, and not the amount of oxygen transported by the blood. In commercial fish operations where the fish are poisoned by sulfide during harvest and then transported, the case may be slightly different. The blood acidosis caused by sulfide poisoning may be greatly magnified by the physical exertion of the fish during these operations. The resulting drop in blood pH may in turn reduce the O_2-carrying capacity of the blood to the point that insufficient amounts of oxygen are delivered to the tissue

(Noble et al., 1970; Riggs, 1960). The use of bottled oxygen should be a routine practice during transport after suspected cases of sulfide poisoning.

Long-range effects. Fish farmers report lingering effects, lasting up to a month, after suspected cases of sulfide poisoning. Symptoms include poor feeding and growth, reduced catch rate in fee-fishing operations, and an increased suceptibility to disease. These observations cannot be explained by the results of this study, but they are consistent with the case histories of human subjects. General neurological disorders in humans often continue for more than a month after acute exposure to sulfide (National Research Council, 1979). The symptoms may include headache, dizziness, fatigue, poor memory and depression. These effects would cause other problems in a large culture pond where fish must travel long distances to and from a feeding area (Randolph and Clemens, 1976). A fish that is unable or unwilling to travel to and from the feed area each day as a result of neurological disorders, would experience a steadily deteriorating condition due to starvation.

Practical Recommendations

From November through March, when water temperatures are low, the total dissolved sulfide rarely exceeded 2 mg/l in the sediment of commercial culture ponds in Oklahoma, and during that period the maximum concentration of sulfide was found deep in the sediment (Chapter I). This means that the exposure of fish to harmful levels of H_2S are very unlikely during routine harvesting operations in the colder weather.

The situation is different in the warm weather. Total dissolved sulfide concentrations may be as great as 18.1 mg/l in July, and the maximum concentration of dissolved sulfide is very near the surface of the sediment from July through September (Chapter I. At this time of the year, concentrations of un-ionized sulfide as great as 0.1 to 0.5 mg/l could be found in the water during harvest, depending on the sulfide concentration in the sediment, the amount of sediment disturbed, and the depth and pH of the water.

The exposure of fish to hydrogen sulfide during the warmer months can be reduced in several ways. First, the fish should be landed in areas of the pond that have low sulfide concentrations in the sediment (Chapter I). Second, whenever practical, fish should be landed at an area that has relatively deep water. This will result in both less sediment being disturbed and a greater dilution of the sulfide that is released from the sediment. Third, the handling time of the fish in the sediment-water mixture should be kept to a minimum. This research has shown that exposure of fish to 0.1 mg/l H_2S at 10°C for only five minutes can cause measureable damage, and the effect is five times as great at 20°C. Thus, fish should be moved to deeper water or to near the inlet as soon as possible after landing them.

Two pond treatments can reduce the effects of hydrogen sulfide. First, the application of lime, especially in areas of the country with low soil and water pH's, may reduce the toxicity of sulfide by raising the pH. Second, potassium permanganate applied to the pond during harvest will oxidize the hydrogen sulfide in the water.

Fish that are exposed to hydrogen sulfide have reduced cytochrome oxidase activities, and an increase in blood lactate. If they are weighed, loaded into trucks, and transported immediately, a fatal metabolic acidosis may result. Three precautions should be taken to avoid this. First, the physical exertion of the fish should be kept to a minimum at all stages of the operation to reduce the increase in blood lactate. Second, the fish should be held for several hours, and preferably overnight, in cool water before being transported. This will allow the poisoned enzyme to recover and blood lactate to return to normal levels. Third, the fish should be transported with bottled oxygen if exposure to sulfide is suspected, and they should be transported at as low a temperature as is practical. Reducing the loading densities in transport tanks may reduce the physical exertion of the fish, increase the oxygen of the water in the tank, and help to improve the condition of the fish at the destination.

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Table 1. Cytochrome oxidase activity in four tissues of three fish species. Fish were collected in August when the water temperature averaged 28°C for the month.

Species	n	Total Length (cm)	Mean Cytochrome Oxidase Activity			
			Liver	Muscle	Brain	Gill
Ictalurus punctatus	10	11.6	0.156	0.017	0.057	0.032
			(0.026)	(0.005)	(0.010)	(0.005)
Ictalurus punctatus	7	42.5	0.173	0.036	0.081	0.045
			(0.023)	(0.031)	(0.010)	(0.008)
Ictiobus cyprinellus	5	44.8	0.107	0.048	0.052	0.028
			(0.042)	(0.015)	(0.008)	(0.006)
Lepomis cyanellus	7	12.8	0.155	0.032	0.086	0.051
			(0.042)	(0.007)	(0.009)	(0.012)

Cytochrome oxidase activity is expressed as $\Delta O.D._{550} \cdot min^{-1}$ for a 1:7500 tissue dilution in 16.1 μM cytochrome c, run at 24.5 ± 0.5°C; numbers in parentheses indicate the standard deviation.

Table 2. August and March cytochrome oxidase activity in tissues of channel catfish fingerlings (10-20 cm total length) and food fish (40-47 cm total length).

Tissue	Mean Cytochrome Oxidase Activity	
	August 3-4	March 9-12
Fingerlings		
Gill	0.032 (10, 0.005)	0.025 (10, 0.006)
Brain	0.057 (10, 0.010)	0.041 (10, 0.004)
Liver	0.157 (10, 0.026)	0.084 (10, 0.017)
Muscle	0.017 (10, 0.005)	0.037 (9, 0.015)
Food Fish		
Gill	0.045 (7, 0.008)	0.022 (5, 0.005)
Liver	0.173 (7, 0.023)	0.026 (5, 0.005)
Muscle	0.036 (7, 0.031)	0.030 (5, 0.004)

Cytochrome oxidase activity is expressed as $\Delta O.D._{550} \cdot min^{-1}$ for a 1:7500 tissue dilution in 16.1 μM cytochrome c, run at 26 ± 2°C; numbers in parentheses indicate the sample size and standard deviation.

Table 3. Effect of pH on hydrogen sulfide inhibition of cytochrome oxidase *in vitro*. Brain homogenate from a 15 cm channel catfish acclimated to 20°C and assayed at 22.5°C in 16.2 μM cytochrome *c* at a 1:7500 final tissue dilution.

pH	Total Sulfide	Un-ionized Sulfide	% Control Cytochrome Oxidase Activity
5.0	10^{-6}M	0.98 X 10^{-6}M	34.6
7.5	10^{-6}M	0.14 X 10^{-6}M	55.3

Un-ionized sulfide is based on a pK_1 of 7.04 (Weast, 1972).

Table 4. Cytochrome oxidase activity in eight tissues of fathead minnows (5 to 7 cm total length), before and after lethal exposure to hydrogen sulfide. Fish were acclimated to 20°C and exposed to 20 mg/l total dissolved sulfide at 20°C and pH 8.0 (1.0 mg/l H_2S). Individual test fish were removed from the sulfide solution when respiration ceased (13 to 23 minutes).

	Control Fish		Test Fish		
Tissue	n	Activity	n	Activity	% Decrease
Brain	10	0.082 (0.015)	5	0.046 (0.006)	44
Gill	5	0.045 (0.006)	5	0.021 (0.001)	53
Heart	10	0.230 (0.053)	5	0.141 (0.027)	39
Eye	5	0.013 (0.002)	5	0.009 (0.001)	31
Liver	10	0.096 (0.026)	7	0.061 (0.021)	36
Kidney	10	0.101 (0.032)	5	0.049 (0.008)	55
Testes	5	0.025 (0.007)	5	0.028 (0.007)	-12
Muscle	5	0.030 (0.010)	5	0.073 (0.006)	27

Cytochrome oxidase activity expressed as $\Delta O.D._{550} \cdot min^{-1}$, for a 1:7500 final tissue dilution; numbers in parentheses are standard deviations.

Table 5. Cytochrome oxidase activity in six tissues of fingerling channel catfish (10-15 cm), before and after lethal exposure to hydrogen sulfide. Fish were acclimated at 20°C and exposed to 20 mg/l total dissolved sulfide at 20°C and pH 8.0 (1.0 mg/l H_2S). Individual fish were removed when respiration ceased (9 to 15 min). All groups had a sample size of five.

	Cytochrome Oxidase Activity		
Tissue	Control	Test	% Decrease
Brain	0.051 (0.006)	0.037 (0.009)	28
Gill	0.027 (0.005)	0.013 (0.002)	52
Heart	0.308 (0.019)	0.106 (0.021)	66
Liver	0.136 (0.018)	0.092 (0.022)	32
Kidney	0.077 (0.014)	0.043 (0.012)	44
Muscle	0.026 (0.004)	0.012 (0.003)	54

Cytochrome oxidase activity expressed as $\Delta O.D._{550} \cdot min^{-1}$ for a 1:7500 final tissue dilution; numbers in parentheses are standard deviations.

Table 6. Effect of temperature on uptake of hydrogen sulfide. Groups of 10 to 15 cm channel catfish acclimated to 10°C and exposed to 0.5 mg/l un-ionized sulfide, or acclimated to 20°C and exposed to 0.1 mg/l un-ionized sulfide; enzymes assayed at 24.5 ± 1.5°C.

Time exposed (min)	% Control Cytochrome Oxidase Activity			
	Brain		Gill	
	10°C	20°C	10°C	20°C
0	100 (8)	100 (5)	100 (3)	100 (4)
5	44 (4)	88 (5)	52 (3)	72 (4)
10	48 (4)	58 (5)	39 (2)	41 (4)
20	42 (4)	40 (4)	41 (2)	33 (4)
30	42 (4)	40 (5)	39 (2)	26 (2)

Two replicate determinations were made on each of (n) brains, and (n) replicate determinations were made on the pooled tissue samples, at each time.

Table 7. Changes in blood lactate, and brain and gill cytochrome oxidase activity in 10 to 15 cm channel catfish, during exposure to 0.1 mg/l un-ionized sulfide. Fish were acclimated and exposed at 20°C; enzymes were assayed at 23°C.

	Blood Lactate	% Control Enzyme Activity	
Time	mg/100ml	Brain	Gill
0	11.6 (4)	100 (5)	100 (4)
10	28.4 (2)	58 (5)	41 (4)
20	20.0 (3)	40 (5)	33 (4)
30	38.1 (4)	40 (5)	26 (2)

Numbers in parentheses are the sample size for blood lactate and brain enzyme activity and the number of replicates on a pooled gill tissue sample.

Table 8. Blood parameters of three species of fish sampled in August and March.

	Ictalurus punctatus (fingerling)	Ictalurus punctatus (adult)	Lepomis cyanellus	Ictiobus cyprinellus
Summer				
Date	8-3-78	8-4-78	8-15-78	8-18-78
n	10	11	6	6
Length (cm)	11.6 (10-15)	42.5 (40-47)	12.8 (12-14)	44.8 (41-48)
Hb (gms/100ml)	5.7 (0.8)	8.4 (1.9)	7.4 (0.9)	7.5 (2.7)
MetHb (gms/100ml)	0.09 (0.06)	0.35 (0.13)	0.36 (0.10)	0.07 (0.07)
% MetHb	1.7 (1.0)	4.3 (1.6)	4.9 (1.6)	1.8 (3.2)
Hct (%)	19.4 (3.3)	34.0 (9.7)	28.2 (2.1)	25.3 (8.9)
Winter				
Date	3-8-79	3-8-79	3-8-79	3-8-79
n	12	5	9	7
Length (cm)	20.4 (17-23)	44.0 (42-47)	15.0 (13-16)	50.1 (45-53)
Hb (gms/100ml)	5.7 (0.1)	5.9 (1.8)	9.0 (0.7)	11.8 (1.8)
MetHb (gms/100ml)	0.26 (0.13)	0.35 (0.15)	0.60 (0.42)	0.12 (0.07)
% MetHb	4.6 (2.7)	7.1 (5.0)	9.2 (3.2)	1.0 (0.6)
HCT (%)	19.3 (3.2)	21.4 (7.0)	40.1 (4.2)	40.3 (7.0)

Numbers in parentheses are standard deviations, or range in total length.

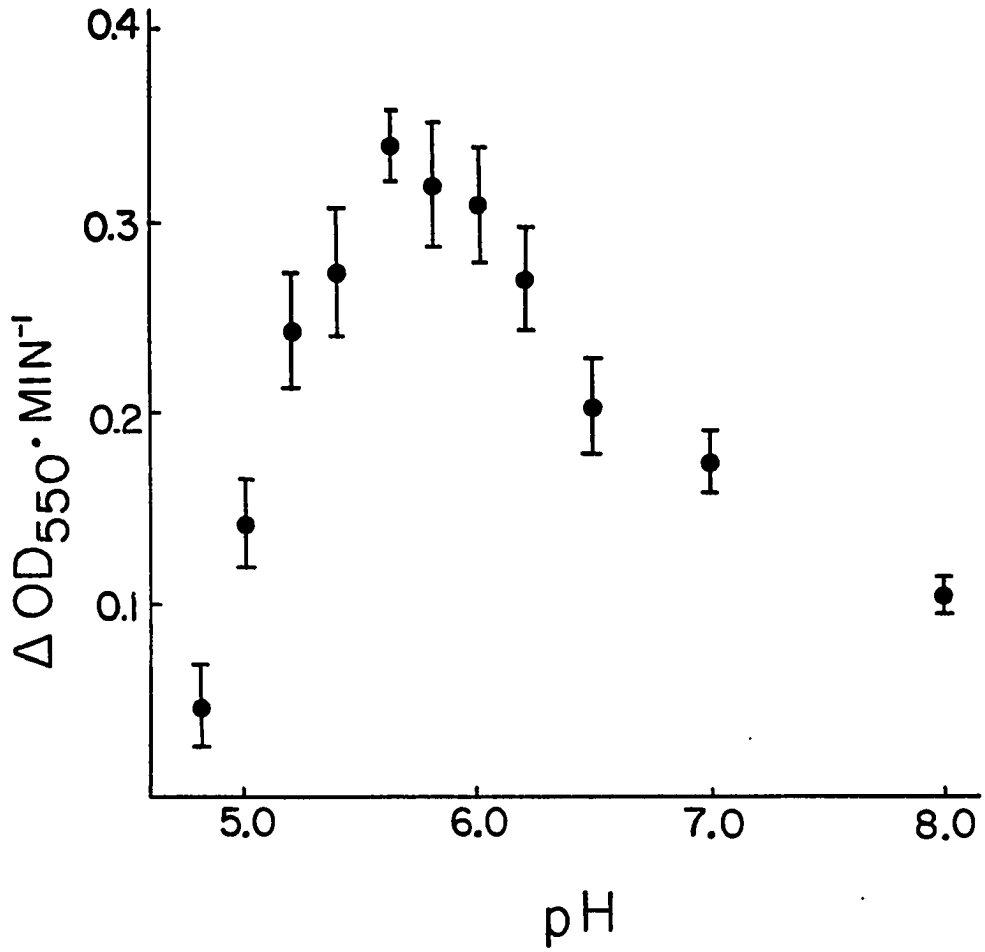

Fig. 1. Optimum pH for cytochrome oxidase activity. Mean and standard deviation of two replicate determinations on each of five fathead minnow brain homogenates; 10 μM cytochrome c in a 30 mM citrate buffer; test temperature = 25°C; cytochrome oxidase activity is expressed as $\Delta O.D.^{550} \cdot min^{-1}$ for a 1:6500 final tissue dilution; fish were acclimated to 25°C.

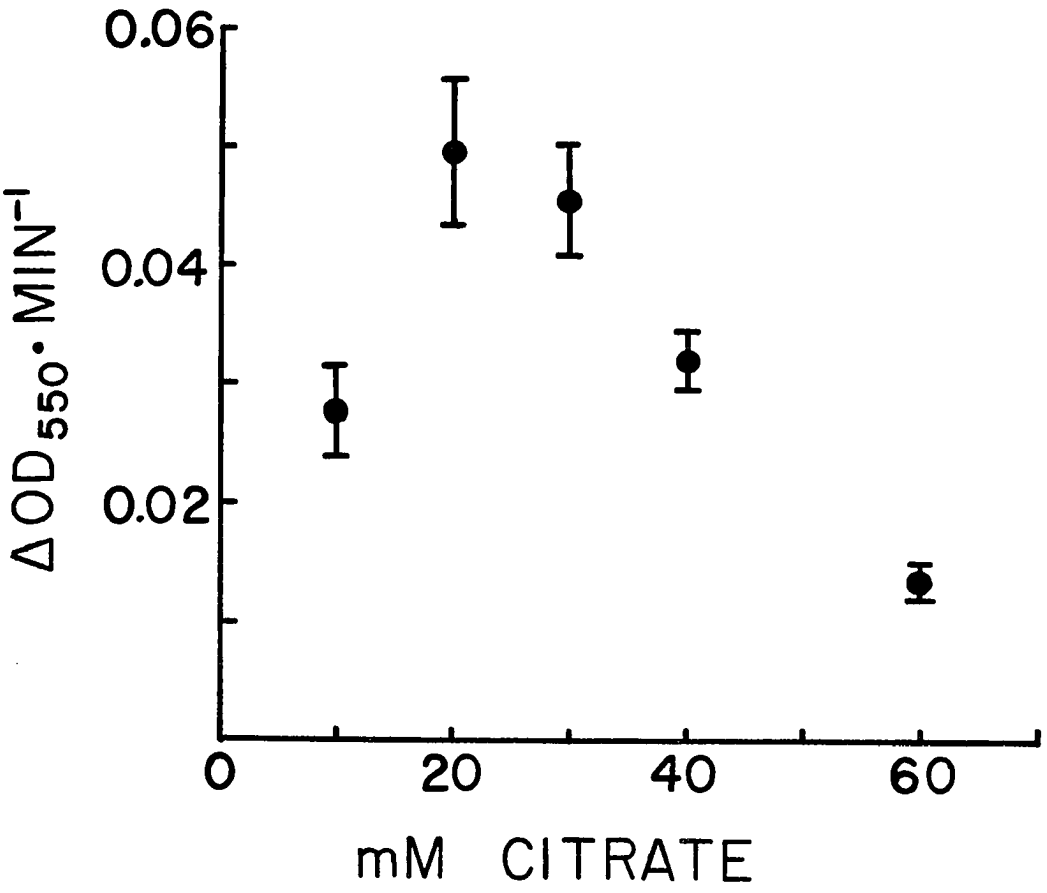

Fig. 2 Optimum buffer molarity for cytochrome oxidase activity. Mean and standard deviation of two replicate determinations on each of five fathead minnow brain homogenates; 10 μM cytochrome c; pH = 5.6; test temperature = 25°C; cytochrome oxidase activity is expressed as $\Delta O.D.^{550} \cdot min^{-1}$ for a 1:6500 final tissue dilution; fish were acclimated to 25°C.

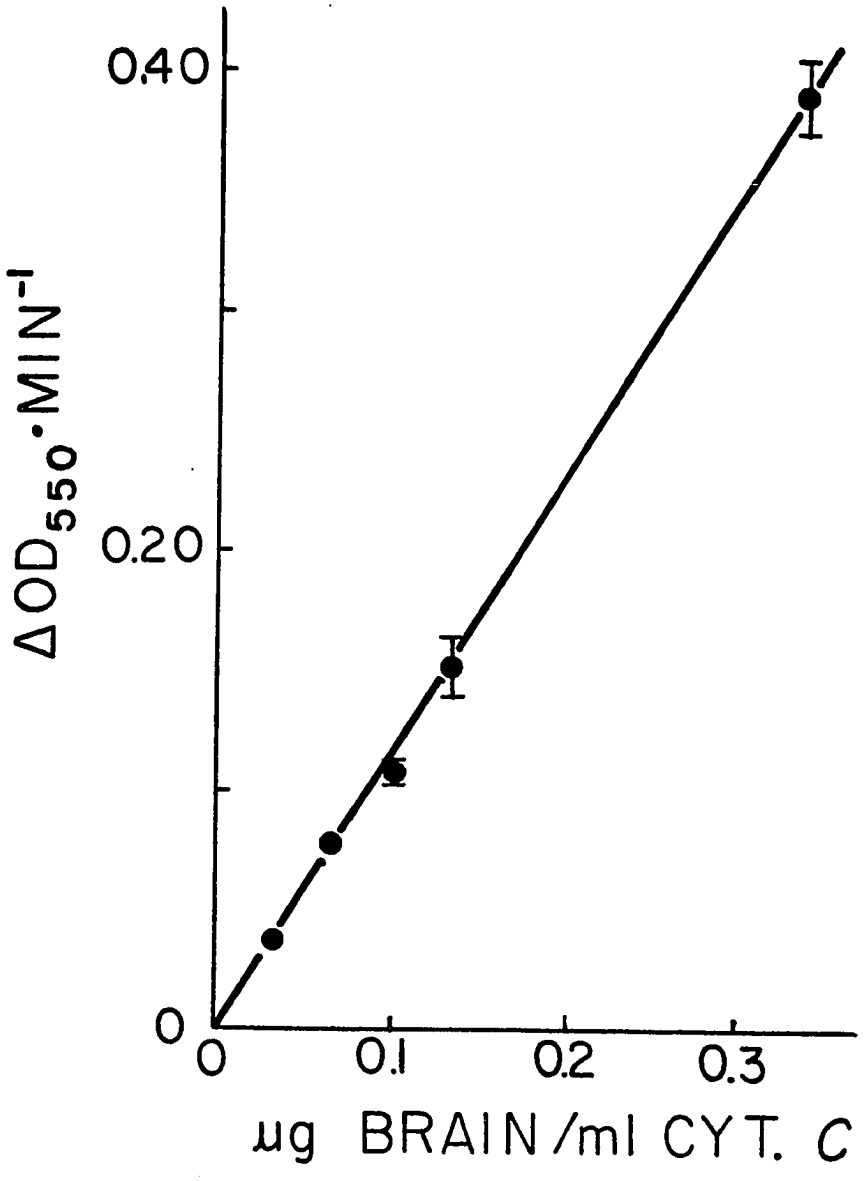

Fig. 3. Effect of tissue dilution on cytochrome oxidase activity. Mean and standard deviation of five determinations on a fathead minnow brain homogenate; 16.2 µM cytochrome c in a 20 mM citrate buffer; pH = 5.6; test temperature = 24°C; fish were acclimated to 25°C.

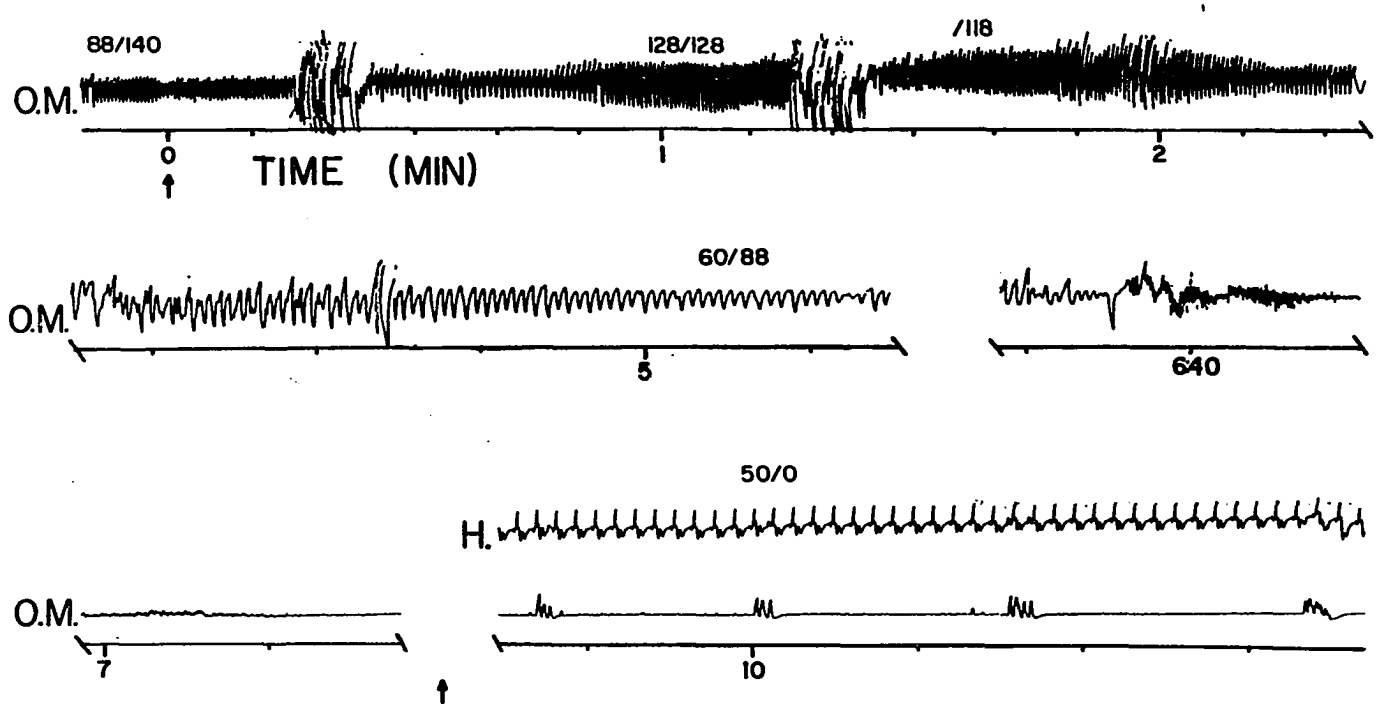

Fig. 4. Response of opercular muscle (O.M.) and heart (H) to hydrogen sulfide. Recordings are from a 20 cm channel catfish acclimated to and tested at 20°C; 0.5 mg/l un-ionized sulfide, pH = 8.0, added at time 0 (↑); the fish was removed from the sulfide after 8 minutes of exposure (↑); numbers over tracings are heart rate/opercular rate; recording of heart rate is shown only on lower tracing but was measured entire time.

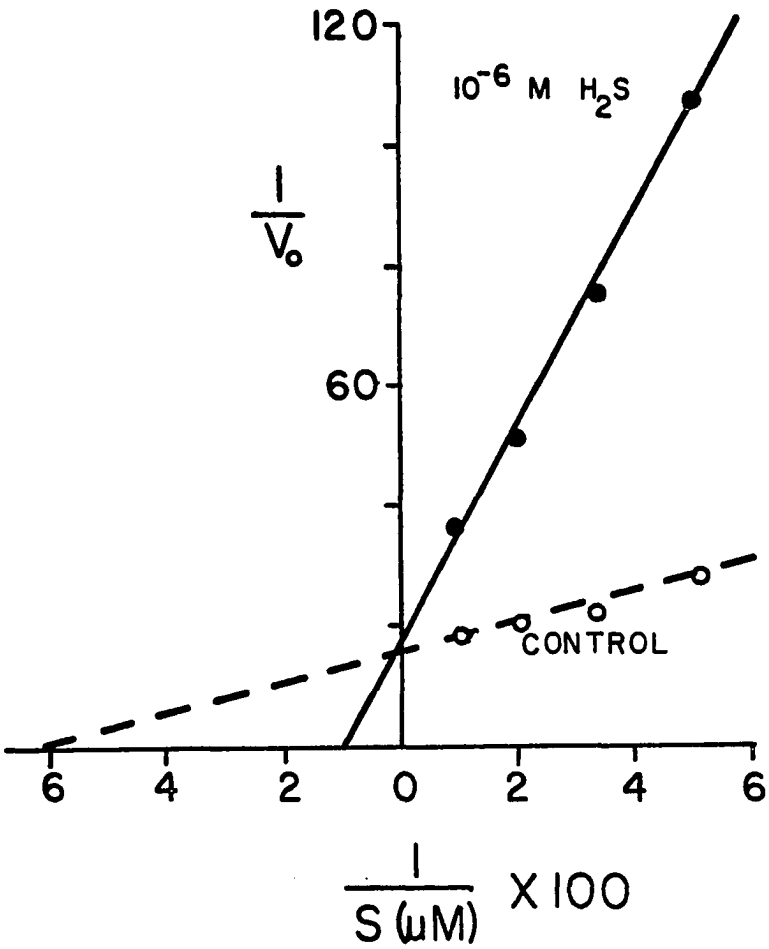

Fig. 5. Lineweaver-Burke plot of control and sulfide-inhibited cytochrome oxidase. A fathead minnow brain homogenate tested at 25°C; 20 mM citrate at pH = 5.6; 1:25,000 final tissue dilution; six replicate determinations for each control point, and three replicates for each test point.

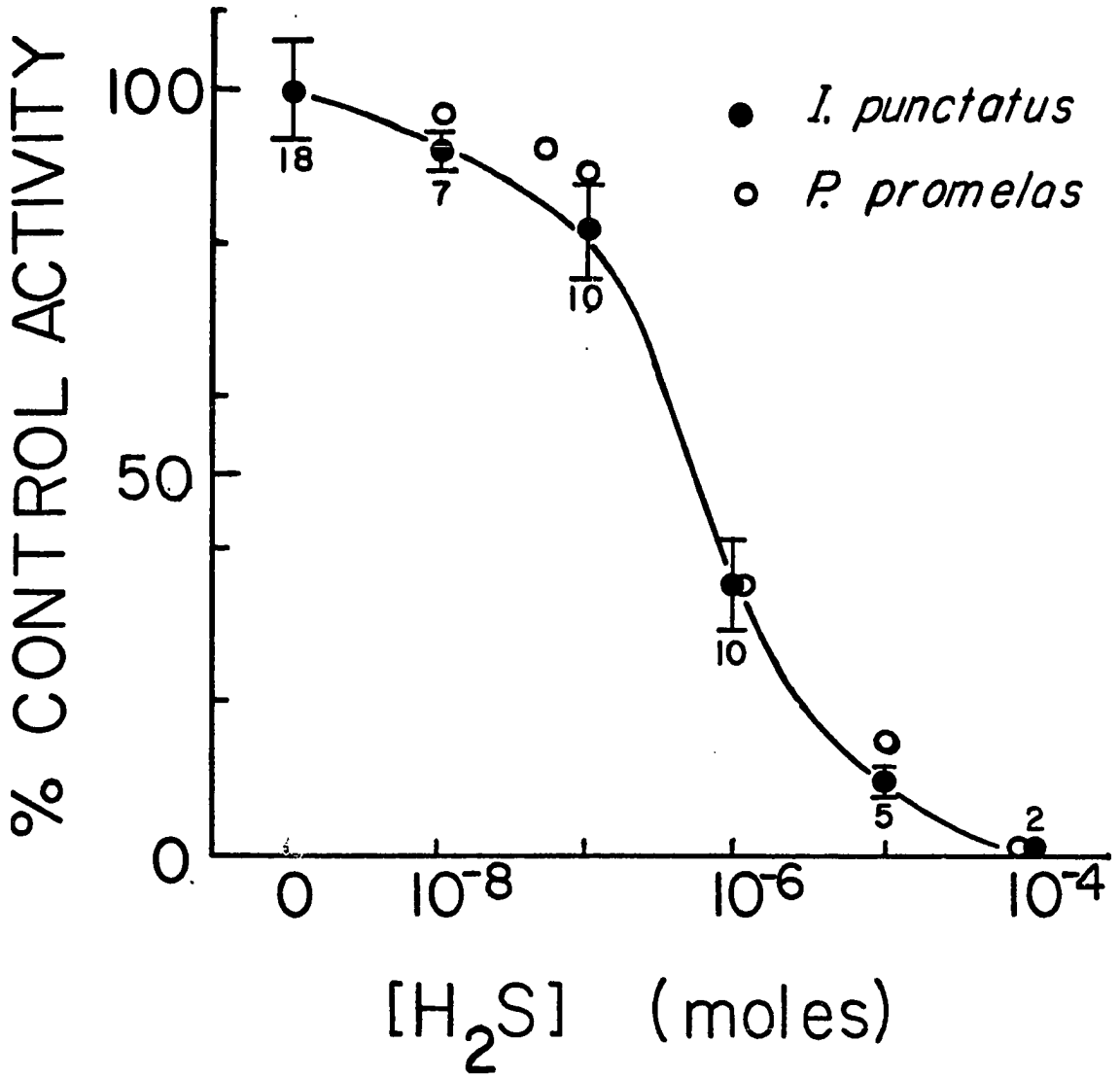

Fig. 6. Effect of sulfide concentration on cytochrome oxidase activity in vitro. Mean, n and standard deviation for a channel catfish brain homogenate; mean of two replicates for the fathead minnow brain homogenate; both fish were acclimated to 25°C; 10 μM cytochrome c in a 20 mM citrate buffer, pH = 5.6, temperature = 25°C.

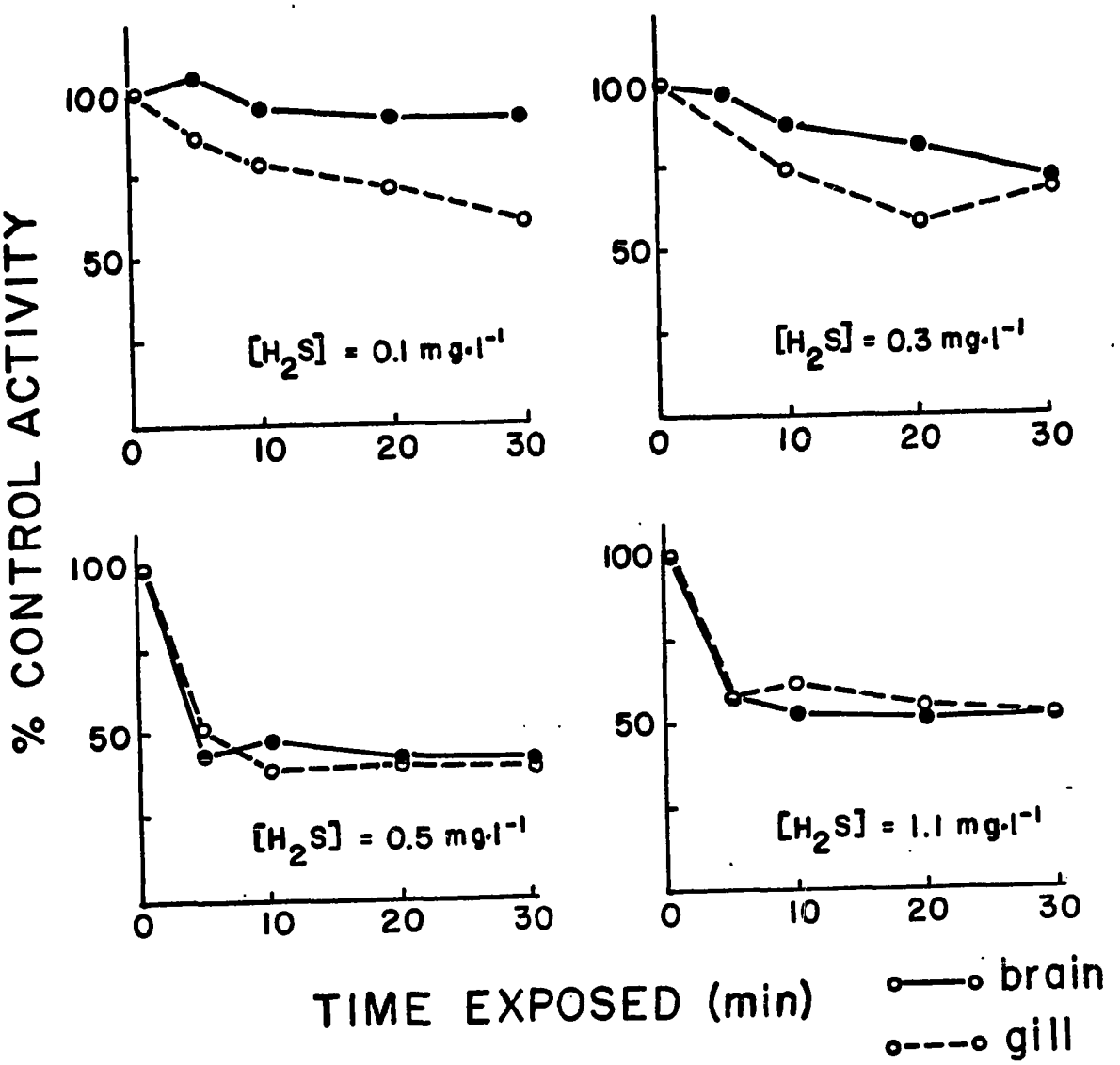

Fig 7. Effect of exposure time at 10°C on in vivo cytochrome oxidase activity of 10-15 cm channel catfish brain and gill, at four different un-ionized sulfide concentrations. Fish were acclimated to 10°C; there were two replicate determinations on each of five brains at each time, and four replicates on the pooled gill tissue from five fish at each time; enzymes were assayed at 27.5 ± 1.5°C.

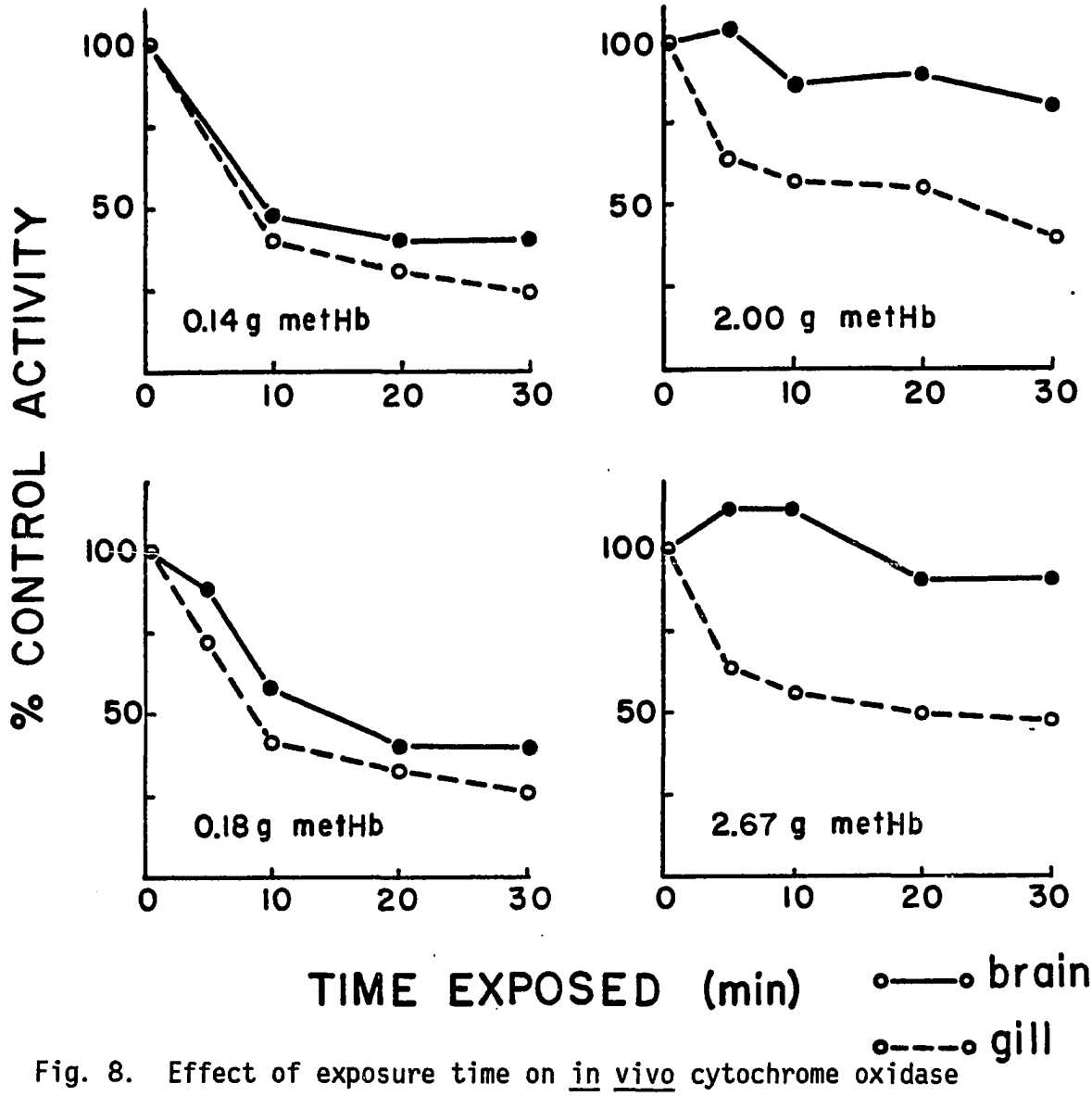

Fig. 8. Effect of exposure time on in vivo cytochrome oxidase activity of 10-15 cm channel catfish brain and gill, in groups of fish with four different methemoglobin concentrations. Fish were acclimated to 20°C and exposed to 0.1 mg/l un-ionized sulfide at 20°C; enzymes were assayed at 24 ± 1°C; means of two replicate determinations on each of five brains at each time; means of four replicates on pooled gill tissue at each time.

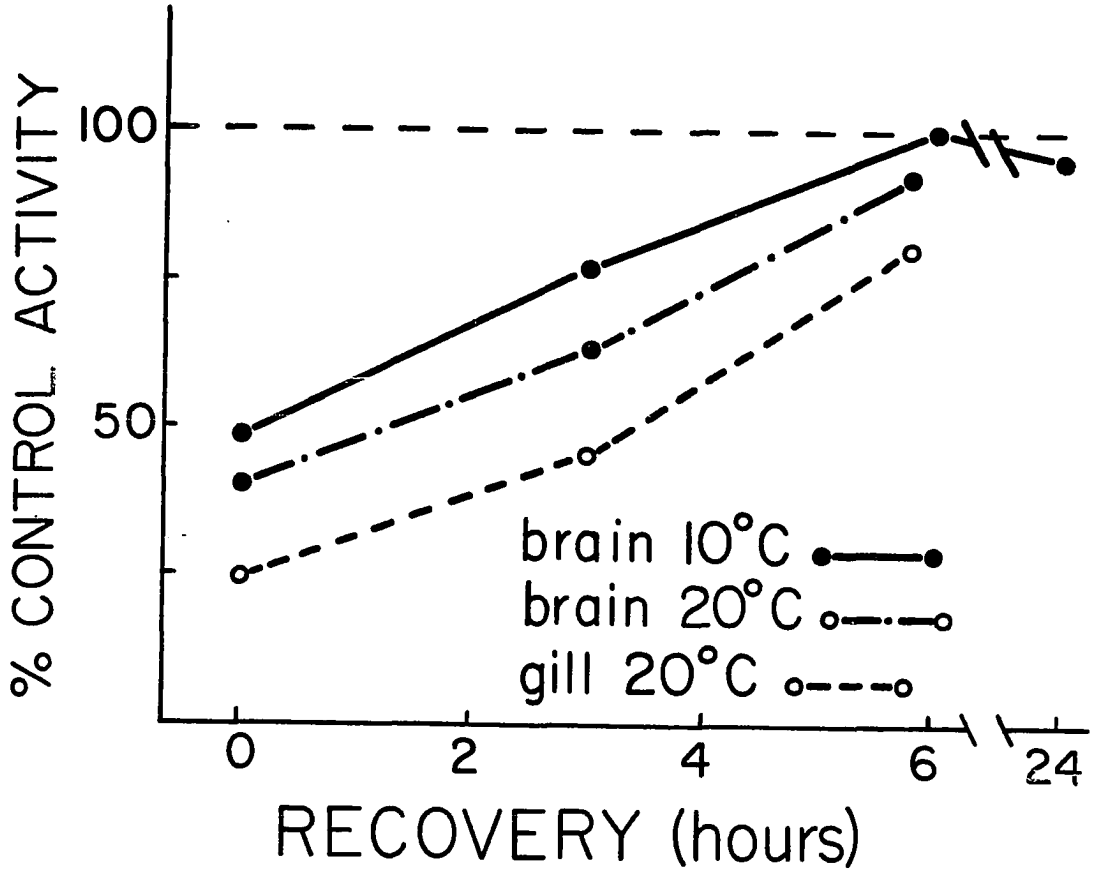

Fig. 9. In vivo recovery of cytochrome oxidase activity in 10-15 cm channel catfish brain and gill. Fish sampled at 0, 3, 6 and 24 hours after exposure to sulfide; fish were acclimated, exposed, and allowed to recover at the same temperature; enzymes were assayed at 24°C; means of replicate determinations on five brains and four replicate determinations on a pooled gill tissue sample.