RESPONSES OF FISHES TO A LOW pH ENVIRONMENT

THESIS

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By

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Data were collected from natural and introduced fishes present in Ferndale Lake, a small (120 ha) sport fishing reservoir in Camp County, east Texas. Levels of pH measured in the lake during the study period ranged from 3.5 to 5.3. Monthly field surveys and experimental manipulations were designed to evaluate quantitatively the signs of stress at various biological levels. Lethal limits to low pH were quantified for largemouth bass (Micropterus salmoides) and bluegill (Lepomis macrochirus) to be pH 3.8 and 4.0, respectively. Mean blood pH ($\pm$ 1 SD) of 59 bluegill was 7.41 ($\pm$ 0.16), with no significant difference ($P < 0.05$) among groups from Ferndale Lake and Moss Lake (Cooke Co., Texas) under experimental conditions, even when severe stress was externally apparent. In a dual-trough horizontal pH gradient, bluegill behavioral avoidance was observed at pH levels below 7.0. Individual testing of 40 bluegill in pH gradient of 5.2 to 7.6 resulted in median occupation of pH 7.1, with an interquartile range of pH 6.9 to 7.3. Decreased community structure and population "well being" compared to early studies cannot be attributed entirely to recent acidic condition. Separating potential stress due to lake conditions from that due to heavy biotic predation by sport fishing in a small reservoir is difficult.

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CHAPTER I

INTRODUCTION

Constituents of water dictate the success of fish as they are a part of the milieu within which the organism is bathed. Recently, professionals have begun determining the effects of alterations in water quality on fish which might be exposed to such fluctuations so as to better understand fish needs and implement best management practices. Realization that aqatic systems possess great potential for both sport and commercial fisheries, and the growing need for such systems to meet demands of our rapidly expanding population have increased the importance of protection and management for a high sustained yield.

Studies of low pH freshwater systems, with particular emphasis on resident fish populations, began about half a century ago. Of particular interest in these early studies was the relationship between pH and the presence or absence of various sport fishes within the system (Creaser, 1930) and with the effects of fish transfer into a system at a low pH. (Brown and Jewel, 1926; Wiebe, 1931).

A majority of more recent research began with the discovery that the pH of many freshwater systems is decreasing as a result of acid pollution. Poorly buffered systems experiencing acid precipitation have undergone drastic reductions in pH (Dillon, et al., 1978; Jeffries et al., 1979). A majority of the hundreds of affected lakes occur in Canada, northeastern United States and northwestern Europe, the numbers

increasing with increasing proximity to industrial centers (EPA, 1979; Dillon et al., 1978). This relation is explained by analyses of acid precipitation which indicate that nitric (HNO_3) and sulfuric acids (H_2SO_4) are main components. They are emitted to the atmosphere as nitrogen oxides (NO and NO_2) and sulfur dioxides (SO_2) by combustion of fossil fuels (Cogbill and Likens, 1974; Likens et al., 1979). These oxides readily react with water vapor to form their respective acids (Vermeulen, 1978), resulting in depression of the pH of rain from an historically natural level of no less than 5.6 to recorded levels more than a hundred times more acidic.

Increased utilization of high sulfur (soft) coals by industries, coupled with construction of taller smokestacks, has increased the area over which acid precipitation impacts (EPA, 1979). Buffering capacity of soils and rocks making up the geology of a particular drainage and lake basin will determine the sensitivity of that system to acid rainfall (Dillon et al., 1978). Some are extremely susceptible, while others are able to neutralize the acid (Johnson, 1979), eventually deteriorating buffered systems with time (Glass et al., 1979). The hazards associated with acid rain are drastic enough to warrant President Carter's mandate of a ten-year program to monitor acid rain and its effects in his 1978 Environmental Message to Congress, with international attention given the problem by the United State-Canada Research Consultation Group.

Acid mine drainage, the leaching of iron-sulfur pyrites and marcasites from bituminous coal mine tailings by rain runoff and streams, has been recognized as a source of acid pollution in coal mining areas of the world (King et al., 1974). Acid mine drainage is usually confined within

temporary ponds of the local watershed (King et al., 1974; Parsons, 1977; Koryak et al., 1979), the communities of which are severly altered by the acidification and associated changes in water chemistry (Lind and Campbell, 1970; Warner, 1971; Parsons, 1977; Nur, 1979). Increased concentrations and solubilities of heavy metals, often associated with acid mine drainage (Warner, 1971) and acid rain (Beamish and Van Loon, 1977), pose an additional threat to aquatic communities.

Aquatic organisms respond in many ways to stressful levels of pH, the degree of response increasing with increasing acidity. Number of species present and abundance of each correlate directly with pH level (Warner, 1971; Almer, et al., 1974). Acidification, regardless of cause, reduces the diversity and biomass of phytoplankton (Yan, 1979; Yan and Stokes, 1978) aufwuchs (Stickney and Campbell, 1972), zooplankton (Patalas, 1971; Sprules, 1975, 1975a; DeCosta and Janicki, 1978; Janicki and Decosta, 1979), and zoobenthos (Koryak et al., 1972; Tomkiewicz and Dunson, 1977). In these instances, as is the case with fish inhabiting acidified waters, difference in pH sensitivities among species results in communities with the most sensitive species disappearing first, followed by others slightly more tolerant disappearing with increased acidity, while the most tolerant species assume a dominance in abundance (Beamish and Harvey, 1972; Dunson and Martin, 1973; Beamish, 1974).

Acid tolerance of several fish species varies among species and methods (Trama, 1954; Beamish, 1972; Daye and Garside, 1975; Robinson, et al., 1976; Matthews and Hill, 1977). Tropical South American fish are some of the most tolerant fish tested, surviving indefinitely at pH levels as low as 3.5 (Dunson et al., 1977). Interactions with other environmental

variables, such as dissolved oxygen and temperature, affect the ability of fish to tolerate low pH (Robinson et al., 1976; Falk and Dunson, 1977; Neville, 1979).

Mount (1973) reported an inability of fathead minnows to acclimate to low pH levels. Robinson et al. (1976) found low pH tolerance to differ among strains of brook trout (Salvelinus fontinalis). Hybridization produced offspring of intermediate acid tolerance in one cross, while offspring of another cross exhibited increased acid tolerance. These authors suggested that increased tolerance to low pH could be artificially selected, producing strains which would be more likely to survive in acidic lakes.

Two non-mutually exclusive mechanisms have been suggested to explain fish mortality at low ambient pH levels. (1) Acid induces depletion of body sodium, resulting in the breakdown of the ion exchange process (Packer and Dunson 1970; Dunson et al., 1977; Cameron, 1978). Normally, hydrogen ions leave the body through gill lamellae in exchange for sodium ions uptaken from the water (Evans, 1975). Net increases in blood acidity occur with failure of sufficient cation exchange. Tissue anoxia, resulting from reduced oxygen-carrying capacity of low pH blood, is reported as a probable cause of death (Packer and Dunson, 1972; Hargis, 1976). (2) Excess mucous secretion in response to low pH exposure may form a diffusion barrier, decreasing oxygen transfer across the gills, which could lead to anoxia and subsequent death (Janssen and Randall, 1975; Ultsch, 1978; Packer, 1978; Ultsch and Gros, 1979). Electron microscopic examinations by Daye and Garside (1976) of surficial tissues of brook trout exposed to sub-lethal pH levels revealed alteration of epithelial tissue occurring

at pH 5.2 and lower, due to corrosion of the cells and hypertrophy of mucous. They reported an inverse relationship between degree of tissue damage and level of pH below 5.2. According to Daye and Garside, specific epithelial tissues, in order of their increasing sensitivity to acid conditions, include gill lamellae, opercular integument, cornea, lining of the nares, body integument, lens of the eye and lining of the esophagus. Fish in situ may be able to reach a homeostasis between repair of cells and destruction caused by external medium. Achieving such a balance would require a large expenditure of energy (Daye and Garside, 1976). Diversion of large amounts of energy toward maintenance could negatively affect growth and reproductive rates, predator avoidance, and incidence of parasites.

Mount (1973) reported reproductive capacity to be significantly reduced at various stages in the life history of fathead minnows (Pimephales promelas) at pH levels below 6.6. Similar reports were made for brook trout (Salvelinus fontinalis) and flagfish (Jordanella floridae) from life history studies by Menendez (1976) and Craig and Baksi (1977), respectively. Other investigators concur with results from the above studies, showing reductions in the number of viable eggs per spawn (Ruby et al., 1977; Panek and Cofield, 1978), hatchability of eggs, survival and growth rates of newly hatched alevins (Krishna, 1953; Johanson and Kihlstrom, 1975; Kwain, 1975; Trojnar, 1977, 1977a; Daye and Garside, 1977, 1979; Carrick, 1979), and growth rates of adults (Jacobsen, 1977). Incidence of spawning shows a decrease with lowered pH, and Craig and Baksi (1977) noted that spawning flagfish exposed to rapid pH depression would immediately cease spawing activities. Conclusions from these studies support a lower

acceptable pH level of 6.5, established by the Water Pollution Control Act of 1969, as a more desirable freshwater quality criterion than the lower safe pH level of 5.0 accepted by the European Inland Fisheries Advisory Commission (1969) for European waters.

Observations by Dunson and Martin (1973) and Cooper (1973) indicate that some fish may survive in systems with average pH below laboratory-determined lethal levels by congregating at fresh water inflows. Behavioral avoidance by fish of many suboptimal or adverse environmental factors has been demonstrated (Sprague, 1964, 1968; Hemmings, 1966; Hoglund and Astrand, 1973; Scherer, 1975; see Larrick et al., 1978 for review), while some fish have been shown to preferentially select one level of temperature over others (Beitinger, 1976, 1977; Beitinger et all, 1975) with data from such studies utilized as fresh water quality criteria. Jones (1948) and Hoglund (1961) reported avoidance responses for sticklebacks (Gasterosteus aculeatus) below pH 5.4 and roaches (Rutalus rutalus) below 5.6. Red shiners (Notropis lutrensis) were reported to avoid pH levels below neutral, in a series of two-choice tests (Matthews, 1977). Such reactions by fish could be advantageous to their survival, provided pH variation exists within their natural habitat.

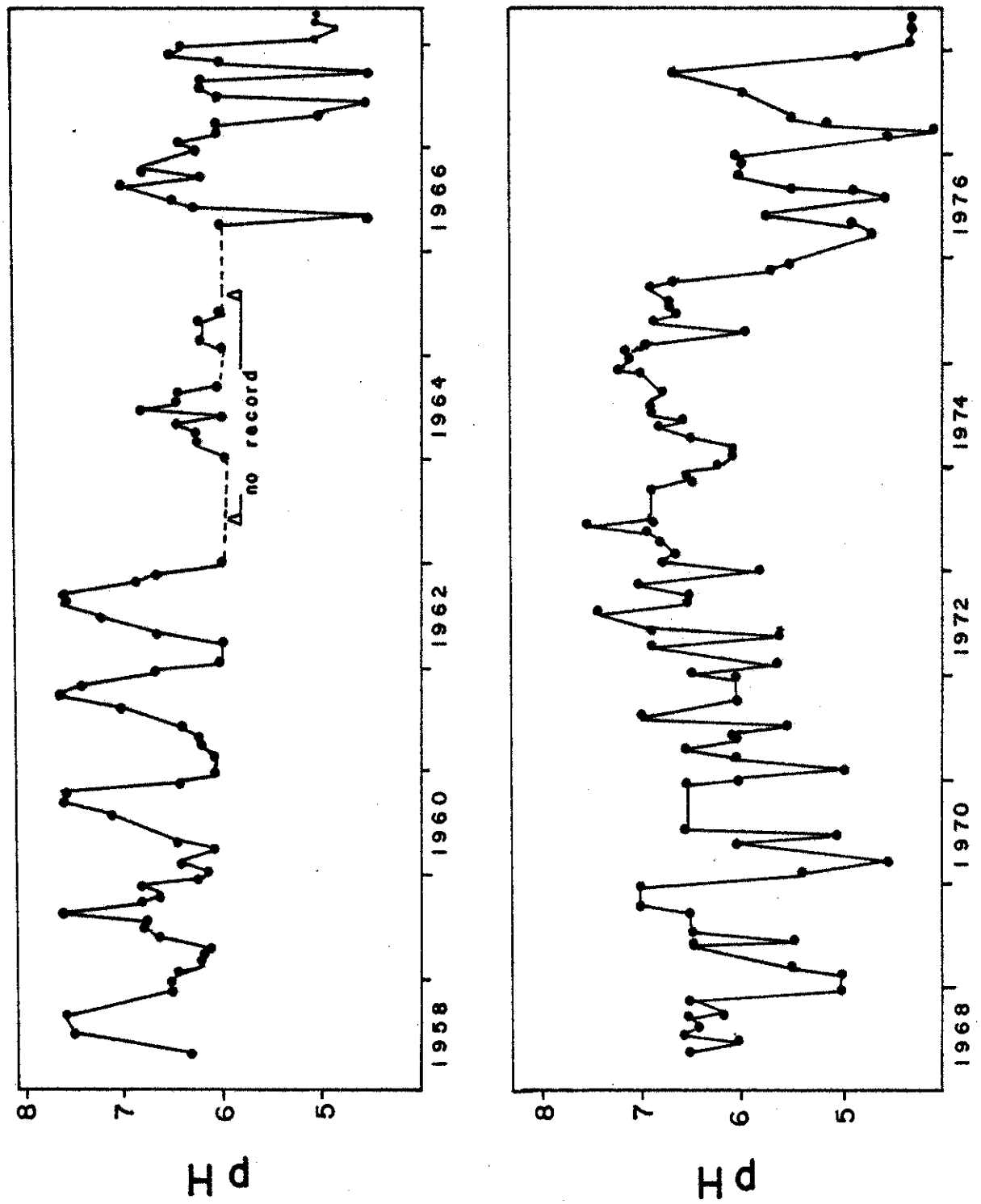
My study was initiated in September 1978 as an examination of the fish community in a critically acidic east Texas sport fishing reservoir. Ferndale Lake resulted from an impoundment in 1909 of North Lilly Creek, about seven miles southeast of Pittsburg, in Camp County, and covers about 120 hectares in the Pineywoods region of the state. Historically, Ferndale Lake has experienced pH levels more acidic (5.0 to 6.5) than the 6.6 to 8.5 pH range for undisturbed lakes (Silvey and Harris, 1947).

Temporal fluctuations have been recorded since that study, with a net decline in pH to levels as low as 3.8 recorded in the course of my study (Figure 1). The current pH of Ferndale Lake would classify it as critically acidic (Beamish and Harvey, 1972; Beamish, 1974).

The cause of low pH in Ferndale probably can be attributed to several interacting factors. Soils in that area of Texas are acidic, with little or no buffering capabilities. Acid brine springs associated with oil field operations were found within the North Lilly Creek drainage, the prinicipal feeder stream into Ferndale. Localized rains with pH as low as 3.3 were measured. I have measured pH in North Lilly Creek as low as 2.9, and other intermittently flowing tributaries of North Lilly Creek have had levels below 4.0 and as low as 3.4.

I have taken a multiple-level approach to delineate the responses of fish inhabiting Ferndale Lake to such a stressful environment. Energy required to overcome or avoid stressful conditions could be expected to be diverted from such processes as growth and reproduction, resulting in a decline in these two processes. Data were collected from behavioral and physiological experimentation designed to determine if pH in Ferndale Lake is sufficiently low as to result in stress to fish. Indicators of fish "well being" and reproductive effort were monitored through one season to determine if stresses affecting fishes could be detected by such indices.

Figure 1--Record of pH maintained by Ferndale Lake Club personnel showing wide fluctuations and downward trend.


pH
8
7
6
5
1958
1960
1962
1964
1966
no record
pH
8
7
6
5
1968
1970
1972
1974
1976

CHAPTER II

MATERIALS AND METHODS

My research design consisted of an integrated series of field observations and collections, laboratory analyses, and controlled experimentation to assess the responses of fish exposed to low pH of Ferndale Lake at the community, population, and individual levels. Fish community composition and species abundance data were gathered by collecting and identifying fish. A boat equipped by Coffelt Electronics with a pulsed alternating-current electroshocking system was used for obtaining a majority of fish samples. Linear shoreline transits were shocked 15-20 min. each month from September 1978 through August 1979 (December and January were omitted due to equipment failures). Stunned fish were collected with long-handled nets (5 cm mesh), identified to species, counted, and either returned to the lake or held for further analyses. Additional methods employed to collect fish community data included angling, beach seining, minnow traps, SCUBA, and nightime spotlight observation. Weekly creel records maintained by Ferndale Lake Club were also examined.

The well-being of populations of largemouth bass (Micropterus salmoides), the principal sport fish, and sunfish (Lepomis spp.), were examined monthly using several indicators. Largemouth bass, bluegill (Lepomis macrochirus), and redear sunfish (Lepomis microlophus) were weighed (g) and measured (total length to mm). Condition factors (K_{TL})

were calculated as follows:

$$K_{TL} = (W)\ (10^5)\ L^{-3}$$

where L is the total length in mm and W is the weight in g.

Monthly samples of bluegill and redear sunfish were frozen and returned to the Physiological Ecology Laboratory at North Texas State. Ovaries were removed and weighed to the nearest 0.01 g. A gonadosomatic index (GSI) was calculated for each fish as percentage of total body weight represented by gonad wet weight. Percentage of each fish sample represented by females in reproductive condition (determined by visible egg development) was determined for monthly samples.

A series of lethality tests was designed to determine the lower lethal pH limit of juvenile bluegill and largemouth bass. Bluegill were collected from Ferndale by electroshocking on 9 November 1978 (11.0 to 15.0 ^{o}C, pH = 4.7), 13 December 1978 (6.0 to 8.0 ^{o}C, pH = 4.6), and 29 May 1979 (24.0 to 26.0 ^{o}C, pH = 4.2). Juvenile largemouth bass were netted from the club's brood pond on 25 June 1979 (33^{o}C, pH - 7.1) All tests were conducted on site. Test chambers were 37-l glass tanks filled with lake water adjusted to desired pH levels by titration with 10% V/V hydrochloric acid. A Markson digital pH meter (0.01 unit resolution) was used to measure pH in tanks. A constant flow of water pumped from the lake through a water bath held test tank temperatures within 1^{o}C of lake water. Individual tanks were gently aerated with airstones. After a minimum recovery period of 8 h, fish were randomly distributed among tanks. Fish and pH levels were checked after 0.5, 1, 2, 4 and 8 h, and every 8 h thereafter. Mortalities, determined by a loss of equilibrium,

were removed and tabulated. Resistance times were determined by plotting percentage survivorship against time elapsed for each exposure level tested.

Inability of acid-exposed fish to maintain blood pH, as a possible cause of death, was examined by measuring blood pH of bluegill after exposure to various controlled conditions. Thirty-three bluegill (19-110g) were collected from Ferndale Lake (pH = 4.3) by electroshocking. Fish were allowed 12 h for recovery. Six 37-l tanks were filled with lake water and partially submerged in the lake as a constant temperature bath ($29^{o}C$). Water pH was adjusted in paired tanks to 3.5, 5.5 and 7.5. NaCl was added to one tank of each pair to increase salinity from 1.4 ppt (lake concentration) to 5.5 ppt, as measured by YSI salinity meter. Fish were randomly distributed among tanks and exposed to test conditions for eight hours to test for difference in blood pH due to more available sodium ions for exchange with hydrogen ions across the gills. Fish were then removed from tanks and blood samples drawn from hemal arch, severed anterior to the caudal peduncle. Sampling micropipets were sealed for handling. Blood pH was measured to 0.001 unit with Instrumentation Lab pH microelectrode. Accuracy of measurements was controlled by calibration with pH 6.840 and 7.384 standard buffers before each reading. Precision of this technique was ascertained when enough blood could be obtained from a fish for replicate readings. Significant differences between treatment groups were determined by analysis of variance. In addition, values were compared to those from fish collected from Ferndale and held *in situ* (pH 4.1, $31^{o}C$) and a group collected from Moss Lake,

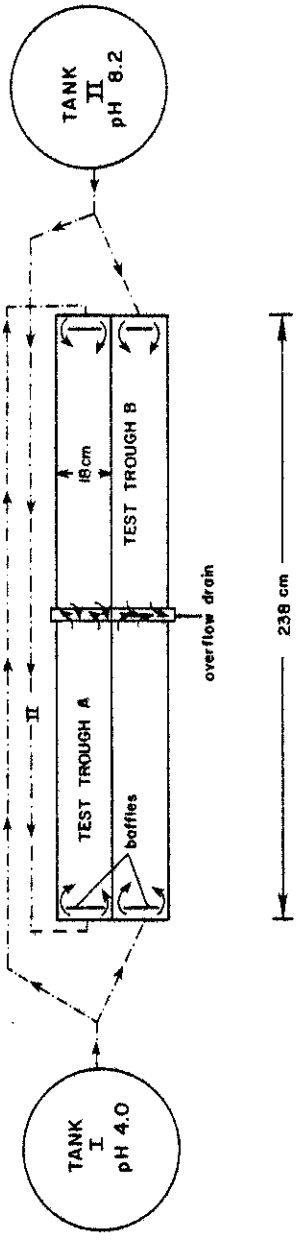
Cooke County, Texas, and held in tanks at pH 6.8 (25°C) for 4 days prior to blood pH measurements.

Behavioral responses of juvenile bluegill were observed in a dual horizontal pH gradient trough modified from Sprague (1964). Turbulence of flow through chambers (238 long, 18 cm wide and 20 cm deep) was abated by employment of plastic mesh baffles near each inflow port. Water levels were maintained at 18 cm by central surface-spanning drains. Two 1350-l stainless steel tanks served as gravity feeding reservoirs for stock water from Ferndale Lake. Water in stock tank I (Figure 2) remained at ph 4.0, while water in stock tank II was buffered to pH 8.2 with dibasic potassium phosphate. Water in both tanks was oxygen-saturated at 27°C. Flow from each tank was divided and introduced at opposite ends of adjacent troughs. Steepness of each pH gradient was controlled by manual adjustments of flow rates at each inflow port and monitored with a calibrated Markson digital pH meter (resolution 0.01 units). Flow at each of the four ports varied less than 10 ml/min during tests.

Test juvenile bluegill (1.3 to 7.1g) were collected from Ferndale Lake (pH 4.0) in minnow traps. Subsequent to a 4-h holding period, 50 fish were transferred to each side of the trough and allowed 2 h to become accustomed to the chamber.

Flow from the stock tanks was initiated, and a steep pH gradient (4.0 to 7.5) was established within about 10 minutes. The number of fish occupying each of eight uninterrupted segments of each trough were tabulated at 5-min intervals for 30 min, from a vantage point 2 m above the chamber. Each segment of trough was monitored for constancy of pH level.

Figure 2--Dual-trough horizontal pH gradient used in behavioral studies with schematic of flow.


TANK
II
pH 8.2
18cm
TEST TROUGH B
TEST TROUGH A
baffles
overflow drain
238 cm
II
I
TANK
I
pH 4.0

The procedure was repeated with an additional 100 fish in a shallow gradient. Data were subjected to chi-square goodness of fit analyses to determine if observed frequency distributions departed from a null hypothesis of expected uniform distributions.

A final trial was conducted to determine if intraspecific behavioral responses may have affected distributions in group trials. Forty juvenile bluegill were tested individually in a gradient of pH 5.2 to 7.6. Each fish was allowed 10-30 min to explore the trough and establish a stationary position within the gradient. The pH was measured at the positions selected by each test fish.

CHAPTER III

RESULTS

Community Response

Nineteen fish species representing seven families were collected during the 12 monthly samples collected along each of three shoreline transits. Numbers following each common name in Table I indicate the abundance of each fish as determined by their presence in each transit sample. The lowest number (1) indicates the most common species (present in all 36 sample transits) and the highest number (5) the rarest species (present in fewer than 9 sample transits). Intermediate values (2, 3 and 4) were assigned to very common (27-35 transits), common (18-26 transits), and uncommon (9-17 transits), respectively.

Population Response

Condition factors (K_{TL}), calculated as a measure of well-being of the largemouth bass population, had a mean $\pm$ SD of 1.27 $\pm$ 0.15. This value is low compared to values reported for Texas bass by Prentice in 1978. Condition factors reported by Cooper (1950) for largemouth bass in Ferndale (pH 5.0-6.0) were significantly higher (t test, $P<0.001$) than those from this study (table II). Raw data from all tagged bass returned to the lake and lake conditions during sampling dates are included in the appendix.

Gonadosomatic indices (GSI) for bluegill and redear sunfish populations indicated ovarian maturity for monthly samples of May, June, and

TABLE I

RELATIVE SPECIES ABUNDANCE

Family	Species	Common Name	Abundance
Centrarchidae	*Micropterus salmoides*	largemouth bass	2
	M. punctatus	spotted bass	5
	Lepomis macrochirus	bluegill	1
	L. microlophus	redear sunfish	1
	L. megalotis	longear sunfish	3
	L. gulosus	warmouth	3
	L. cyanellus	green sunfish	5
	Pomoxis nigromaculatus	black crappie	3
Ictaluridae	*Ictalurus punctatus*	channel catfish	3
	I. furcatus	blue catfish	5
	I. natalis	yellow bullhead	3
	I. nebulosus	brown bullhead	5
	Pylodictus olivaris	flathead catfish	5

TABLE I--Continued

Family	Species	Common Name	Abundance
Cyprinidae	*Notemigonus crysoleucas*	golden shiner	5
	Pimephales promelas	fathead minnow	4
Catostomidae	*Erimyzon oblongus*	creek chubsucker	3
Esocidae	*Esox americanus*	grass pickerel	5
Lepisosteidae	*Lepisosteus oculatus*	spotted gar	5
Amiidae	*Amia calva*	bowfin	5

TABLE II

COMPARISON OF CONCITION FACTORS (K_{TL}) OF
LARGEMOUTH BASS, MICROPTERUS SALMOIDES,
FROM VARIOUS SOURCES

Site	n	K(TL)	±SD
Ferndale Lake (1978-1979)	329	1.27	0.15
*Ferndale Lake (1947-1949)	109	1.35	0.35
**Texas (statewide)	2631	2.02	N/A
**Texas: Pineywoods region	559	1.80	N/A
**Texas: Sabine-Sulphur-Cypress-Neches River Systems	794	1.81	N/A

*K(TL) calculated from K(SL) values reported by Cooper, 1950 M.S. thesis
**from Prentice, 1978. Texas Parks and Wildlife Department.
N/A information not available

TABLE III

MEAN CONDITION FACTORS FOR FERNDALE SUNFISH, *LEPOMIS* SPP.

Species	n	K(TL)	±SD
Lepomis macrochirus	217	1.81	0.17
L. microlphus	206	1.61	0.15
L. megalotus	31	1.55	0.15
L. gulosus	28	2.24	0.15

July (Figures 3 and 4). "Student's" t test showed no significant difference at the 0.05 level between bluegill and redear sunfish overall mean GSI for the spawning season. Length of the spawning period, estimated by elevated GSI, appeared to be shorter for the bluegill population sampled in 1979 than in Estes' (1949) study. GSI for the population examined by Estes peaked at about 77% of maximum monthly means from my study, but they were at a level indicative of spawning activity from January through August as opposed to late April to August in my 1979 samples (Figure 3).

Physiological Response

Lethality testing of juvenile bluegill exhibited similar responses for the first two trials. Median resistance times and pH levels were strongly correlated (r=1.0), with exposure levels above pH 3.9 producing fewer than 50% mortality after 48 h in test I (fish mean weight $\pm$ 1 SD = 2.66 $\pm$ 1.21) and after 96 h in test II (fish mean weight $\pm$ SD = 2.82 $\pm$ 1.40). Resistance time plots for each exposure are combined for tests I and II in Figure 5. In a third test no mortalities occurred over the entire exposure range, with a minimum of 3.75 (fish mean weight $\pm$ SD was 4.6 $\pm$ 2.50). Testing of juvenile largemouth bass (mean weight $\pm$ SD of 3.50 $\pm$ 1.0) produced similar response curves, but indicated that this species is more resistant in that pH levels of 3.8 and higher allowed for more than 50% survivorship after 96 h exposure (Figure 6).

Bluegill exposed to experimentally controlled levels of pH and salinity ranged in total length (TL) from 103 to 183 mm. ANOV revealed no significant effects by level of exposure pH or salinity on measured blood pH, even though individuals in the low pH exposure

Figure 3--GSI for bluegill females in Ferndale Lake. Bar extends to the mean with ± 1 SD indicated by vertical line. Number of females examined is above each bar. Single points represent data from 1948 blugill (Estes, 1949).

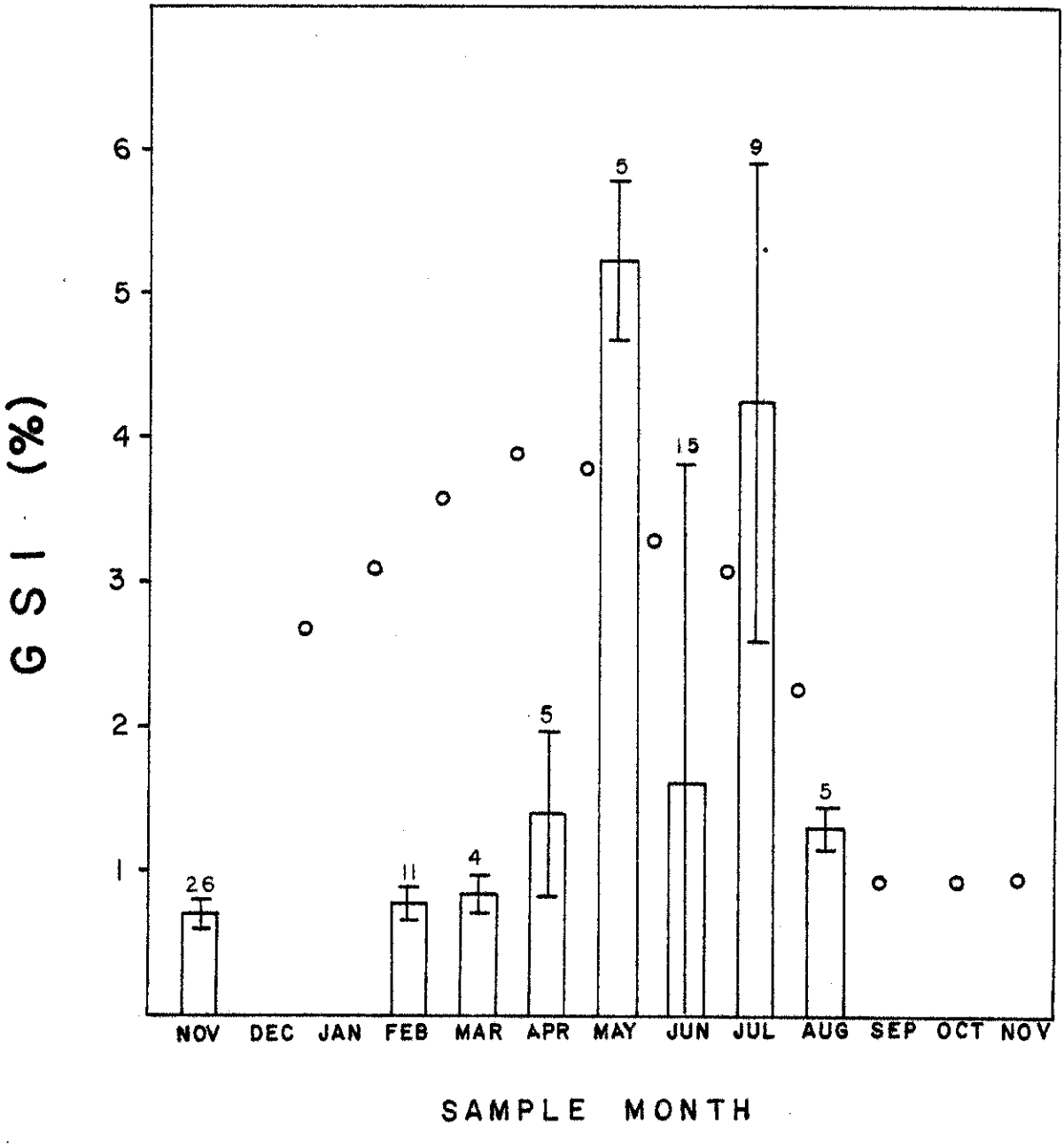

GSI (%)
6
5
4
3
2
1
26
11
4
5
5
15
9
5
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
SAMPLE MONTH

Figure 4--GSI for redear sunfish females in Ferndale Lake. Bar extends to the mean with ± 1 SD indicated by vertical line. Number of females examined is above each bar.

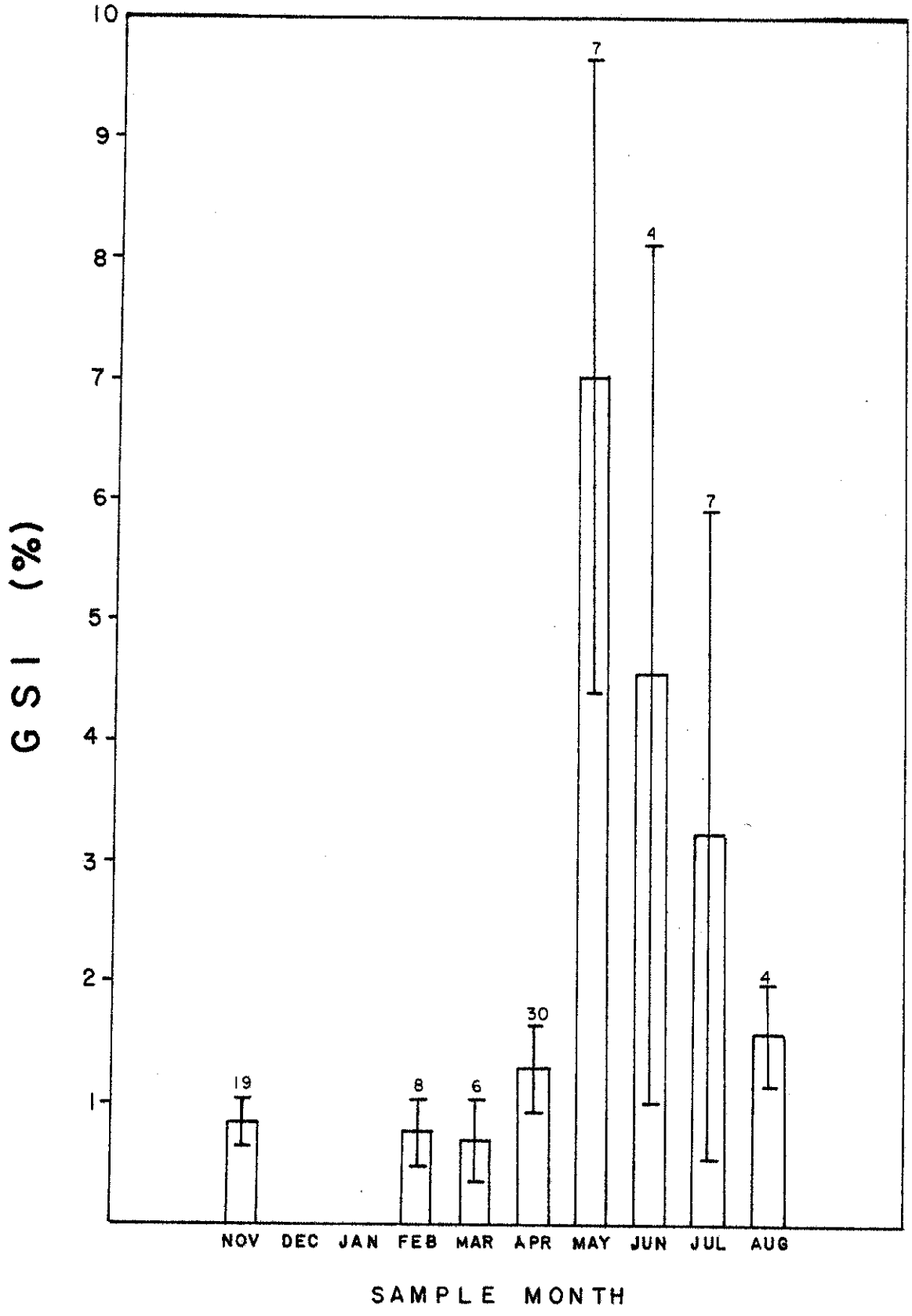

GSI (%)
10
9
8
7
6
5
4
3
2
1
19
8
6
30
7
4
7
4
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SAMPLE MONTH

Figure 5--Resistance times for juvenile bluegill at various pH levels and at two test temperatures. Open points are from a 48 hour test at 7°C and solid points represent fish exposed for 96 hours at 13.5°C. Exposure pH is given for each curve.

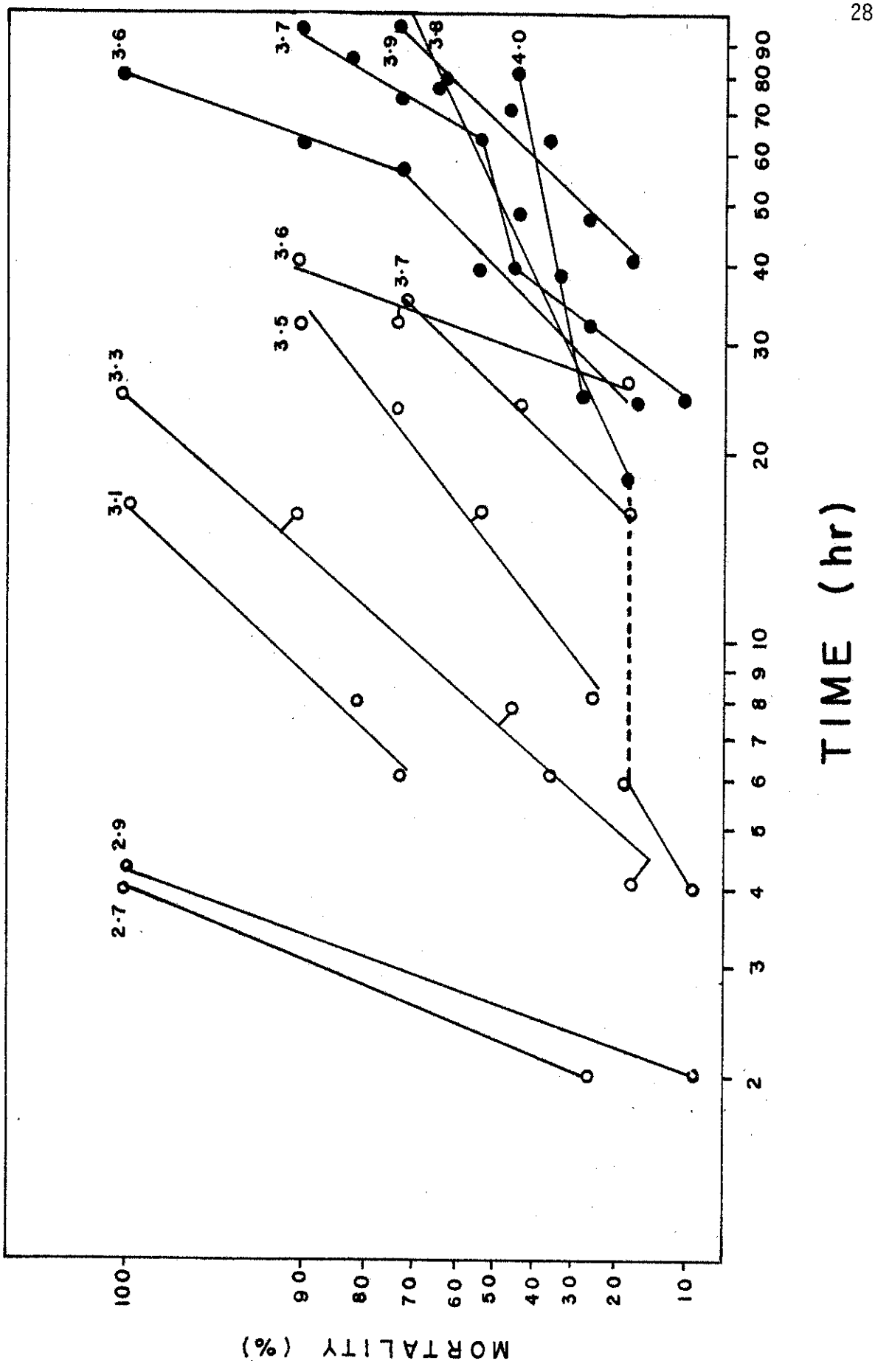
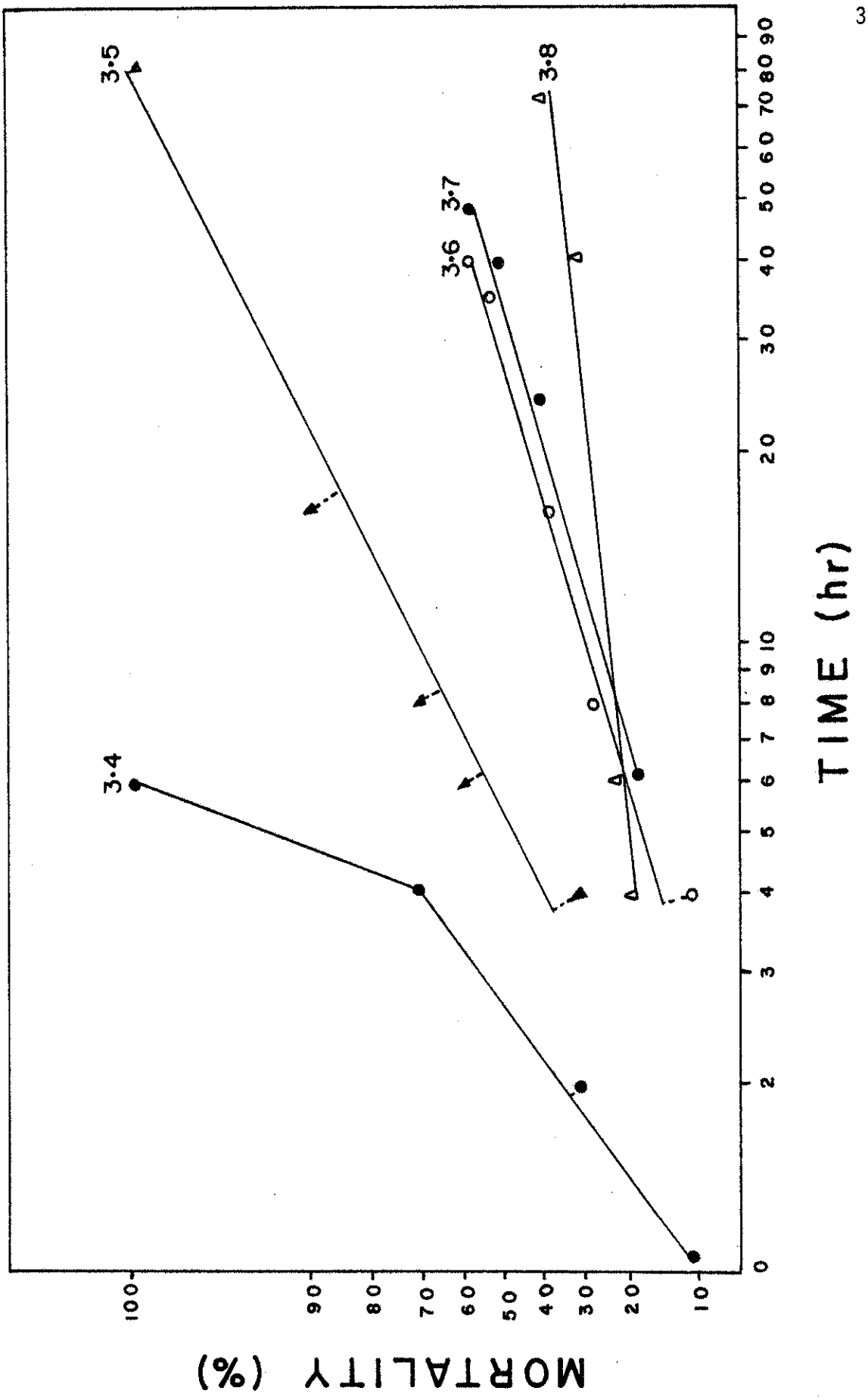

MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
TIME (hr)
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
2·7
2·9
3·1
3·3
3·5
3·6
3·7
3·8
3·9
4·0

Figure 6--Resistance times for largemouth bass juveniles at five levels of pH at 31°C. Exposure pH is given for each curve.


MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
TIME (hr)
0
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
3·4
3·5
3·6
3·7
3·8

groups showed externally obvious signs of severe stress, including erratic swimming, excess mucus secretion, and loss of equilibrium. Mean $\pm$ SD blood pH of all 33 test fish was 7.46 $\pm$ 0.15. When compared to blood pH of two other groups of Ferndale bluegill (110 to 220 mm TL) and one group of bluegill from Moss Lake (102 to 182 mm TL) by ANOV, no significant difference was observed (Figure 7). The mean $\pm$ SD blood pH of all 59 bluegill was 7.41 $\pm$ 0.16.

Behavioral Response

Juvenile bluegills actively avoided the inflow of acidic water (ca. pH 4.0) by moving toward the end of the chamber receiving an inflow of water at pH of 8.0. Occasionally, small schools swam into low pH water but rapidly returned to less acidic water. Observed distributions (Figure 8) of bluegill positions in the four trials were significantly different from uniform (chi-square values ranged from 61.6 to 92.4; $P < 0.001$). In gradual gradients (pH 6.3 to 7.8 available), only 50 of 600 observed fish positions (8.4%) corresponded to pH levels below 7.0 (Figure 8, A and B). During steep gradient testing (pH 4.0 to 7.5 available), 180 of 600 observed positions (30%) occurred in the 12 sections of the trough (75% of water volume) with pH less than 7.0 (Figure 8, C and D).

Although water with pH ranging from 5.2 to 7.6 was available to 40 bluegill tested individually, the median of the pH range occupied by these fish was 7.1, and the interquartile range extended from pH 6.9 to 7.3 (TABLE IV).

Figure 7--Blood pH of bluegill from (I) altered exposure conditions experiment, Ferndale (II) Moss Lake bluegill held at pH 6.8, (III) Ferndale bluegill held at pH 6.4, (IV) Ferndale bluegill held *in situ*, pH 4.0. Mean $\pm$ 1 SD are indicated by horizontal line and bar respectively, with vertical line extending for the range of values measured. Number of fish in each group is above the bars.

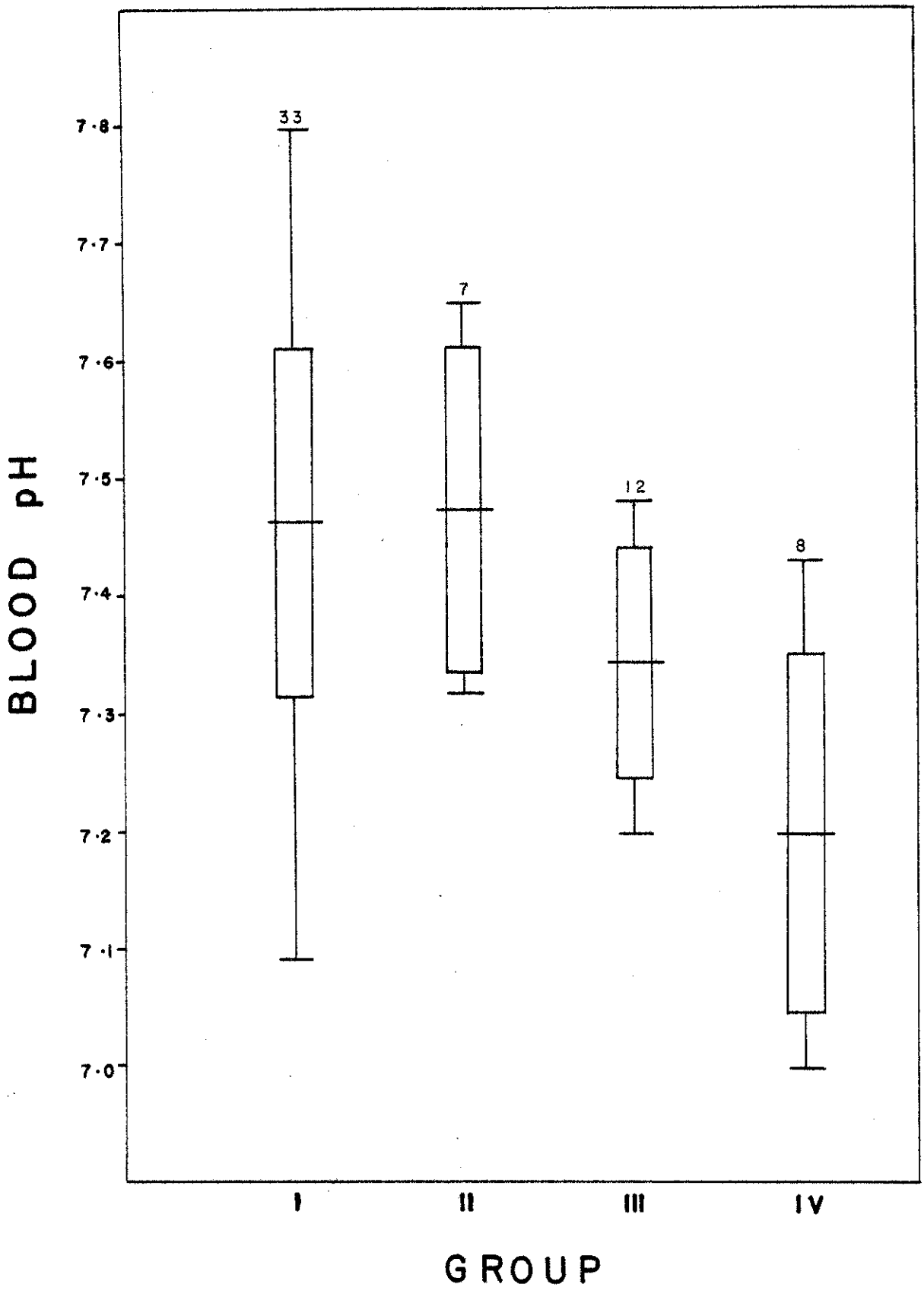

BLOOD pH
7·8
7·7
7·6
7·5
7·4
7·3
7·2
7·1
7·0
33
7
12
8
I
II
III
IV
GROUP

Figure 8--Distribution of fish in shallow (A and B) and steep C and D) pH gradients. Each bar represents one isovolumetric section of chamber.

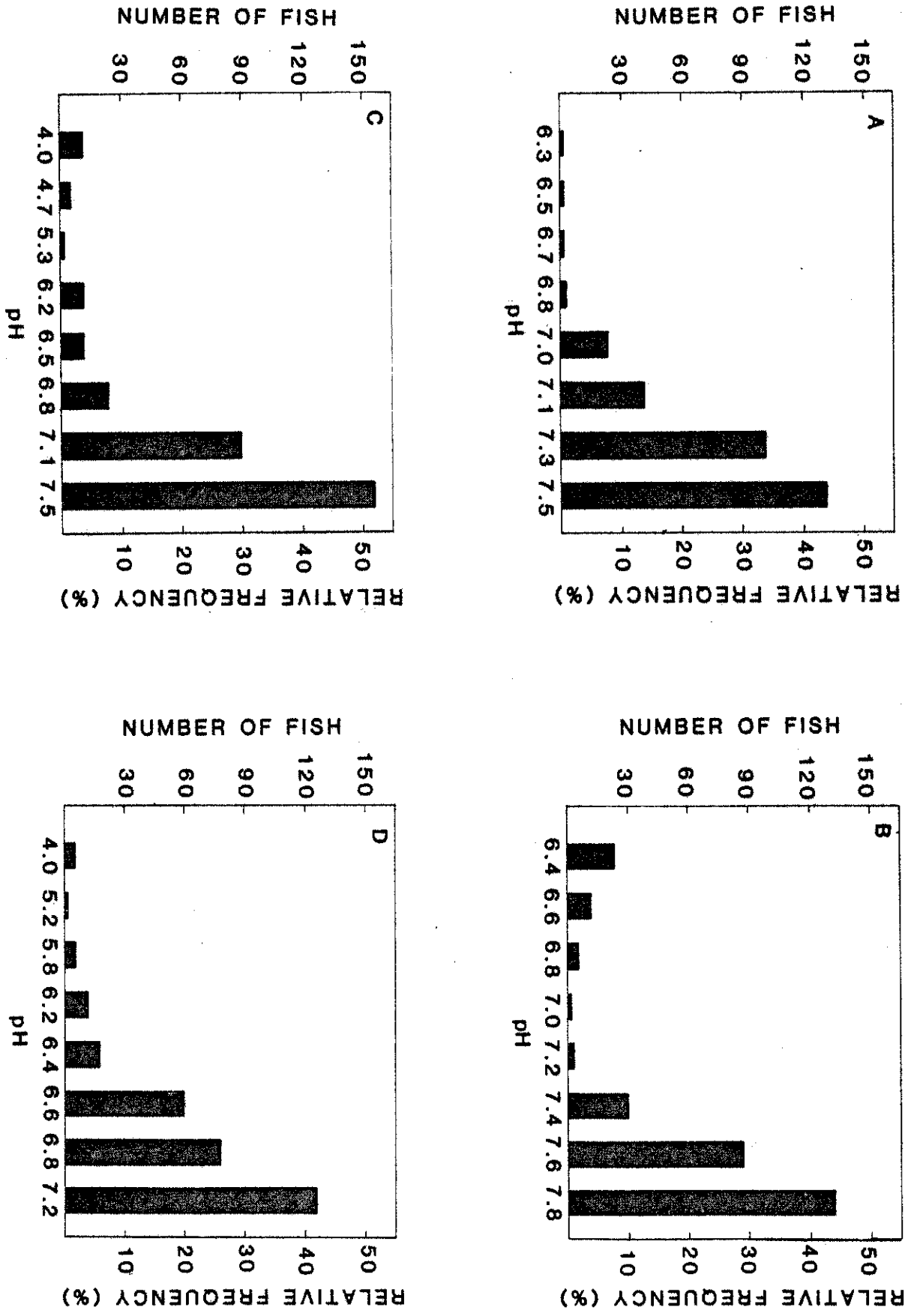

A
NUMBER OF FISH
30
60
90
120
150
6.3 6.5 6.7 6.8 7.0 7.1 7.3 7.5
pH
RELATIVE FREQUENCY (%)
10
20
30
40
50
B
NUMBER OF FISH
30
60
90
120
150
6.4 6.6 6.8 7.0 7.2 7.4 7.6 7.8
pH
RELATIVE FREQUENCY (%)
10
20
30
40
50
C
NUMBER OF FISH
30
60
90
120
150
4.0 4.7 5.3 6.2 6.5 6.8 7.1 7.5
pH
RELATIVE FREQUENCY (%)
10
20
30
40
50
D
NUMBER OF FISH
30
60
90
120
150
4.0 5.2 5.8 6.2 6.4 6.6 6.8 7.2
pH
RELATIVE FREQUENCY (%)
10
20
30
40
50

TABLE IV

POSITIONS OF 40 INDIVIDUALLY TESTED BLUEGILL IN A pH GRADIENT RANGING FROM 5.2 TO 7.6

pH	Number of Fish	%
5.5	1	2.5
5.9	1	2.5
6.1	1	2.5
6.4	2	5.0
6.6	1	2.5
6.8	3	7.5
6.9	4	10.0
7.0	4	10.0
7.1	11	27.5
7.2	8	20.0
7.3	3	7.5
7.6	1	2.5

CHAPTER IV

DISCUSSION

The structure of the fish community in 1978-79 can be compared with historical listing of species made by Silvey and Harris in 1947. Yellow bass (Morone chrysops), smallmouth bass (Micropterus dolomieu), and white crappie (Pomoxis annularis) were among the most common species reported by Silvey and Harris (1947), but were absent from my collections. They also listed green sunfish as common, while my samples included only one representative. Only rarely did Lepomis hybrids appear to exhibit any green sunfish phenotypic characteristics, such as the typical cheek coloring or pigmented spot on the anal fin. Most hybrids were warmouth X redear sunfish or bluegill X longear sunfish. Absence of yellow bass and smallmouth bass probably can be attributed to annual stocking of largemouth bass which would compete for resources, while competition by congeneric species could explain elimination of white crappie and green sunfish populations. Silvey and Harris also mentioned that the sucker (Catostomidae) and minnow (Cyprinidae) families were represented by numerous species. The relative absence of these families from my samples (one sucker and two minnow species) is noteworthy. Family Catostomidae is reported to contain the fish species most sensitive to pH, and often is the first to disappear from a lake experiencing a declining pH (Beamish, 1972, 1974; Beamish and Harvey, 1972; Trojnar, 1977) along with some minnows (Almer et al., 1974).

Only occasional grass pickerels, including juveniles, occurred in monthly samples. The closely related pike (Exox lucius) has been shown to be affected by pH 6.8 and lower, with 97% mortality at pH 4.2 (Johansson and Kihlstrom, 1975). Infrequent collection of bowfins, gars, and some catfish species may be partially an outcome of my collection method and not an accurate reflection of their abundance in the lake.

Management practices can influence the community structure of a small impoundment through introductions (both passive and active) and eliminations (both desirable and undesirable species). These factors, coupled with competitive and predatory interactions, make comparison of my data with historical data difficult to interpret relative to effects of a single environmental parameter such as pH (Echelle et al., 1971). If taken as an instantaneous observation, however, the observed low species diversity in Ferndale Lake is consistent with that in other acid lakes (Connell and Orias, 1964; Smith and Frey, 1971; Beamish, 1974).

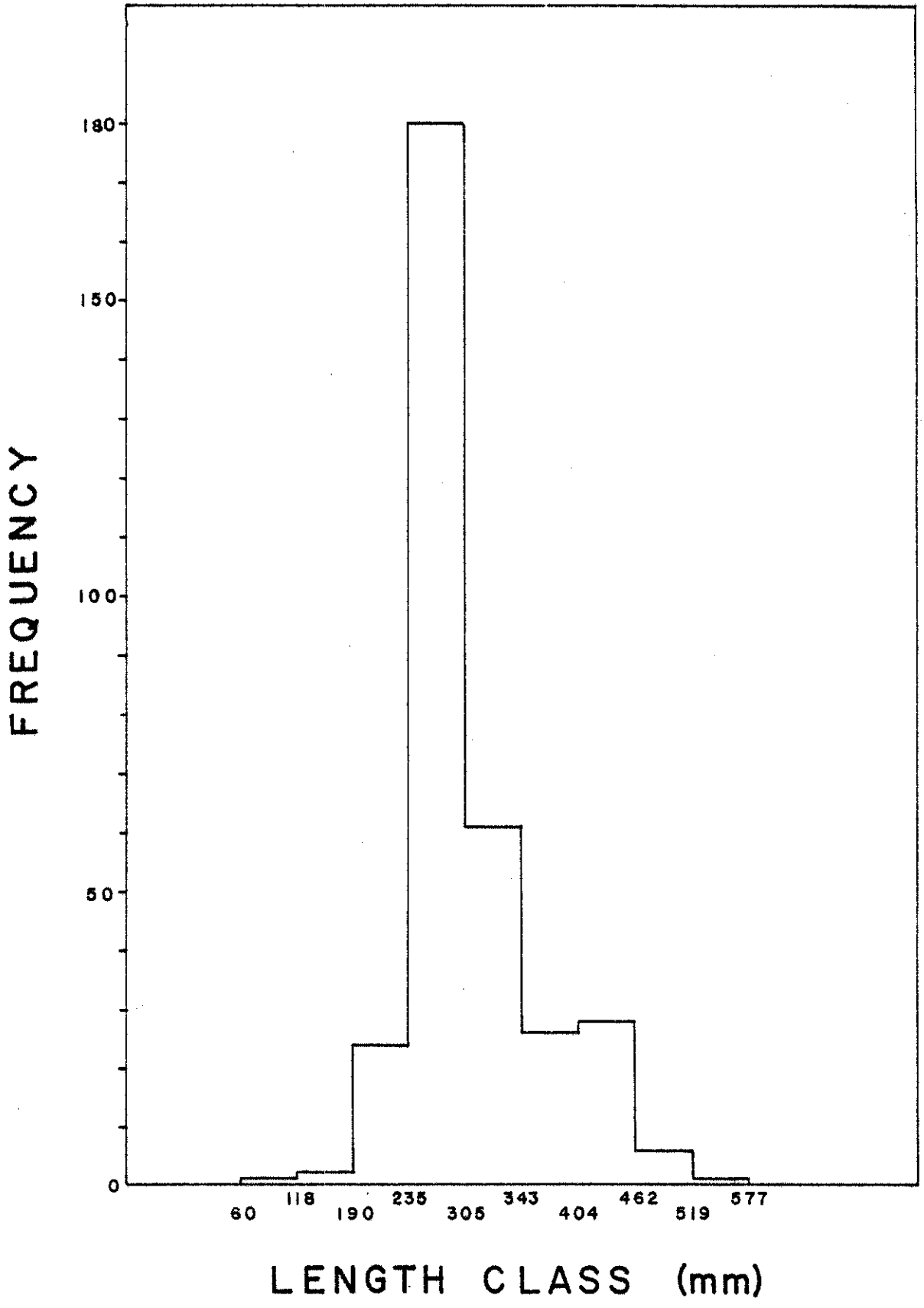
Population density fluctuations occur in any system but are more prevalent in heavily fished small lakes, where condition factors and growth rates may be reduced by selective fishing (Drenner, personal communication; Favro, et al., 1979). My data support the hypothesis that fishing pressure, through 12-in (305 mm) "Keeper size" regulations, influenced the size class distribution in the bass population. The highest density of specimens collected measured just under this limit (Figure 9). In situations such as this, the "excess" of smaller bass resulting from size-selective predation (i.e. returning small individuals) will be under strict competition for food, resulting in a lower mean condition factor for this size class. At the other extreme, the overly

cropped larger bass will have little competion for food, and may use the abundant smaller bass as suitable prey (Paloheimo, 1979). If this hypothesis holds, the larger bass of the population should posess an elevated K. Subjecting my data to a $\underline{t}$ test revealed this trend for bass. Fish under 305 mm had a $K_{TL} \pm$ SD of 1.22 ± 0.15, which was significantly lower than that of bass exceeding 305 mm in total length (1.35 ± 0.17; P 0.001). Nevertheless, the larger bass of Ferndale treated separately still had a much lower mean condition than fish from other impoundments in the Pineywoods region of east Texas (Prentice and Durocher, 1978).

There is little mention of water pH in historical Ferndale data. Silvey and Harris (1947) reported a pH of 5.1, and club records show a wide variation, with a definite decreasing tendency over the past three decades. Condition factors for largemouth bass were significantly higher in Cooper's 1947-48 sampling than in 1979 ($t=3.3$, $p<0.001$), corresponding with the higher pH. His data may also reflect the same trend of size-selective fishing pressure. The bluegill and redear sunfish had a lower mean K than values reported as being representative of this geographical area, while warmouth had a mean K well within reported ranges (Carlander, 1977).

Bluegill and redear sunfish were used to examine stress manifestations on a more interpretable level. Neither of these species is regularly stocked or heavily fished, making them more representative of unexploited populations. Gonadalsomatic indices (GSI) are useful and widely published for fish as a measure of the energy commitment made by a species toward production of gametes (Gunderson, 1980, Carlander, 1977). Both of these sunfish species are reported to spawn throughout the spring

Figure 9--Length class distribution of 327 largemouth bass tagged in Ferndale Lake, 1978-1979.


FREQUENCY
180
150
100
50
0
60
118
190
235
305
343
404
462
519
577
LENGTH CLASS (mm)

and summer in southern states (Carlander, 1977) and in Ferndale lake during 1948 (Estes, 1949), when the pH was higher. Generally, early and late spawns produce fewer surviving individuals than spawns in mid-summer (Carlander, 1977). The shorter spawning period evident from my data can be interpreted to be an example of "bet hedging," in which the highest reproductive effort is put into the most optimal period for survival of hatched eggs and fry (Pianka, 1978). In a stressed population it would be energetically conservative to reproduce at a time so as to buffer offspring from seasonal trauma. The overall mean bluegill GSI for the period sampled was lower in the 1979 study than in the 1948 study; however, the mean GSI was significantly higher in my study during mid-summer months (t test; $P < 0.001$). Gunderson (1980) showed that a positive correlation exists between GSI and the natural rate of mortality (M). Considering my data in light of such a correlation, it could be concluded that in months during which GSI is highest, M is also highest. For small southwestern lakes, midsummer is accompanied by maximum water temperatures and minimum dissolved oxygen levels. Additional stress from an acid environment could push M and GSI higher during this period.

Fish in Ferndale Lake are chronically exposed to levels of pH that cause mortalities in experimental situations. Only one minor fish kill, following surface freezing of a large portion of the lake, was seen or reported for the study period. Levels of pH in the lake were depressed to levels experimentally determined to result in high mortality rates. Bluegill collected from the lake when its pH level was 4.2 exhibited 100% survival at exposure levels of pH 3.8 through 4.2 for 96 hr. In previous testing when lake pH was 4.6, exposure to pH 3.8 resulted

in 80% mortality. These data seem to agree with evidence given by McWilliams (1980) for ability of brown trout (Salmo trutta) to acclimate to low pH, despite reports to the contrary by other investigators for other trout species (Robinson, et al., 1976; Falk and Dunson, 1977; Mount, 1977, Daye, 1980). In any respect, the progressive decline in pH likely eliminated less tolerant individuals of the population. A continual dying-off or stressing of any degree could go unnoticed in a small lake with a large turtle population, as is present in Ferndale Lake (Prete and Glidewell, unpublished data), due to heavy predation by the turtles on the stressed or dead fish. Testing of largemouth bass having no history of acid exposure revealed that at least this group of fish was relatively tolerant of low pH conditions.

The response curves (Figures 5 and 6) generated from lethality tests are useful predictors of resistance times of fish to various levels of acid exposure. This information would be useful in systems which receive acid spills or other intermittent inflows of acid water, even if well buffered, by allowing a prediction of the mortality rate during the recovery period for the system (Warren, 1971).

The mechanisms responsible for death from acute acid exposure are still uncertain. My data from blood pH measurements of low pH stressed and control bluegill would indicate that for acute exposures, failure of the blood buffering capabilities is not the cause of death. Even fish that were severely stressed maintained their blood pH at levels that were not significantly different from fish that were not stressed.

Fish stressed during tests and a small percentage (ca. 2%) among monthly samples had excess secretion of surficial mucus. Large quantities

of mucus were evident, especially on eyes, around the gills, and along lateral line canals and fins. This could affect the fish by indirectly causing its death through reduced predator-avoidance capabilities (Daye and Garside, 1976; Major, 1979). Gill lamellae, the site of gas exchange, could become blocked by mucus, resulting in respiratory failure and, ultimately, hypoxic death (Ultsch and Gros, 1979; Fromm, 1980). Even in sublethal exposure, energy required to balance hypertrophic mucosal cells could result in reduced growth and/or reproduction, and hence adversely affect the species' success in the community. In any respect, the most sensitive species would disappear in relatively few generations of intermittent acute exposures (Beamish, 1974). Moderately tolerant populations could be expected to reflect the progressive stress from chronic exposure in their population structure or their condition coefficients. Only the most tolerant individuals would contribute to the next generation. A practical method of rearing super-tolerant strains of sport and forage fishes for stocking lakes susceptible to acidic conditions would be of great benefit (Robinson, et al., 1976).

Avoidance by bluegill of pH less than 7.0 suggests that even relatively minor depression in pH from neutrality, either directly or through parallel alteration of other aspects of the water chemistry, elicits active behavioral avoidance as a first line of defense. Bluegill avoidance levels agree closely with those reported by Matthews (1977) for red shiners (Notropis lutrensis) tested in a similar apparatus, but indicate a more sensitive response than Jones (1964) reported for sticklebacks (Gasterosteus aculeatus).

Individually tested fish positioned themselves in water with pH levels well above that available in the lake, and simultaneously coincident with the optimal pH of enzyme activity (Winer and Schwert, 1958; Hazel, et al., 1978). Assuming that behavioral preference/avoidance responses to pH are linked to physiological optima, as has been demonstrated for temperature in fish by Brett (1972) and Beitinger and Fitzpatrick (1979), my data would suggest that pH levels selected by fish would be those promoting maximum efficiency of enzyme pathways (Hochachka and Lewis, 1971). The energy loss from hypercatabolic conditions would be channeled away from other functions. Or, if more optimal pH microhabitats are available, an energetic cost would be assumed in location and defense of such preferred habitat. Again, the energetic expenditure would necessarily be deducted from some other budget, to the detriment of the individual, or on a long-term basis, the population.

Regardless of the level approached by an investigator to study cause-and-effect relationships between environmental stresses and exposed biota, ultimately it must be realized that effects perceived at one level are transferred to and from more basic and more complex organizational levels. This is to say that if energy is expended by an individual organism through active behavioral responses or passive physiological processes, this energy is a part of the fixed energy available to that organism, and once channeled to an end, is no longer available for any other process. Stresses, therefore, while not altering the net energy flow in a closed ecosystem must definitely alter energy

relationships and apportionment to biotic and abiotic sectors, leading to an interpreted decline in that ecosystem.

My study is to some degree a pilot study encompassing a broad range of fisheries biology and fish physiology, as related to pH as a stress factor. I hope that through the literature review and baseline data provided herein it will be obvious that many unanswered questions have emanated from the focal point of this problem, with Ferndale Lake providing an ideal study site for further investigations.

Analyses of community structure in Ferndale are only superficial and largely qualitative at this time. A more quantitive assessment of the fish community, including predatory, competitive, and parasitic interactions, would give a good overall picture of chronic stress effects. Indices were used to evaluate the physical condition and reproductive effort of a few fish species. Other approaches might prove more accurate in providing a measure of these population characteristics. Direct growth measurements could be utilized to gauge chronic stress, and would be feasible in controlled laboratory experiments, using in situ growth chambers and with additional tagging efforts. Reproductive effort could be approached at a more basic energetic level, employing calorimetric techniques and comparative sampling from non-perturbed impoundments. Perhaps the largest vacuum lies with physiological processes involved in maintenance of blood pH, as well as mechanistic analyses of the failure of other processes. Importance of pH relative to other environmental factors could be determined by modifying the basic testing apparatus to allow for measuring the magnitude of influence from multiple simultaneous factors. I have mentioned only a subsample of the questions which have made themselves

obvious to me in attempting to keep within the confines of fish biology. The number of questions lying just beneath the surface of a system such as Ferndale is overwhelming.

APPENDIX I

VALUES OF pH AT THREE ROUTINE SAMPLING STATIONS WITH LAKEWIDE MINIMUM AND MAXIMUM pH DURING STUDY PERIOD

Date	pH					T(°C)
	Sta* 1	2	3	Min	Max	
20 IX 78	3.9	3.8	3.6	3.6	4.3	28-30
10 XI 78	4.7	4.5	4.7	4.5	5.3	14-19
13 XII 78	4.6	4.6	4.5	4.5	4.7	6-9
15 I 79	4.4	4.3	4.3	4.3	4.5	5-9
14 II 79	4.2	4.0	3.9	3.6	4.3	10-16
1 III 79	4.3	4.2	4.2	3.8	4.3	10-12
5 IV 79	4.1	4.1	4.0	4.0	4.1	15-17
7 V 79	4.1	4.2	4.3	4.1	4.3	22-23
24 V 79	4.2	4.2	4.2	4.2	5.0	24-25
30 V 79	4.2	4.2	4.2	4.2	4.2	23-24
4 VI 79	4.3	4.3	4.3	4.3	4.3	26-29
20 VI 79	4.3	4.2	4.2	4.2	4.3	30-32
29 VI 79	4.3	4.3	4.3	4.3	4.3	30-32
27 VII 79	4.2	4.1	4.0	3.7	4.2	27-29
2 VIII 79	4.0	3.9	3.9	3.5	4.0	29-30
20 VIII 79	4.1	3.9	3.8	3.8	4.1	32-33
27 IX 79	4.5	4.3	4.6	4.2	4.8	28-30

*see APPENDIX III for location of sampling stations

APPENDIX II

TAGGED FERNDALE LARGEMOUTH BASS, *MICROPTERUS SALMOIDES*
NOVEMBER 1978 THROUGH SEPTEMBER 1979
TAGS NUMBERED (PHIN) 0-338

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
00	445	1340	1.52
01	423	1120	1.48
02	450	1400	1.54
03	370	720	1.42
04	412	960	1.37
05	315	428	1.37
06	294	340	1.34
07	282	260	1.16
08	310	348	1.17
09	324	460	1.35
10	304	380	1.35
11	277	250	1.18
12	309	400	1.36
13	296	312	1.20
14	269	260	1.34
15	264	220	1.20
16	309	402	1.36
17	244	194	1.34
18	271	232	1.17
19	450	1350	1.48
20	420	1010	1.36
21	393	860	1.42
22	327	420	1.20
23	303	350	1.26
24	332	515	1.41
25	314	455	1.47
26	285	315	1.36
27	295	365	1.42
28	337	515	1.35
29	324	403	1.18
30	275	270	1.30
31	279	277	1.28
32	295	345	1.34
33	280	300	1.37

APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
34	278	268	1.25
35	284	312	1.36
36	299	350	1.31
37	-	-	-
38	-	-	-
39	-	-	-
40	-	-	-
41	-	-	-
42	260	275	
43	278	300	1.40
44	278	307	1.43
45	260	268	1.52
46	-	-	-
47	-	-	-
48	-	-	-
49	-	-	-
50	285	312	1.35
51	341	540	1.36
52	233	130	1.03
53	273	241	1.18
54	333	495	1.34
55	280	280	1.28
56	275	235	1.13
57	350	560	1.31
58	306	416	1.45
59	290	320	1.31
60	282	270	1.20
61	300	358	1.33
62	285	319	1.38
63	285	317	1.37
64	290	327	1.34
65	280	269	1.23
66	288	285	1.19
67	315	400	1.28
68	309	415	1.41
69	287	324	1.37
70	304	350	1.25
71	267	210	1.10
72	286	290	1.24
73	442	1310	1.52
74	430	1060	1.33
75	330	490	1.36

APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
76	287	287	1.21
77	-	-	-
78	475	1695	1.58
79	312	418	1.38
80	295	316	1.23
81	360	632	1.35
82	338	520	1.35
83	275	240	1.15
84	290	309	1.27
85	300	380	1.41
86	290	284	1.16
87	275	268	1.29
88	300	305	1.13
89	290	330	1.35
90	297	305	1.16
91	270	265	1.35
92	440	1330	1.56
93	460	1490	1.53
94	460	1580	1.62
95	460	1485	1.53
96	475	1785	1.67
97	255	204	1.23
98	292	308	1.24
99	355	605	1.35
100	336	463	1.22
101	-	-	-
102	498	2160	1.75
103	425	1290	1.68
104	355	728	1.62
105	288	292	1.22
106	330	442	1.23
107	232	200	1.60
108	269	244	1.25
109	270	240	1.22
110	300	324	1.20
111	263	235	1.29
112	255	210	1.27
113	262	228	1.27
114	282	269	1.20
115	250	175	1.12
116	296	275	1.06
117	338	512	1.32

APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
118	309	284	0.96
119	284	288	1.76
120	420	1100	1.48
121	280	310	1.41
122	380	462	0.84
123	285	282	1.22
124	290	303	1.24
125	280	275	1.25
126	300	342	1.26
127	295	355	1.38
128	287	260	1.10
129	304	370	1.32
130	310	365	1.23
131	318	435	1.35
132	290	278	1.14
133	288	200	1.25
134	215	100	1.00
135	231	130	1.05
136	300	300	1.11
137	325	445	1.30
138	226	140	1.21
139	265	215	1.15
140	-	-	-
141	296	320	1.23
142	368	668	1.34
143	351	588	1.36
144	418	1150	1.57
145	305	410	1.45
146	316	408	1.29
147	298	320	1.21
148	279	340	1.30
149	258	197	1.15
150	292	362	1.45
151	271	258	1.30
152	245	180	1.22
153	283	300	1.32
154	300	323	1.20
155	303	350	1.26
156	266	245	1.30
157	286	282	1.21
158	288	300	1.25
159	330	395	1.20
160	302	326	1.18

APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
161	296	310	1.19
162	294	292	1.15
163	282	260	1.16
164	317	404	1.27
165	282	300	1.34
166	296	245	0.94
167	291	321	1.30
168	377	148	1.40
169	274	255	1.24
170	253	167	1.03
171	315	390	1.25
172	276	222	1.06
173	306	319	1.11
174	300	330	1.22
175	445	1480	1.68
176	310	330	1.11
177	288	289	1.21
178	288	290	1.21
179	432	1000	1.24
180	296	306	1.18
181	425	1005	1.31
182	297	312	1.19
183	278	261	1.21
184	449	1270	1.40
185	265	219	1.18
186	298	305	1.15
187	430	1250	1.57
188	430	1100	1.38
189	306	337	1.18
190	275	250	1.20
191	272	240	1.19
192	515	1670	1.22
193	289	305	1.26
194	285	258	1.11
195	284	284	1.24
196	290	284	1.16
197	300	303	1.12
198	355	553	1.24
199	336	458	1.21
200	362	680	1.43
201	302	355	1.29
202	276	250	1.19

APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
202	276	250	1.19
203	285	239	1.03
204	294	280	1.10
205	280	260	1.18
206	299	300	1.12
207	283	272	1.20
208	268	199	1.03
209	301	331	1.21
210	338	492	1.27
211	283	254	1.12
212	277	268	1.26
213	289	293	1.21
214	299	318	1.19
215	285	260	1.08
216	310	364	1.22
217	310	334	1.12
218	304	314	1.12
219	428	950	1.21
220	444	1110	1.27
221	386	688	1.20
232	433	1002	1.23
223	343	486	1.20
224	361	584	1.24
225	430	884	1.11
226	275	274	1.31
227	290	313	1.28
228	332	448	1.22
229	293	290	1.15
230	287	304	1.28
231	287	287	1.21
232	460	1240	1.27
233	310	345	1.16
234	315	399	1.28
235	285	295	1.27
236	290	250	1.03
237	307	332	1.15
238	281	240	1.08
239	305	342	1.21
240	495	1840	1.52
241	310	372	1.25
242	299	304	1.14
243	367	670	1.36
244	385	755	1.32

APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
245	287	272	1.15
246	233	93	0.73
247	309	347	1.18
249	280	278	1.27
250	284	230	1.00
251	246	180	1.21
252	235	239	1.84
253	461	1589	1.61
254	266	180	0.96
255	192	83	1.17
256	82	68	1.23
257	320	466	1.42
258	310	388	1.30
259	328	424	1.20
260	256	216	1.29
261	268	292	1.52
262	226	136	1.18
263	206	104	1.19
264	199	92	1.17
265	-	-	-
266	341	468	1.18
267	266	220	1.17
268	290	294	1.21
269	217	108	1.06
270	196	99	1.13
271	231	144	1.17
272	222	115	1.05
273	195	76	1.02
274	325	419	1.22
275	332	506	1.38
276	351	588	1.36
277	321	386	1.17
278	348	546	1.29
279	221	124	1.15
280	236	147	1.12
281	185	71	1.12
282	200	91	1.14
283	219	120	1.14
284	277	164	0.77
285	382	983	1.76
286	342	519	1.30
287	348	540	1.28

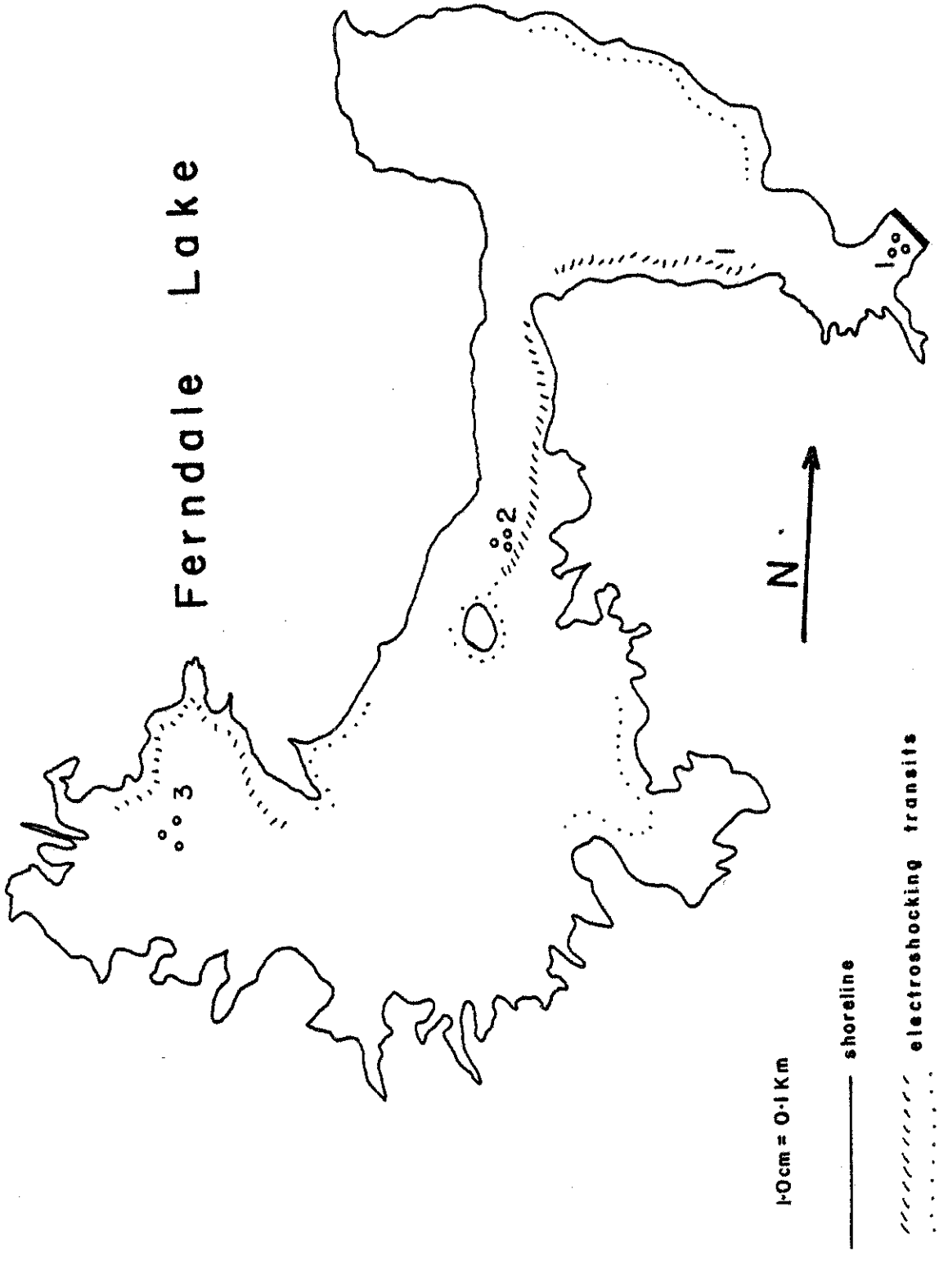
APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
288	310	372	1.25
289	269	224	1.15
290	296	236	0.91
291	303	324	1.16
292	310	359	1.21
293	269	238	1.22
294	420	1100	1.48
295	317	373	1.17
296	441	1180	1.37
297	418	1030	1.41
298	339	531	1.36
299	300	346	1.28
300	300	346	1.28
301	577	1871	0.97
302	375	712	1.35
303	294	358	1.41
304	360	627	1.34
305	345	544	1.32
306	287	262	1.11
307	327	422	1.21
308	296	316	1.21
309	292	312	1.25
310	292	370	1.49
311	290	300	1.23
312	297	334	1.27
313	286	286	1.22
314	507	2200	1.68
315	452	1350	1.46
316	448	1430	1.59
317	295	308	1.70
318	315	375	1.19
319	315	377	1.21
320	477	1720	1.58
321	338	491	1.27
322	328	259	1.30
323	329	417	1.17
324	354	637	1.43
325	366	764	1.56
326	227	143	1.22
327	342	511	1.28
328	422	904	1.20
329	323	457	1.36

APPENDIX II--Continued

Tag #	Length (TL,mm)	Weight (g)	Condition (K_{TL})
330	-	-	-
331	397	950	1.51
332	223	124	1.11
333	224	124	1.10
334	246	152	1.02
335	224	122	1.08
336	242	166	1.17
337	290	270	1.11
338	229	140	1.17

APPENDIX III



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