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PLASMA ION CONCENTRATIONS IN SELECTED FISHES
FROM FOUR NORTH CENTRAL TEXAS RESERVOIRS
WITH DIFFERENT SALINITIES

THESIS

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Mean salinity concentrations in the four reservoirs (Moss, Ray Hubbard, Texoma and Possum Kingdom) ranged from 0.2 ppt in Moss Lake to 2.01 ppt in Possum Kingdom Lake. Reservoir sodium and chloride concentrations were hypotonic to hypertonic to plasma levels in all species. Interspecific differences were seen in ionic concentrations within each reservoir. Total osmotic and sodium concentrations in carp, Cyprinus carpio, were correlated to their concentrations in the reservoirs. No such relationship was noted for chloride, potassium and calcium. A laboratory study indicated that fish collection by electroshock did not bias plasma ion concentrations. Exposures to wide variations in ionic concentrations did not appear to induce stress in the species studied.

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CHAPTER I

INTRODUCTION

Little research has addressed the relationship between ambient salinity and blood chemistry of freshwater fishes. Aside from temperature, variation in concentration of dissolved materials is the most widespread stressor encountered by aquatic animals, according to Gordon (1982). Salt concentrations measured in nature range from 0 parts per thousand (ppt) in fresh snow melt to 250 ppt in Salt Creek, Texas (Table 1). Average sea water contains about 35 ppt salt with sodium and chloride constituting approximately 42 percent and 48 percent, respectively, of the total. It is not possible to make a clear distinction between sea water and fresh water. However, Wetzel (1975) generally considers fresh water to contain less than 2 ppt salt.

Sea water is hyperosmotic and fresh water is hyposmotic to teleost fishes. The ranges of internal osmotic concentrations of fishes (Table 2) is far less than ranges present in aquatic environments. This implies that regulation of internal ionic and osmotic concentrations is mandatory for survival in nonisosmotic conditions.

Regulation of osmotic and ionic concentrations in fishes is accomplished at three sites: gills, gut, and kidneys. The

Table 1. Osmotic concentrations (mOsm) and salinities (ppt) of selected bodies of water.

	Osmotic Concentration	Salinity
Fresh Snow Melt	0	0
"Average River Water"	1.00	0.035
Moss Lake, Texas	3.20	0.113
Lake Ray Hubbard, Texas	3.78	0.132
Lake Texoma, Texas	48.60	1.700
Possum Kingdom Lake, Texas	57.40	2.010
"Standard" Ocean	1000.00	35.000
Little Manitou Lake, Canada	2000.00	70.000
Great Salt Lake, Utah	6000.00	210.000
Salt Creek, Texas	7100.00	250.000

Source: Gordon (1982), U.S.G.S. (1979)

Table 2. Typical total plasma osmotic pressures, sodium and chloride concentrations in various fishes (Gordon 1982).

Species	Osmotic Pressure mOsm	Na ⁺		Cl ⁻	
		mmol l ⁻¹	%Δ	mmol l ⁻¹	%Δ
Lamprey (<u>Lampetra fluviatilis</u>)	245	110	45	95	39
Channel Catfish (<u>Ictalurus punctatus</u>)	285	128	45	108	38
Carp (<u>Cyprinus carpio</u>)	290	130	45	108	37
Largemouth Bass (<u>Micropterus salmoides</u>)	300	140	47	109	36
Yellow Perch (<u>Perca fluviatilis</u>)	294	154	52	120	39
Freshwater Shark (<u>Carcharinus leucas</u>)	650	245	38	220	35
Hagfish (<u>Eptatretus stouti</u>)	950	520	50	500	48
Marine Shark (<u>Squalus acanthias</u>)	1020	290	28	240	24

gradients for sodium and chloride between freshwater teleost plasma and most freshwater environments is typically 130 to 170 millimolar (mmol) internally to 0.05 to 0.1 mmol externally (Hutchinson 1957; Gordon 1982). The permeable gills and to a lesser extent the pharyngeal spaces, permit continuous osmotic entry of water into the body fluids and continuous outward diffusion of solutes. Water uptake is completely compensated by the kidneys, which in freshwater fishes produce large volumes of dilute urine. Although the urine contains low concentrations of sodium and chloride (<10 mmol), substantial quantities of salt are lost since large volumes of fluid are voided. Freshwater fishes drink little or no water at all (Conte 1969).

The continuous loss of ions is compensated by small amounts of ions from food sources and active uptake mechanisms in the gills. Food probably plays a limited role in ion uptake since most fishes do not eat for extended periods of time during winter, and some freshwater lampreys do not feed at all as adults. Compensation for salt losses is achieved primarily in the gills. Large granular acidophilic cells in fish gills, chloride cells, recently have been shown to be the salt secretory cells in teleosts (Foskett and Sheffey 1982).

These compensatory osmoregulatory mechanisms appear to be efficient enough to allow freshwater fishes of the same

species to thrive in waters with different salinities without adverse effects on plasma ionic and osmotic concentrations. To test this hypothesis, fish species from four Texas reservoirs were compared. The relationships between plasma ionic and osmotic concentrations and the concentrations of these components in the environment were a focus of this study. Four freshwater fish species were compared: largemouth bass (Micropterus salmoides), carp (Cyprinus carpio), channel catfish (Ictalurus punctatus) and flathead catfish (Pylodictus olivarius). These species were selected due to their economic and sport value and the variable responses to salinity that have been reported in previous research. Norton and Davis (1977) noted significantly elevated ($p < 0.05$) plasma chloride and sodium concentrations after transferring freshwater acclimated channel catfish into 7.6 ppt salt water. The ion concentrations decreased significantly when catfish were returned to fresh water. Also these researchers found no significant changes in plasma osmotic concentration in channel catfish over a range of concentrations up to isosmotic.

In field measurements, Natchin and Lavrova (1974) reported that plasma sodium concentrations in carp increased as a function of the external sodium concentrations. They also observed significant differences in plasma sodium, calcium, potassium and magnesium in fishes of different families inhabiting the same body of water. These authors found no

correlation between potassium, calcium and magnesium in fish plasma and the concentrations of these ions in the environment. To test the hypothesis that fishes of different species within the same reservoir have different ionic and osmotic concentrations in their plasma, fishes captured in the field were compared by species within each reservoir.

The degree to which reservoirs might affect plasma ionic and osmotic concentrations in fishes is variable. Substrate dominated Texas reservoirs, such as Possum Kingdom Lake in Palo Pinto County and Lake Texoma in Grayson County, are characterized by high levels of dissolved salts in the water. Salt Creek, a tributary of the Brazos River, drains salt deposits in west Texas and contributes waters to the Brazos River with salinities as high as 250 ppt (U.S.G.S. 1979). Most dissolved materials in surface waters consist mainly of carbonates, sulphates and chlorides of calcium, magnesium, sodium and potassium. Concentrations of these components vary among lakes and reservoirs on temporal and spatial bases. The concentrations of four major cations, Na^+ , K^+ , Ca^{2+} and Mg^{2+} , and four major anions, Cl^- , SO_4^{2-} , CO_3^{2-} and HCO_3^- , usually constitute the total salinity of most fresh waters (Wetzel 1975). These observations are supported by Possum Kingdom Lake and Lake Texoma and two low salinity reservoirs, Lake Ray Hubbard and Moss Lake, which were also selected for this research (U.S.G.S. 1979). Specific ionic and osmotic

concentrations of these four reservoirs were measured during fish sampling to test the hypothesis that differences in salinity existed.

In addition, a laboratory experiment was designed to test the hypothesis that the effects of my fish capture method (electroshock) had no significant effects on the blood chemistry of channel catfish. Capture methods such as angling, seining and netting have been reported to be osmotically stressful in Salmonids (Wedemeyer 1972; Schreck et al. 1976) and Esocids (Soivio and Oikari 1976). However most research with electroshock has addressed non-osmotic consequences (Bouck and Ball 1978; Burns and Lantz 1978). Schreck et al. (1976) reported no changes in plasma calcium and magnesium concentrations in rainbow trout (Salmo gairdneri). Nevertheless Madden and Houston (1976) found exposure to A.C. electroshocking increased plasma chloride and decreased potassium in the same species.

To reiterate, the following major hypotheses were tested in this research:

1. The four reservoirs (Moss Lake, Lake Ray Hubbard, Lake Texoma and Possum Kingdom Lake) have differences in their ionic and osmotic concentrations.

2. There are no differences in plasma ionic and osmotic concentrations of the four test species (largemouth bass, carp, channel catfish and flathead catfish) from reservoirs with highly different salinities.

3. Different fish species residing within the same reservoir have different plasma ionic and osmotic concentrations.

4. Use of electroshock for fish capture does not significantly affect plasma ionic and osmotic concentrations of channel catfish.

CHAPTER II

MATERIALS AND METHODS

Study Sites

Four reservoirs located in north central Texas were sampled: Moss Lake, Cooke County, a 455 ha impoundment of Fish Creek; Lake Ray Hubbard, Dallas and Rockwall Counties, a 9205 ha impoundment of the East Fork Trinity River; Lake Texoma, Grayson County, a 36,017 ha impoundment of the Red River; and Possum Kingdom Lake, Palo Pinto County, a 7163 ha impoundment of the Brazos River (Figure 1). Moss Lake and Lake Ray Hubbard have low salinities (<0.2 ppt, U.S.G.S. 1979) and were selected as "control" reservoirs (Figure 2). Lake Texoma and Possum Kingdom Lake were selected because their salinities are higher than most freshwater reservoirs (>2 ppt, U.S.G.S. 1979).

All fishes were collected in shallow areas (<3 m). Collections along the rock dams of Moss Lake and Lake Ray Hubbard during night were most successful. Collections at Lake Texoma were made in coves immediately west of the dam on the south shore (Figure 3). Collections from Possum Kingdom Lake were made in coves on the south shore near the dam (Figure 3).

Figure 1. Counties in North Central Texas where the four study reservoirs are located.

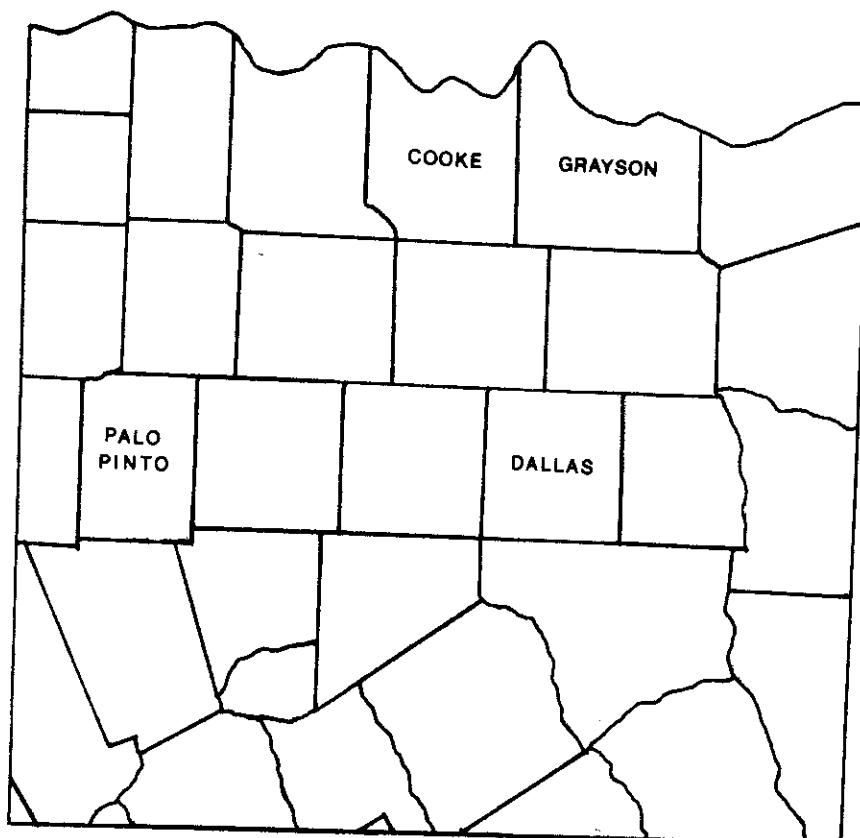
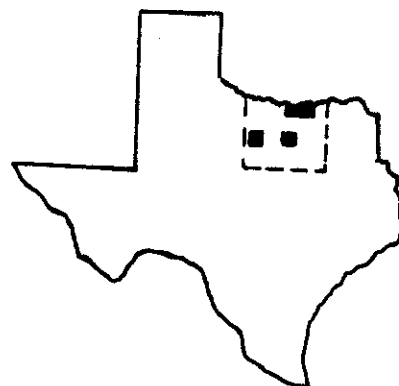
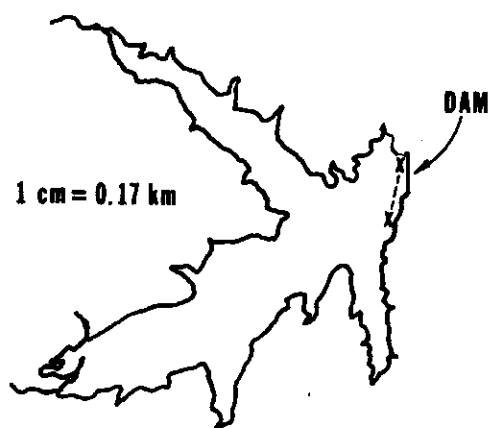
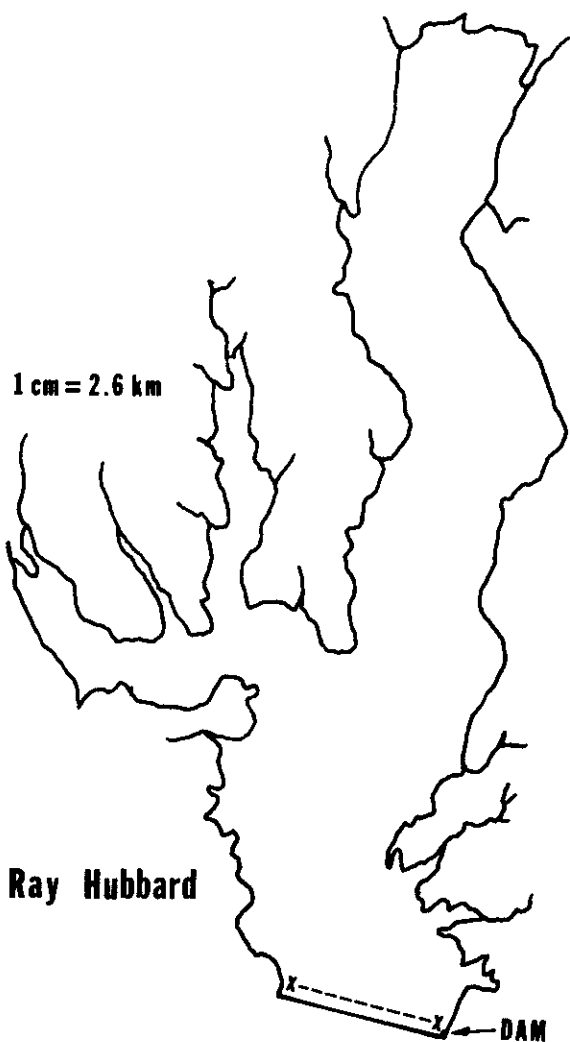


Figure 2. Moss Lake, Cooke County, Texas and Lake Ray Hubbard, Dallas and Rockwall Counties, Texas. Fish collections were made at locations denoted by x----x.

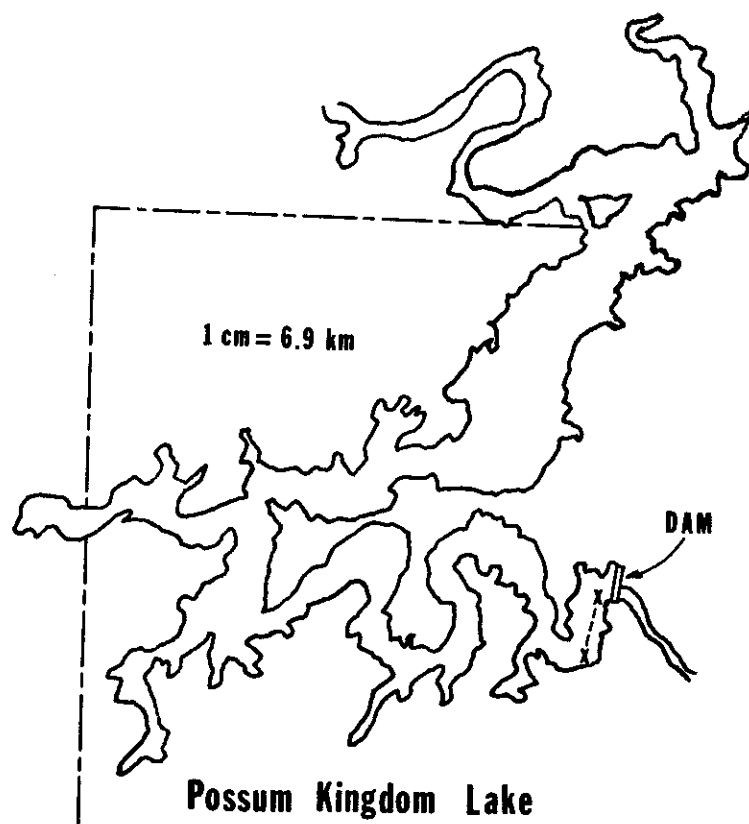
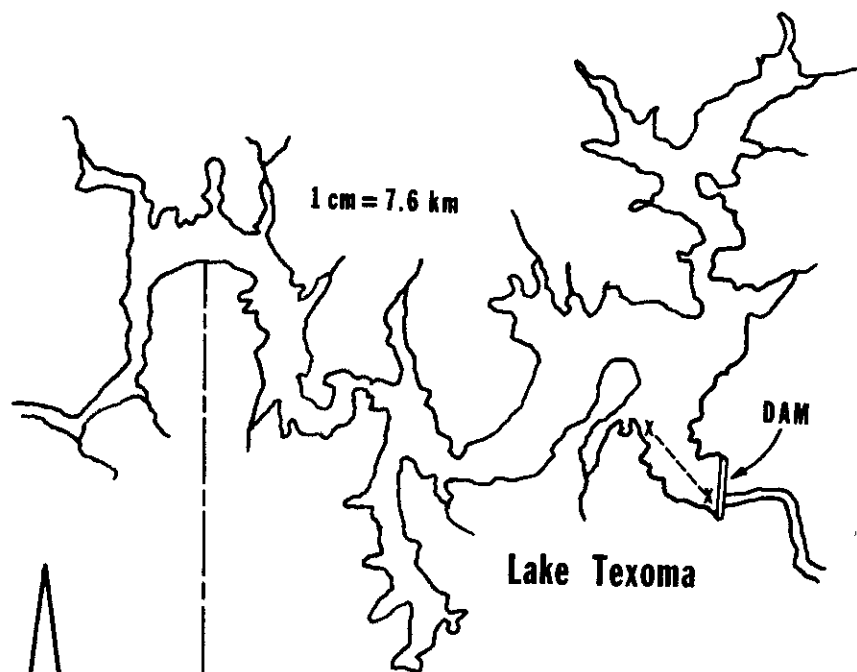


Moss Lake



Lake Ray Hubbard

Figure 3. Lake Texoma, Grayson County, Texas and
Possum Kingdom Lake, Palo Pinto County, Texas. Fish
collections were made at locations denoted by x----x.



Study Species

Three of the four species have considerable economic and sport fishing value. Species were also selected owing to their abundance, ease of capture and large size. Since a 4 ml blood sample was needed for analyses, fishes greater than 300 g body weight were required. Four species met these requirements: carp (Cyprinus carpio), largemouth bass (Micropterus salmoides), channel catfish (Ictalurus punctatus) and flathead catfish (Pylodictus olivarius). Fishes were collected from June to December 1978.

Collection Methods

A boat-mounted Coffelt Electronics Model RF-10 electroshocker was employed to collect fishes. This model was designed for use in water with conductivities of 50 to 5000 umhos cm^{-1} . Pulsed direct current (60 cycles sec^{-1}) was maintained at 250 volts and amperage was adjusted in high water conductivities by decreasing electrode surface area with electrical tape. Currents ranged from 3 to 8 amps. These low currents provide for high dispersion of the electrical field in the water which increases the size of the area in which a fish will be stunned.

Three water samples were taken just under the surface throughout each collection site. Salinity (± 0.1 ppt), temperature ($\pm 0.1^\circ\text{C}$) and conductivity (± 10 umhos cm^{-1}) at the sample locations were determined with a Yellow Springs Instrument Co.

Model 33 salinity-conductivity-temperature meter. Salinity, freezing point depression (Δ), conductivity and osmotic concentration are reported frequently throughout this text and their relationships are presented in Table 3.

Sampling Protocol

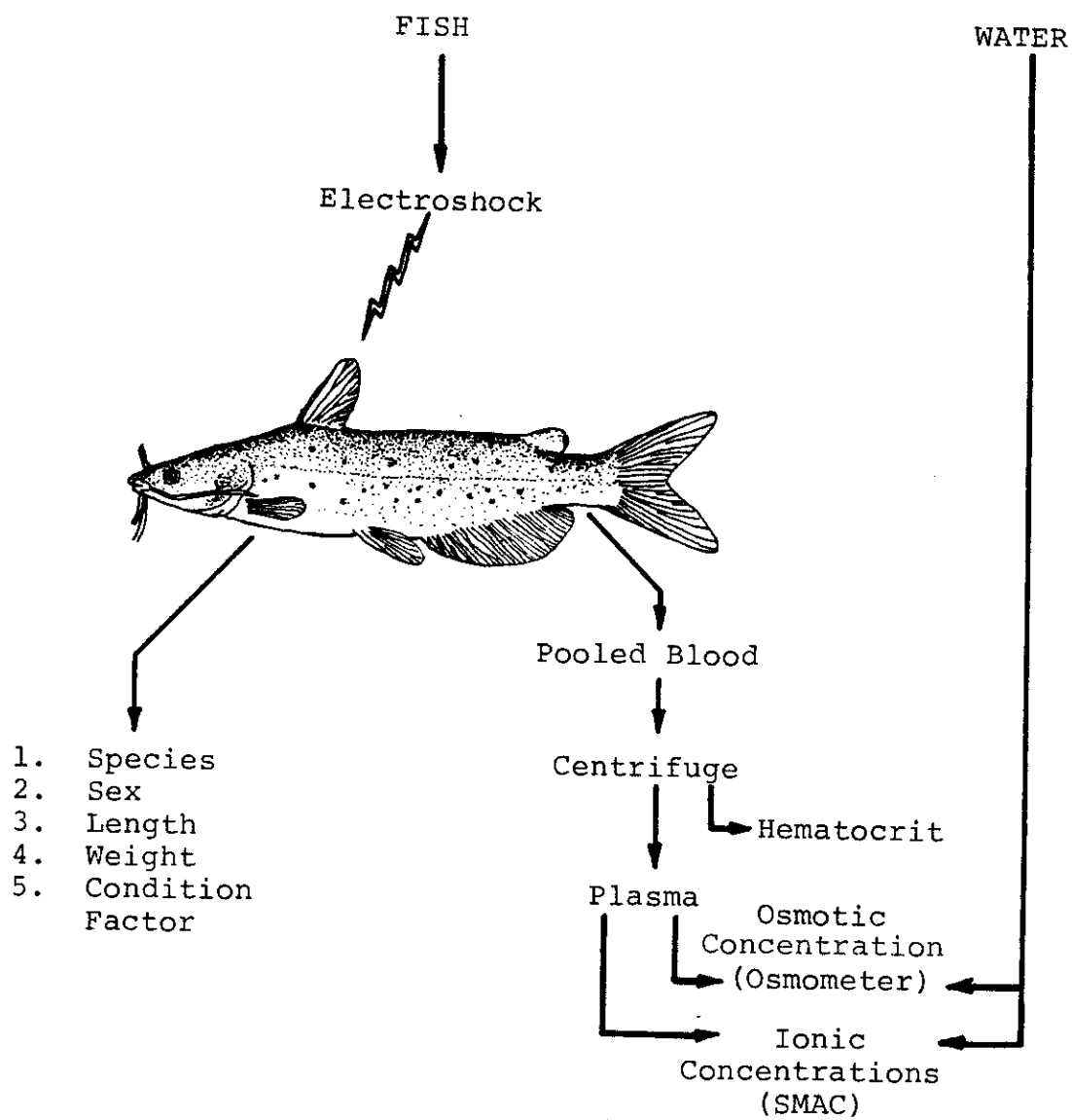
Following capture, fishes were held in a circulating boat live well. On shore, they were placed in aerated 200 l fiberglass containers prior to blood withdrawal. A heparinized 12 ml syringe with an 18 gauge hypodermic needle was inserted into the caudal peduncle immediately posterior to the anal fin. The needle was moved back and forth against the hemal arch to rupture the caudal vein and cause blood to pool. This technique provided adequate blood withdrawal with little erythrocyte hemolysis. A heparinized 1.5 mm x 80 mm microhematocrit tube was filled from the syringe. Jones and Pearson (1976) found significant variations in hematocrits taken from successively drawn blood samples. Therefore my hematocrits were taken from a mixture of the entire blood sample. Microhematocrit tubes were centrifuged at 11,500 rpm for 5 min in the field and read to the nearest percent with a Crit card (Figure 4). The remainder of the blood was centrifuged at 4200 rpm for 5 min. The plasma was removed with Pasteur pipettes, placed in numbered one dram vials, chilled and returned to the laboratory.

Table 3. Equivalencies among four commonly used measures of salinity (Δ = freezing point depression).

Δ	Salinity	Conductivity ($\mu\text{mho cm}^{-1}$)	Osmotic Conc. (mOsm)
0.000	0.00	0	0
0.028	0.52	800	15
0.062	1.14	1700	33
0.121	2.25	3300	65
0.358	6.65	9800	192
0.770	14.33	20400	414
0.948	17.62	24600	509
1.860	34.62	45500	1000

Source: Weast (1982)

Figure 4. Protocol for data acquisition from fishes and water.



Fishes were tagged, placed in ice and returned to the laboratory where standard length, weight and sex were determined. Condition factors were calculated as:

$$K_{SL} = \frac{W(g) \times 10^5}{L^3(mm)}$$

where W = weight in grams and L = standard length in millimeters. Since external sexual dimorphism is not apparent in the four species studied, gender was determined by gonad inspection.

The blood plasma's osmotic concentration (mOsm) was determined on a 0.2 ml sample (± 5 mOsm) the day following collection with a Precision Systems model 2007 osmometer. Finally a 0.6 ml plasma sample was analyzed with a Sequential Multiple Analyzing Computer (SMAC) blood autoanalyzer at Ford Medical Laboratories in Denton, Texas. The variables quantified and sensitivity ranges of the SMAC are listed in Table 4. The precision of the SMAC tests was checked by dividing large samples and analyzing them separately. Use of the SMAC autoanalyzer for plasma analysis in fishes facilitates studies of this type since analysis time and costs are greatly reduced.

Specific ions and osmotic concentration of the water samples were determined with the SMAC autoanalyzer and the osmometer, respectively.

Table 4. The detection limits and precision of variables measured by the SMAC autoanalyzer.

Parameter	Range	Precision
*Sodium	0-160 meq l ⁻¹	± 0.1 meq l ⁻¹
*Chloride	0-130 meq l ⁻¹	± 0.1 meq l ⁻¹
*Potassium	0-10 meq l ⁻¹	± 0.1 meq l ⁻¹
*Calcium	0-15 meq l ⁻¹	± 0.1 meq l ⁻¹
Electrolyte Balance
Phosphorus	0-60 meq l ⁻¹	± 0.1 meq l ⁻¹
Cholesterol	0-5000 mg l ⁻¹	± 1 mg l ⁻¹
Creatinine	0-12 mg l ⁻¹	± 0.1 mg l ⁻¹
Glucose	0-600 mg l ⁻¹	± 1 mg l ⁻¹
Iron	0-50 µg l ⁻¹	± 1 µg l ⁻¹
Total Protein	0-10 g l ⁻¹	± 0.1 g l ⁻¹
Triglycerides	0-4000 mg l ⁻¹	± 1 mg l ⁻¹
Uric Acid	0-100 mg l ⁻¹	± 0.1 mg l ⁻¹
LDL Cholesterol	0-4000 mg l ⁻¹	± 1 mg l ⁻¹
Albumin	0-20 gl ⁻¹	± 0.5 g l ⁻¹
Globulin	0-20 gl ⁻¹	± 0.5 g l ⁻¹
Creatine Phosphokinase	0-1000 U l ⁻¹	± 2 U l ⁻¹
Lactate Dehydrogenase	0-1000 U l ⁻¹	± 2 U l ⁻¹
Alkaline Phosphatase	0-100 U l ⁻¹	± 2 U l ⁻¹
Glucose Oxylate Transaminase	0-100 U l ⁻¹	± 2 U l ⁻¹

Source: Technicon Instruments Co.

*Osmoregulatory ions measured by SMAC.

Electroshock Experiment

Sixty channel catfish weighing 310 to 884 g obtained from Indian Mound Catfish Farm were used to assess the effects of electroshock on blood chemistry. These fish were held without food for 5 days at 23°C in a recirculating 2000 l holding tank. The RF-10 electroshocker was installed in the laboratory and 1.27 cm x 76.2 cm copper tubes were used as electrodes. Blood samples were taken from 10 control fish prior to shocking. Then an electroshock of 250 volts and 5 amps was administered until all 50 fish were obviously stunned (about 15 sec). Blood was taken from groups of 10 fish; the first collected immediately after electroshocking and others at 1, 3, 6 and 24 hr later. Samples were processed as in the field collections. The blood plasma osmolality, hematocrit and chemistry were determined with the osmometer, microhematocrit tubes and SMAC as previously described. Gender was determined by gonad inspection. Three water samples taken at each fish sampling were similarly analyzed.

CHAPTER III

RESULTS

Electroshock Experiment

Parametric one-way analysis of variance (ANOV) and Duncan's Multiple Range Test ($\alpha = 0.05$) demonstrated that the principle osmoregulatory ions of channel catfish (sodium, chloride, calcium and potassium) were not significantly affected ($p < 0.05$) by the experimental electroshock. Mean plasma sodium concentration (Figure 5) for the ten controls was 135.5 meq l^{-1} (range 122 to 143) and increased only slightly after electroshock. Mean plasma chloride (Figure 5) decreased initially from control levels of 114.5 meq l^{-1} (range 101 to 119) but these changes were non-significant ($p < 0.05$). Mean plasma potassium levels (Figure 6) were essentially unchanged following electroshock (controls = 3.38 meq l^{-1} , range 2.0 to 7.3). Plasma calcium concentrations (Figure 6) rose initially from control values of 5.1 meq l^{-1} (range 4.6 to 6.8) but all six groups of fish were not significantly different ($p < 0.05$).

Total osmotic concentraion and hematocrit (Figure 7) increased slightly after electroshock from control values of 264.8 mOsm (range 229 to 280) and 35.3 (range 29 to 41), respectively. These changes from control values were non-

Figure 5. Mean plasma sodium and chloride concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10 for each group) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . These concentrations were not significantly different ($p < 0.05$) among groups.

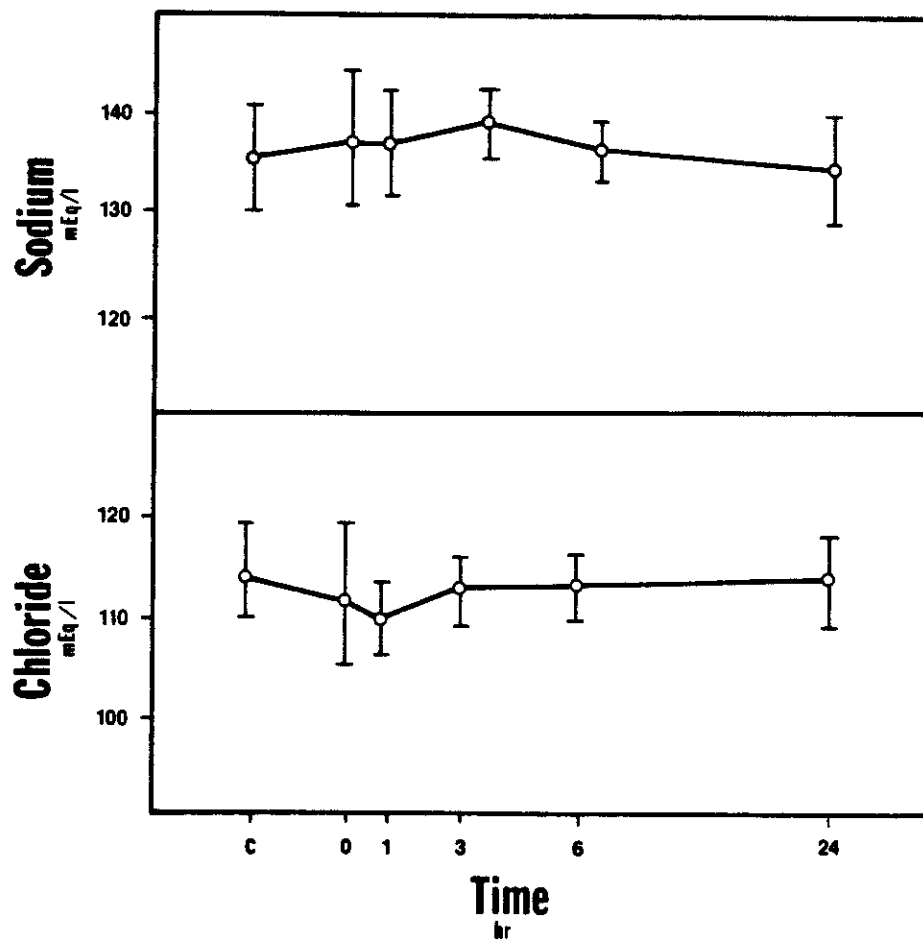


Figure 6. Mean plasma calcium and potassium concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10 for each group) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . These concentrations were not significantly different ($p < 0.05$) among groups.

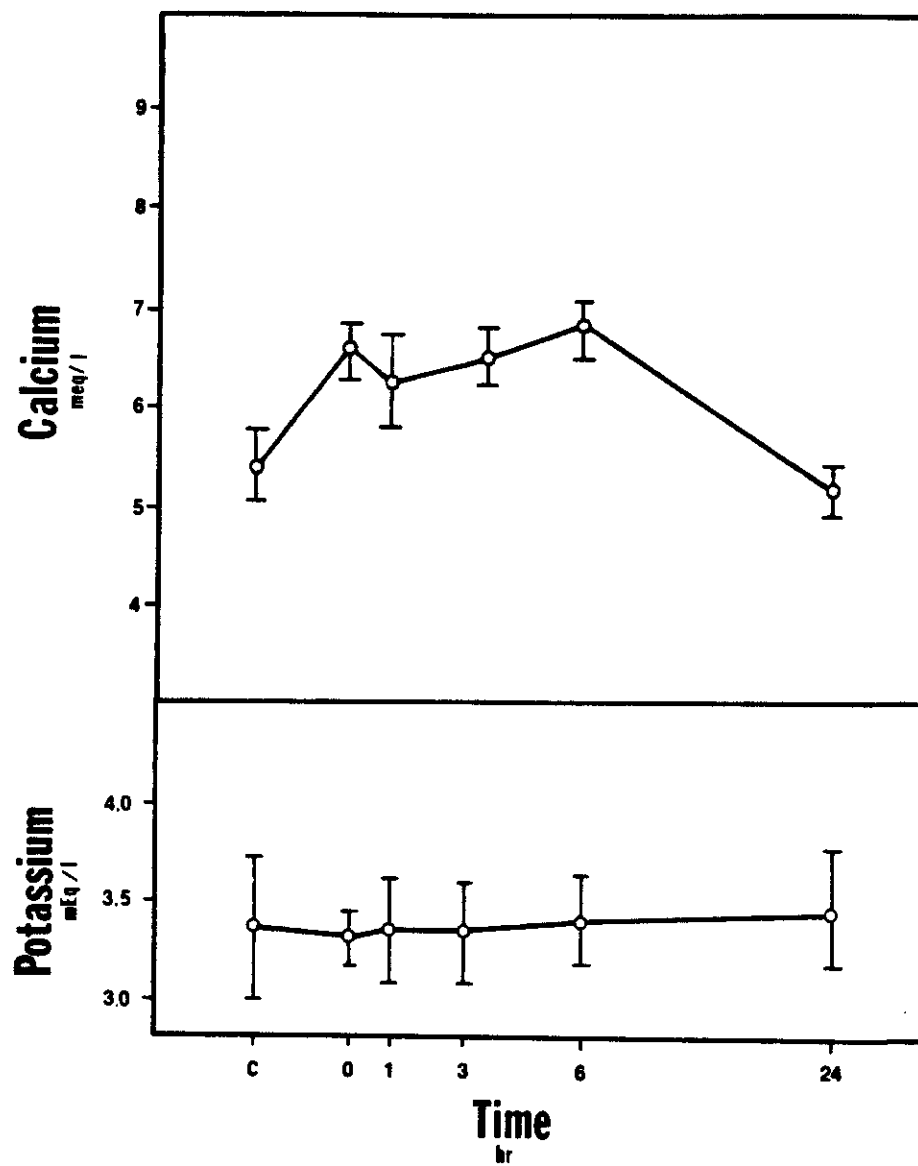
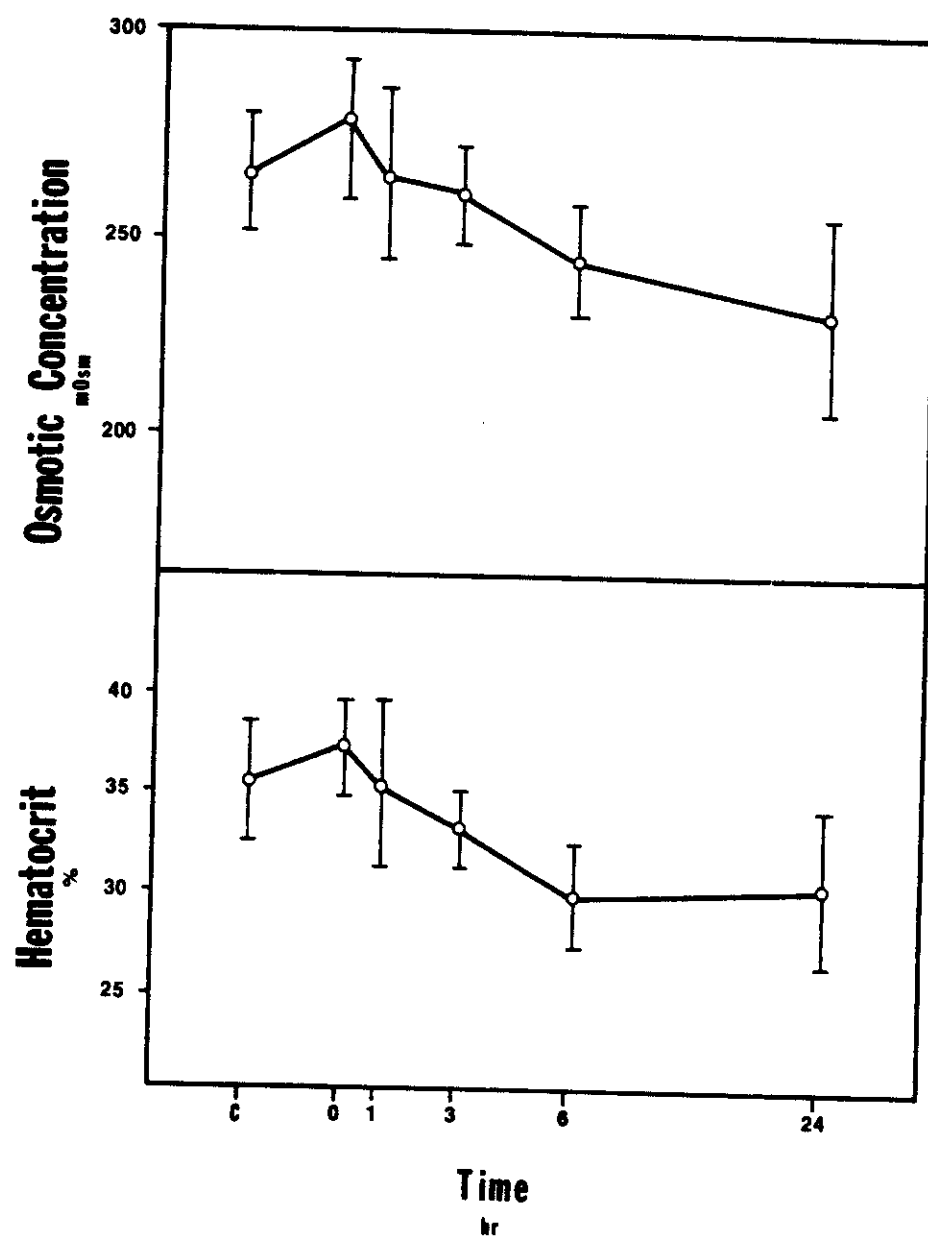


Figure 7. Mean plasma osmotic concentration and hematocrits (\pm ranges) in five groups of channel catfish and one control group (n= 10 for each group) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . These concentrations were not significantly different ($p < 0.05$) among groups.



significant ($p < 0.05$). Plasma protein and glucose oxalate transaminase (GOT) concentrations (Figure 8), indicators of possible tissue damage from electroshock, increased slightly (10% and 11%, respectively) following electroshock. Control levels for GOT were 192.2 activity units (U) l^{-1} (range 86 to 427). One activity unit is that amount of enzyme that will act upon one microgram of substrate. Mean plasma protein concentrations were 27.8 g l^{-1} (range 26 to 31). The increases in GOT and plasma protein levels were not significantly different ($p < 0.05$) from controls.

Water parameters from samples collected at all catfish sampling intervals were not significantly different ($p < 0.05$) from initial values prior to electroshock. Mean initial values were: pH, 7.6; dissolved oxygen, 8.8 mg l^{-1} ; salinity, <1 mOsm; conductivity, 400 μ hos cm^{-1} and temperature, 23°C.

Field Collections

Water Parameters

Mean total osmotic concentrations of the reservoirs during sampling ranged from 3.96 mOsm at Moss Lake to 63.02 mOsm at Possum Kingdom Lake (Table 5). Salinities, reported as osmotic concentration, were not significantly different ($p < 0.05$) within reservoirs among sampling efforts. Similarly sodium concentrations ranged from 9 to 9.5 meq l^{-1} at Moss Lake to 490 to 500 meq l^{-1} at Possum Kingdom Lake (Table 5).

Figure 8. Mean plasma glucose oxylate transaminase (GOT) and protein concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10 for each group) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . These concentrations were not significantly different ($p < 0.05$) among groups.

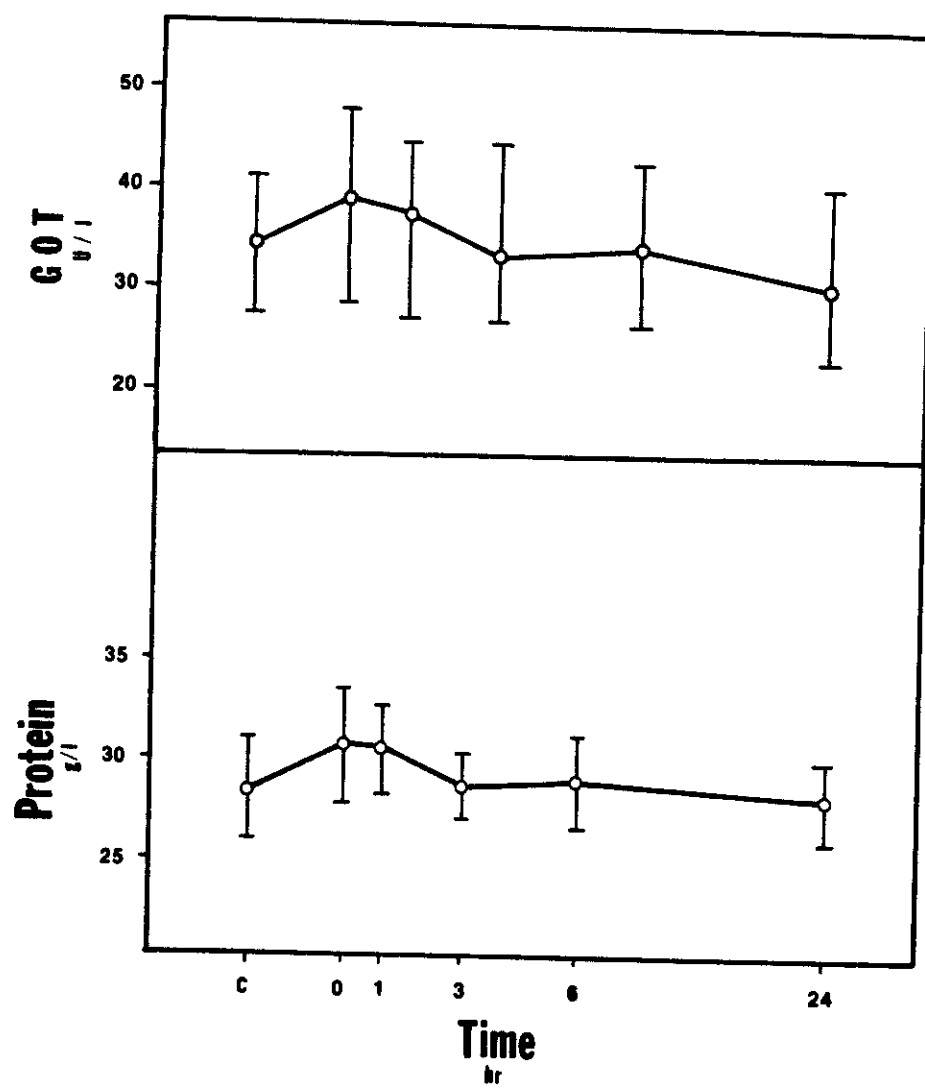


Table 5. Means and ranges (in parentheses) of physical and chemical water parameters taken at time of fish collections.

Location	N	Temp °C	Salinity mOsm	Cond µmhos cm ⁻¹	Na ⁺	Ca ⁺⁺ meq l ⁻¹	K ⁺ meq l ⁻¹	Cl ⁻
Moss Lake								
19 VI	78	3 25	3.96 (3.8- 4.1)	190 (180- 195)	9 (8- 10)	24 (22-26)	3.3 (3.1-3.4)	8.3 (8- 9)
22 VII	78	3 30	4.28 (3.9- 4.5)	205 (185- 217)	9 (8- 10)	23 (22-26)	3.5 (3.2-3.7)	8.5 (8- 9)
12 X	78	3 22	4.17 (3.8- 4.4)	200 (188- 212)	9.5 (8- 11)	23 (20-24)	3.1 (3.0-3.3)	8.7 (8- 10)
Lake Ray Hubbard								
13 VII	78	3 32	5.84 (4.9- 6.3)	280 (270- 295)	19 (15- 22)	20 (20)	3.9 (3.7-4.0)	16 (14- 18)
29 IX	78	3 23	4.38 (3.9- 4.7)	210 (195- 226)	17 (16- 19)	21 (20-23)	3.4 (3.1-3.5)	15 (14- 17)
24 X	78	3 17	5.22 (5.1- 5.4)	250 (228- 268)	17 (16- 19)	21 (20-22)	3.2 (3.1-3.5)	15 (14- 17)
Lake Texoma								
12 IX	78	3 25	52.17 (50.4-55.0)	2500 (2460-2535)	270 (260-290)	58 (49-59)	3.9 (3.6-4.4)	430 (410-440)
4 XI	78	3 19	54.47 (52.1-57.3)	2610 (2512-2700)	265 (260-278)	60 (52-62)	4.1 (3.7-4.4)	450 (402-461)
Possum Kingdom								
17 IX	78	3 25	61.14 (60.4-62.1)	2930 (2900-3010)	320 (315-340)	55 (54-49)	3.6 (3.4-4.1)	500 (488-500)
30 X	78	3 20	63.02 (61.9-66.3)	3020 (2950-3100)	331 (317-338)	58 (54-61)	3.9 (3.6-4.2)	490 (482-500)

Calcium concentration was lowest at Lake Ray Hubbard (20 to 21 meq l^{-1}) and generally reflected the watershed characteristics of each reservoir. Lake Texoma had the highest calcium composition (50 to 60 meq l^{-1}) of the four reservoirs. Potassium was present in Moss Lake at concentrations of 3.1 to 3.5 meq l^{-1} and was at similar concentrations in the other reservoirs (Table 3). Potassium did not comprise more than two percent of the total salinity in any of the reservoirs (range 3.1 to 4.1 meq l^{-1}).

Chloride did not comprise an equal percentage of the total salinity in the four reservoirs (Table 5). It was lowest at Moss Lake (8.3 to 8.7 meq l^{-1}) and highest at Possum Kingdom Lake (490 to 500 meq l^{-1}).

Water temperatures varied significantly ($p < 0.05$) among sampling efforts at all reservoirs. The lowest temperature was 17°C in October at Lake Ray Hubbard and the highest was 32°C in July, also at Lake Ray Hubbard.

Total osmotic concentrations were not significantly different ($p < 0.05$) among Moss Lake and Lake Ray Hubbard (Table 6). However, total osmotic concentration in Lake Texoma and Possum Kingdom Lake varied significantly ($p < 0.05$) from Moss Lake and Lake Ray Hubbard and were different ($p < 0.05$) from each other. Moss Lake and Lake Ray Hubbard were not significantly different ($p < 0.05$) in calcium concentrations (Table 6) but were significantly different from Lake Texoma and Possum

Table 6. Relationships among chemical parameters in the four reservoirs. Solid line connecting two or more mean values represents no significant difference ($p < 0.05$) in that parameter among the reservoirs. The relationships were established by Duncan's Multiple Range Test ($N = 9$).

	Moss Lake	Lake Ray Hubbard	Lake Texoma	Possum Kingdom
Salinity	<u>4.14</u>	<u>5.15</u>	<u>53.32</u>	<u>62.08</u>
Sodium (meq l^{-1})	<u>9.17</u>	<u>17.67</u>	<u>267.50</u>	<u>325.50</u>
Calcium (meq l^{-1})	<u>23.33</u>	<u>20.67</u>	<u>58.50</u>	<u>56.50</u>
Potassium (meq l^{-1})	<u>3.3</u>	<u>3.5</u>	<u>4.0</u>	<u>3.8</u>
Chloride (meq l^{-1})	<u>8.5</u>	<u>15.3</u>	<u>440</u>	<u>495</u>

Kingdom Lake. All four reservoirs were significantly different from each other in sodium concentrations. Potassium concentrations were not significantly different among reservoirs. Differences in chloride concentrations in Lake Texoma and Possum Kingdom Lake were not significant ($p < 0.05$) although they differed significantly from Moss Lake and Lake Ray Hubbard.

Intraspecific Comparisons Within Reservoirs

Largemouth bass.-- A total of 74 largemouth bass from 312 to 1407 g net weight were examined. Mean condition factors (K_{SL}) ranged from 2.16 in Lake Ray Hubbard to 2.88 in Moss Lake (Figure 9) and were not significantly different ($p < 0.05$) in bass among reservoirs.

Total osmotic concentration of largemouth bass plasma was inversely linearly correlated ($R^2 = 0.88$) to water salinity. Mean values for bass ranged from 284.5 to 332.5 mOsm (Figure 10).

Mean plasma potassium concentrations (Figure 11) were significantly higher ($p < 0.05$) in bass from Possum Kingdom Lake (4.8 meq l^{-1} , range 3.9 to 5.7) than those for bass from the other three reservoirs which ranged from 3.5 to 3.7 meq l^{-1} .

The other major osmoregulatory ions were not significantly different ($p < 0.05$) among bass from the four reservoirs. Mean

Figure 9. Intraspecific comparisons of mean condition factors ($K \pm$ ranges) among reservoirs. A = Moss Lake, B = Lake Ray Hubbard, C = Lake Texoma, D = Possum Kingdom Lake, LmB = largemouth bass, C.cat = channel catfish and f.cat. = flathead catfish.

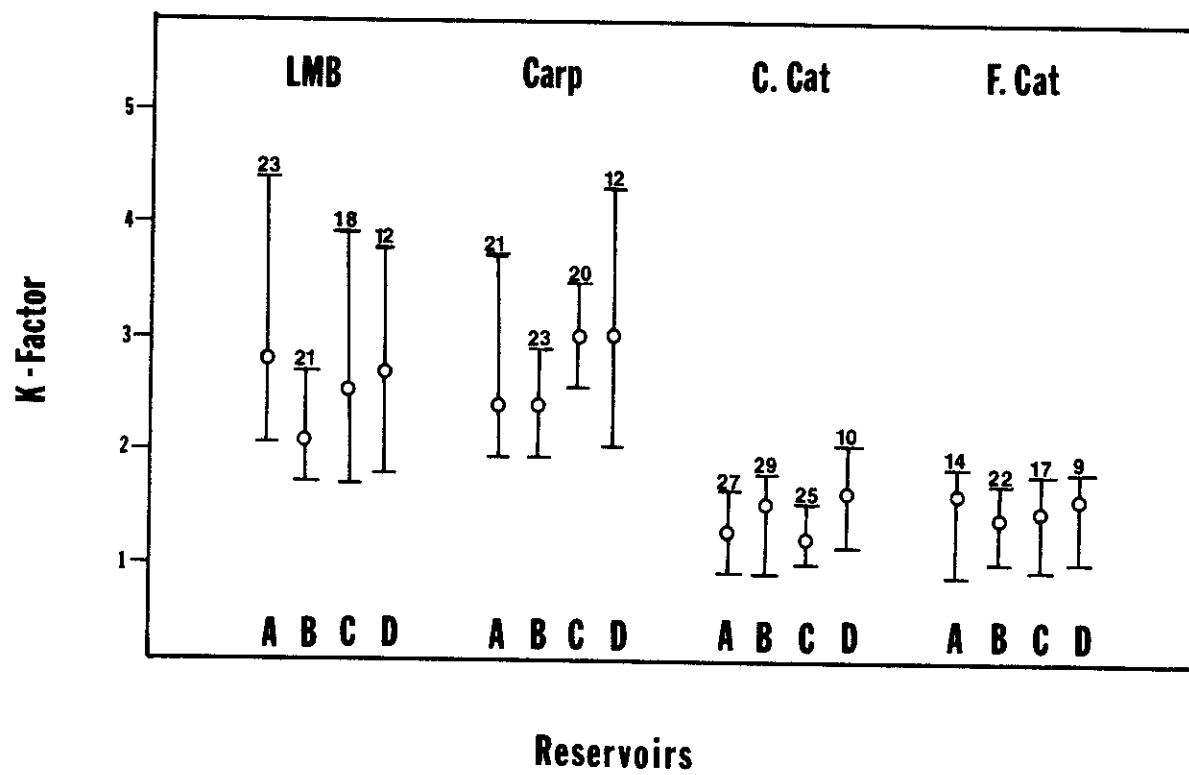
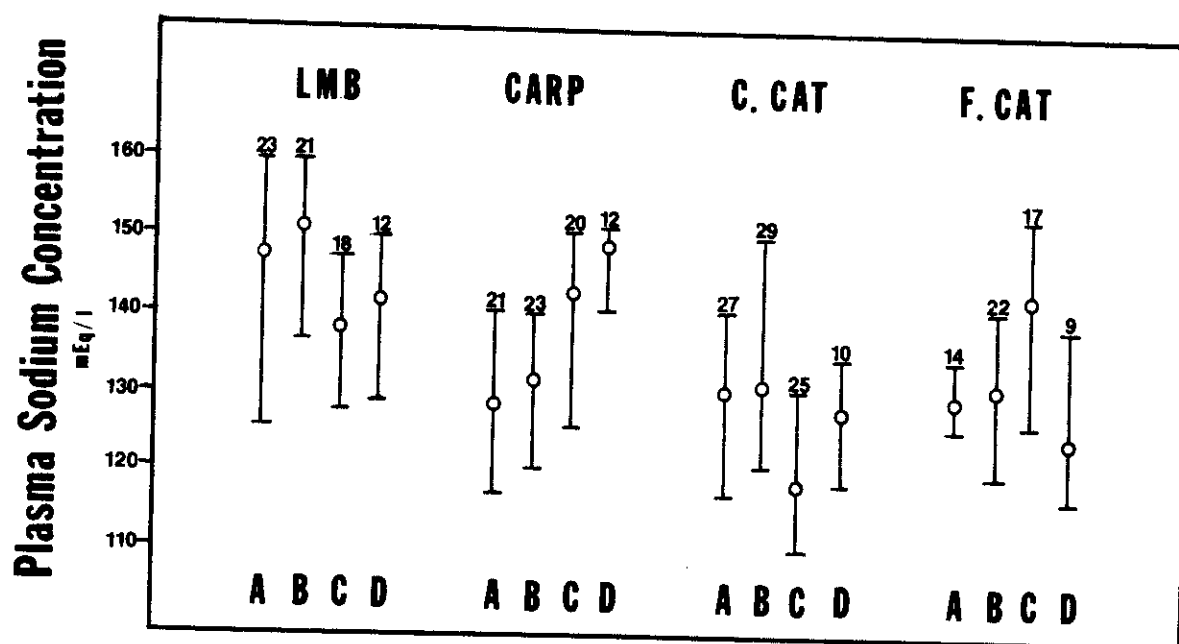
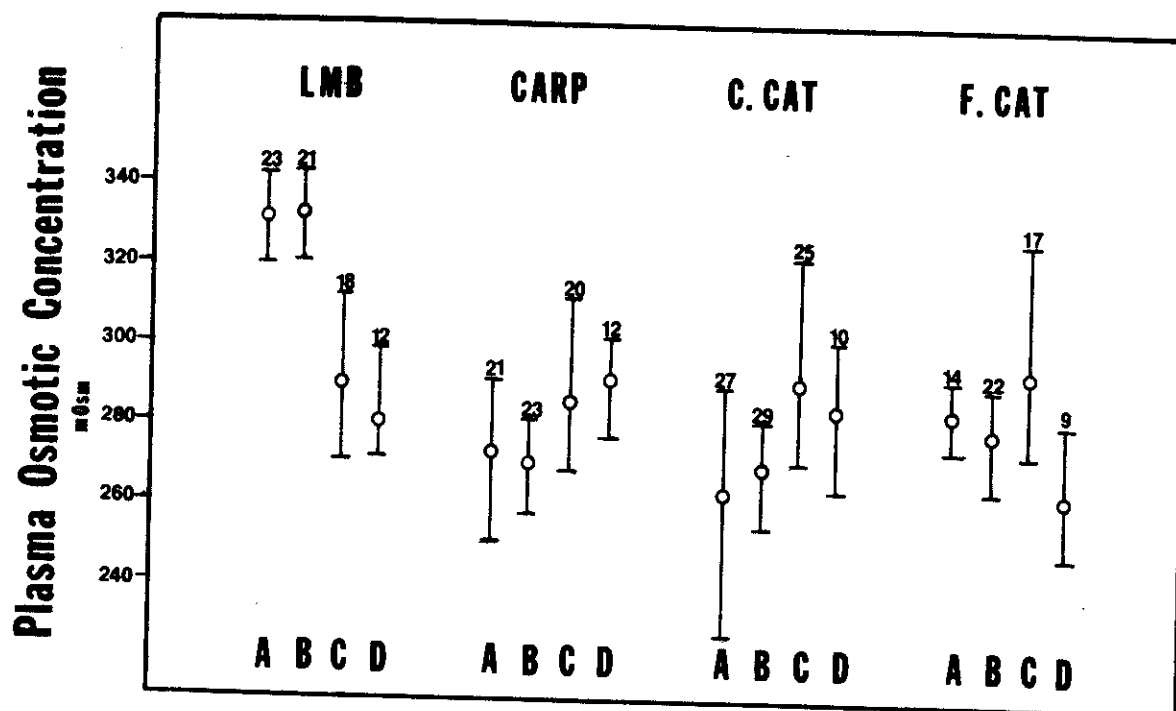
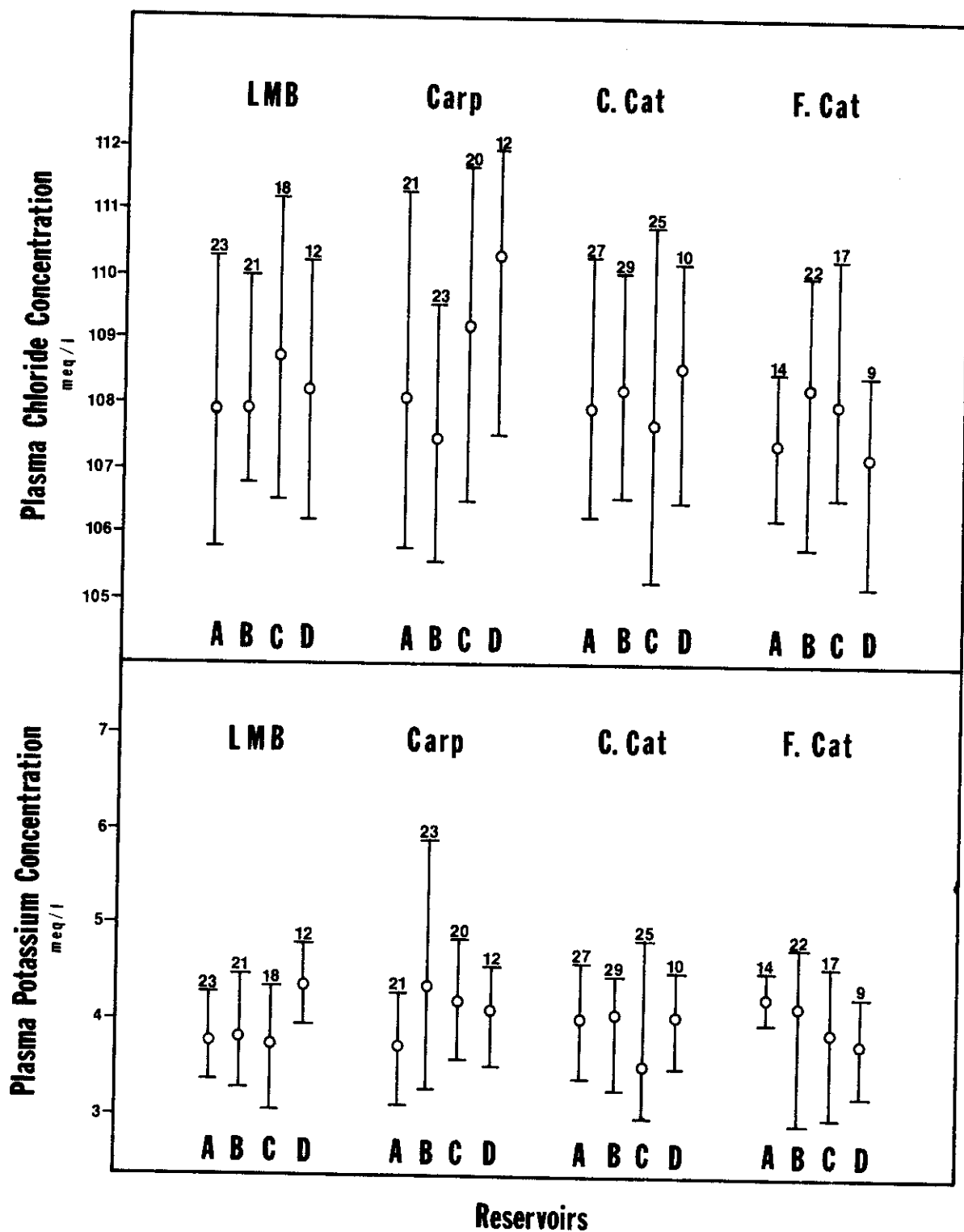


Figure 10. Intraspecific comparisons of mean plasma osmotic concentrations and sodium concentrations (\pm ranges) among reservoirs. Symbols are the same as in Figure 9.



Reservoirs

Figure 11. Intraspecific comparisons of mean plasma chloride and potassium concentrations (\pm ranges) among reservoirs. Symbols are the same as in Figure 9.



plasma sodium concentrations ranged from 137.5 to 151.1 meq l^{-1} (Figure 10). Mean plasma chloride values for bass from these reservoirs ranged from 107.4 to 111.3 meq l^{-1} (Figure 11).

Mean calcium concentrations were nearly identical, varying from 4.6 to 4.8 meq l^{-1} among bass from the four reservoirs (Figure 12).

Hematocrit values (Figure 12) were significantly higher ($p < 0.05$) in bass from Moss Lake and Lake Ray Hubbard than in bass from Lake Texoma and Possum Kingdom Lake. Also hematocrits were linearly correlated ($R^2 = 0.96$) to water temperature (Figure 13).

Male and female largemouth bass did not differ significantly in plasma components.

Carp.--A total of 76 carp, weighing from 321 to 3732 g, were collected. Mean condition factors ranged from 2.45 to 3.08 (Figure 9) and were not related significantly ($p < 0.05$) to salinity.

Plasma sodium concentrations in carp were linearly correlated ($R^2 = 0.97$) to sodium concentrations in the reservoirs (Figure 14). Mean plasma sodium concentrations ranged from 129.1 meq l^{-1} at Moss Lake to 147.8 meq l^{-1} at Possum Kingdom Lake (Figure 10).

Total osmotic concentration of carp plasma was linearly correlated ($R^2 = 0.97$) to total osmotic concentration of the

Figure 12. Intraspecific comparisons of mean hematocrit and plasma calcium concentrations (\pm ranges) among reservoirs. Symbols are the same as in Figure 9.

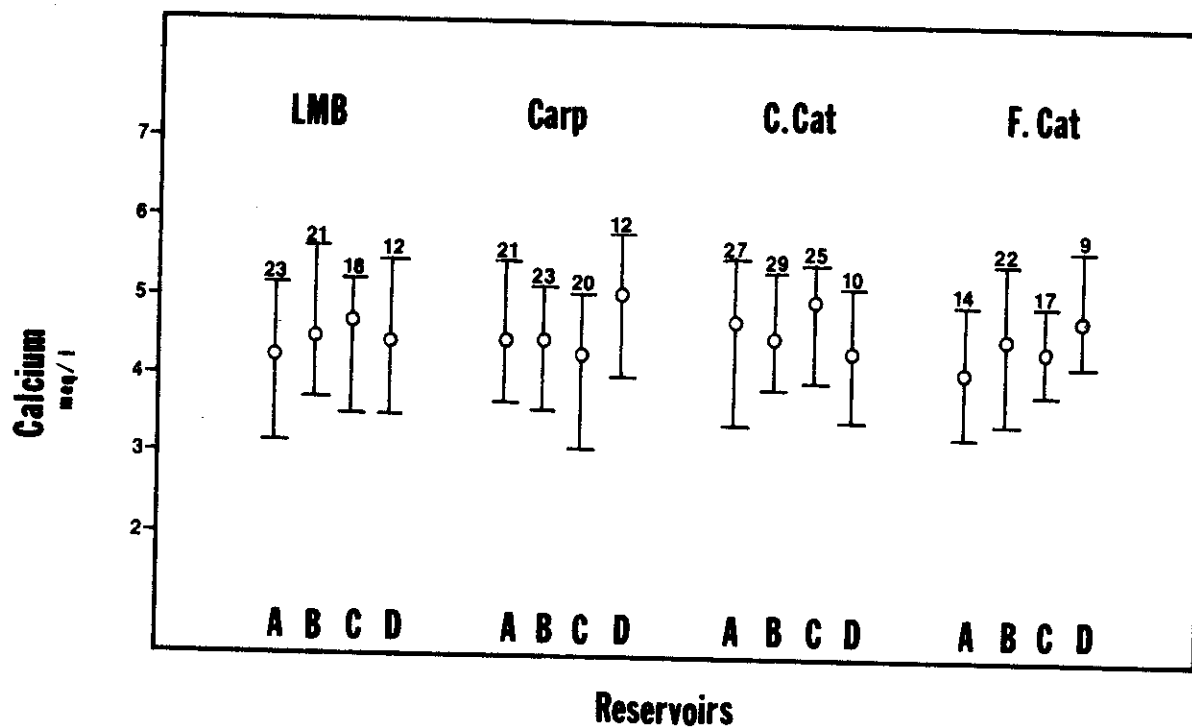
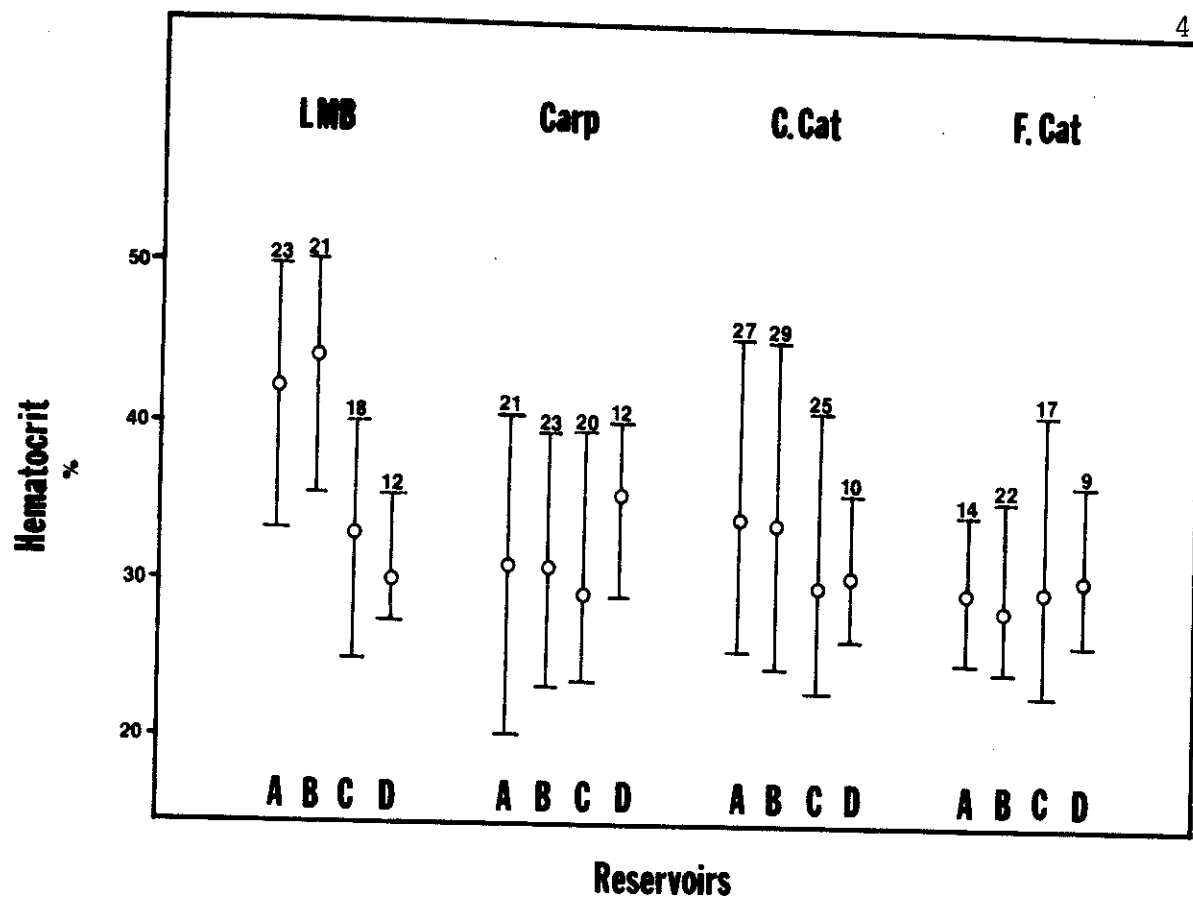


Figure 13. Plot of mean largemouth bass hematocrits versus water temperature at the collection sites. Best fit linear model is given; n for each mean appears beside it.

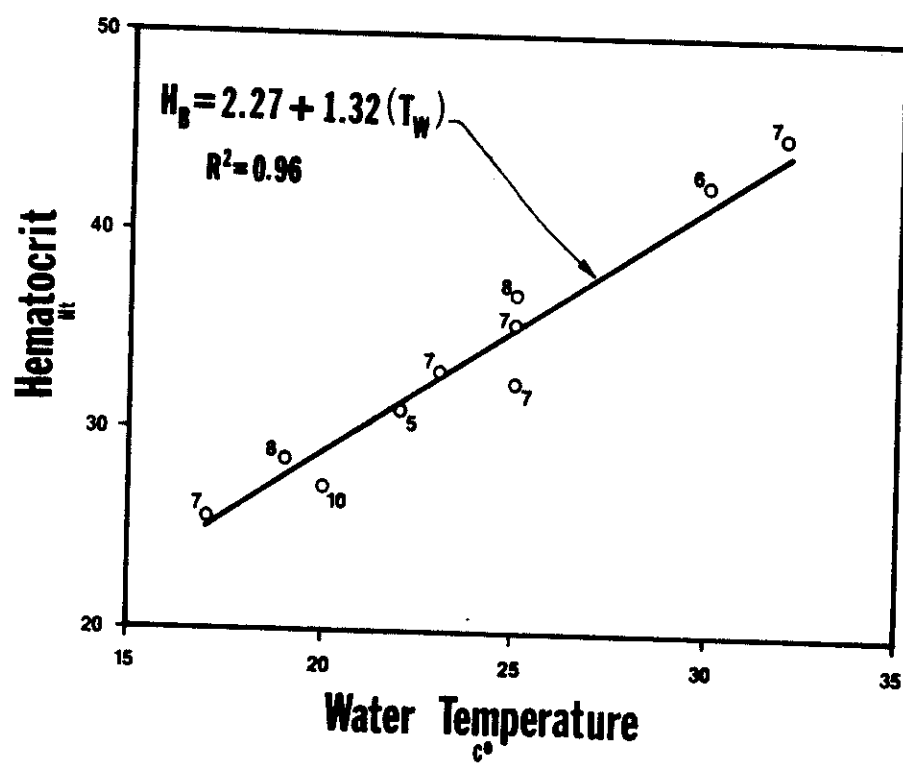
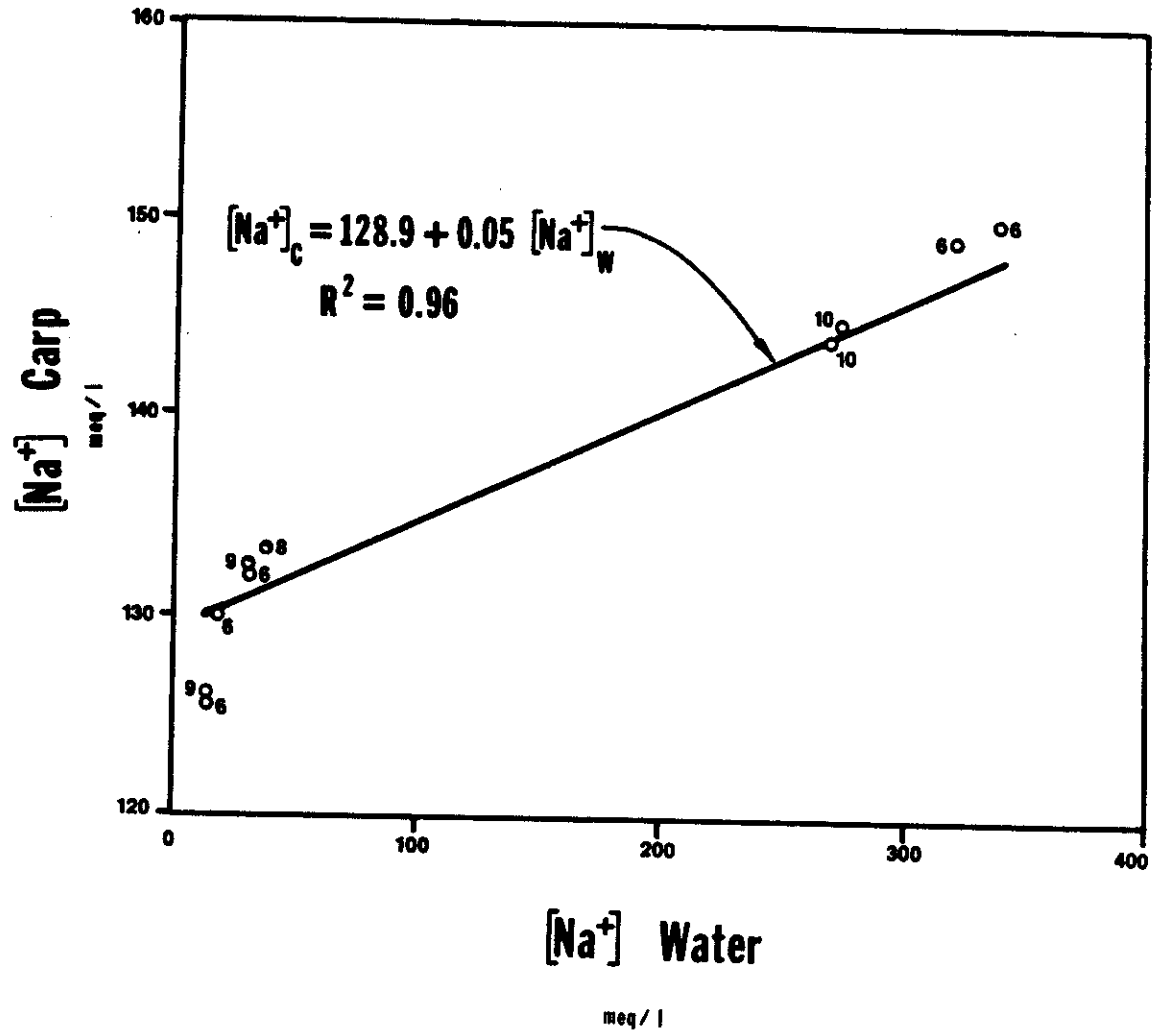


Figure 14. Plot of mean plasma sodium concentration in carp versus sodium concentration in the reservoirs. Best fit linear model is given; n for each mean appears beside it.



reservoirs (Figure 15). Mean plasma osmotic concentrations ranged from 270 to 292 mOsm (Figure 10).

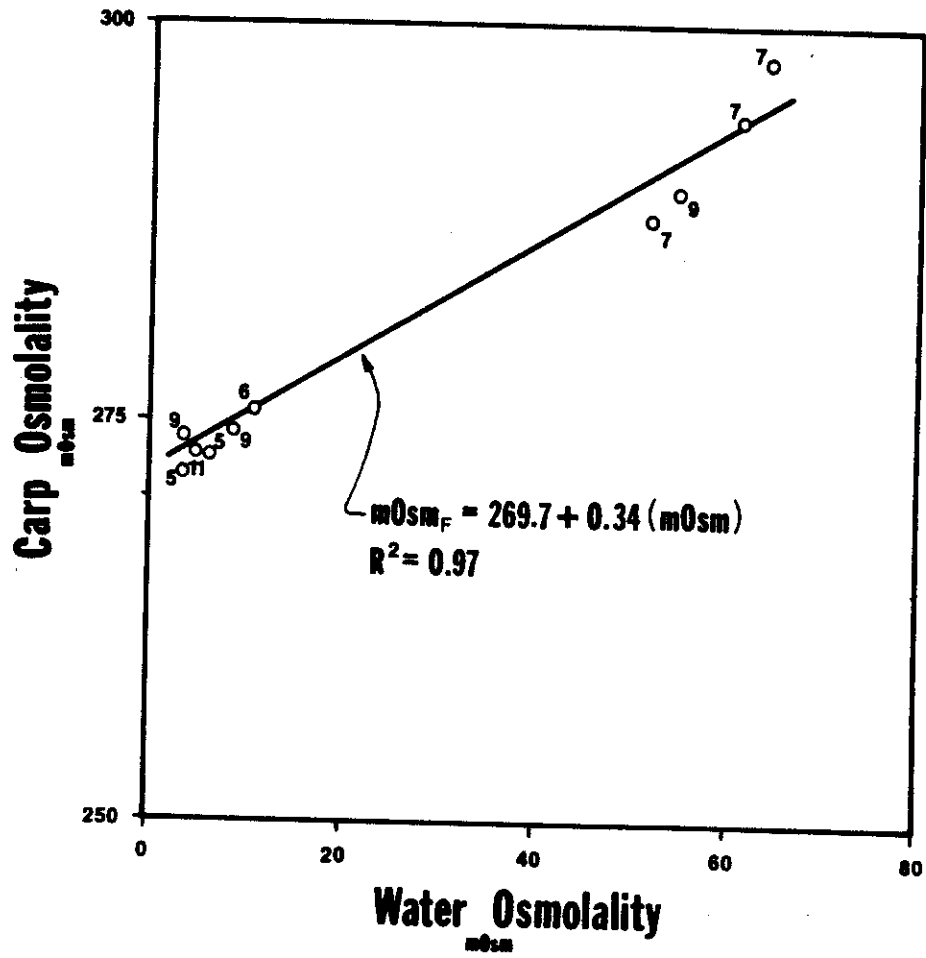
The other osmoregulatory ions were not significantly different ($p < 0.05$) among carp from the four reservoirs. Mean plasma potassium levels for carp ranged from 3.4 to 4.3 meq l^{-1} (Figure 11). Plasma chloride levels ranged from a mean 105.9 to 117.3 meq l^{-1} for carp from the four reservoirs (Figure 11). Plasma calcium values in carp varied only slightly from 4.6 to 4.8 meq l^{-1} among reservoirs. Hematocrits for carp ranged from a mean 28.1 to 35.1 (Figure 12).

With cholesterol as the sole exception, measured blood components were not different in male and female carp. Plasma cholesterol varied significantly ($p < 0.05$) among sexes. Males ranged from 612 to 2120 mg l^{-1} (mean 1280) and females ranged from 1940-4860 mg l^{-1} (mean 2860).

Channel catfish.--A total of 91 channel catfish, weighing 290-3924 g, were collected from the four reservoirs. Mean K-factors (Figure 9), which ranged from 1.42 in Lake Texoma to 1.82 in Possum Kingdom Lake, were not significantly different among channel catfish from the four reservoirs.

No significant differences were observed in any of the four major osmoregulatory ions (sodium, chloride, calcium and potassium) or osmotic concentrations among channel catfish from the four reservoirs. Mean total osmotic concentrations ranged from 263 mOsm at Moss Lake to 290 mOsm at Lake Texoma (Figure

Figure 15. Plot of mean plasma osmotic concentration in carp versus osmotic concentration in the reservoirs. Best fit linear model is given; n for each mean appears beside it.



10). Mean plasma sodium levels ranged from 118.6 meq l^{-1} at Lake Texoma to 132.1 meq l^{-1} at Moss Lake (Figure 10). Mean plasma chloride and potassium concentrations ranged from 107.9 meq l^{-1} at Moss Lake to 110.5 meq l^{-1} at Possum Kingdom Lake and 3.5 meq l^{-1} at Lake Texoma to 4.2 meq l^{-1} at Possum Kingdom Lake, respectively (Figure 11). Mean plasma calcium values ranged from 4.6 meq l^{-1} at Possum Kingdom Lake to 5.0 meq l^{-1} at Lake Texoma (Figure 12). Mean hematocrits of channel catfish from the four reservoirs ranged from 31.5 at Possum Kingdom Lake to 34.8 at Lake Ray Hubbard (Figure 12). These differences were non-significant ($p < 0.05$).

Plasma osmotic and ionic values were not significantly correlated to levels in the reservoirs ($p < 0.05$). Male and female channel catfish did not differ significantly ($p < 0.05$) in plasma components.

Flathead catfish.--A total of 62 flathead catfish, weighing 337-2310 g, were examined. K-factors were not significantly different ($p < 0.05$) among flathead catfish from the four reservoirs. Mean K-factors ranged from 1.63 in Lake Ray Hubbard to 1.87 in Moss Lake.

Plasma concentrations of the four-osmoregulatory ions and osmotic concentrations in flathead catfish from the four reservoirs were not significantly different. Mean osmotic concentrations ranged from 265 mOsm at Possum Kingdom Lake to 288 mOsm at Lake Texoma (Figure 10). Mean plasma sodium

concentrations were lowest in flathead catfish from Possum Kingdom Lake (124.7 meq l^{-1}) and highest in those from Lake Texoma (143.4 meq l^{-1} , Figure 10). Mean plasma chloride and potassium concentrations ranged from 105 meq l^{-1} at Possum Kingdom Lake to 109 meq l^{-1} at Lake Ray Hubbard and from 3.6 meq l^{-1} at Possum Kingdom to 4.5 meq l^{-1} at both Moss Lake and Lake Ray Hubbard, respectively (Figure 11). Mean plasma calcium levels ranged from 4.5 meq l^{-1} at Moss Lake to 4.8 meq l^{-1} at Possum Kingdom Lake (Figure 12).

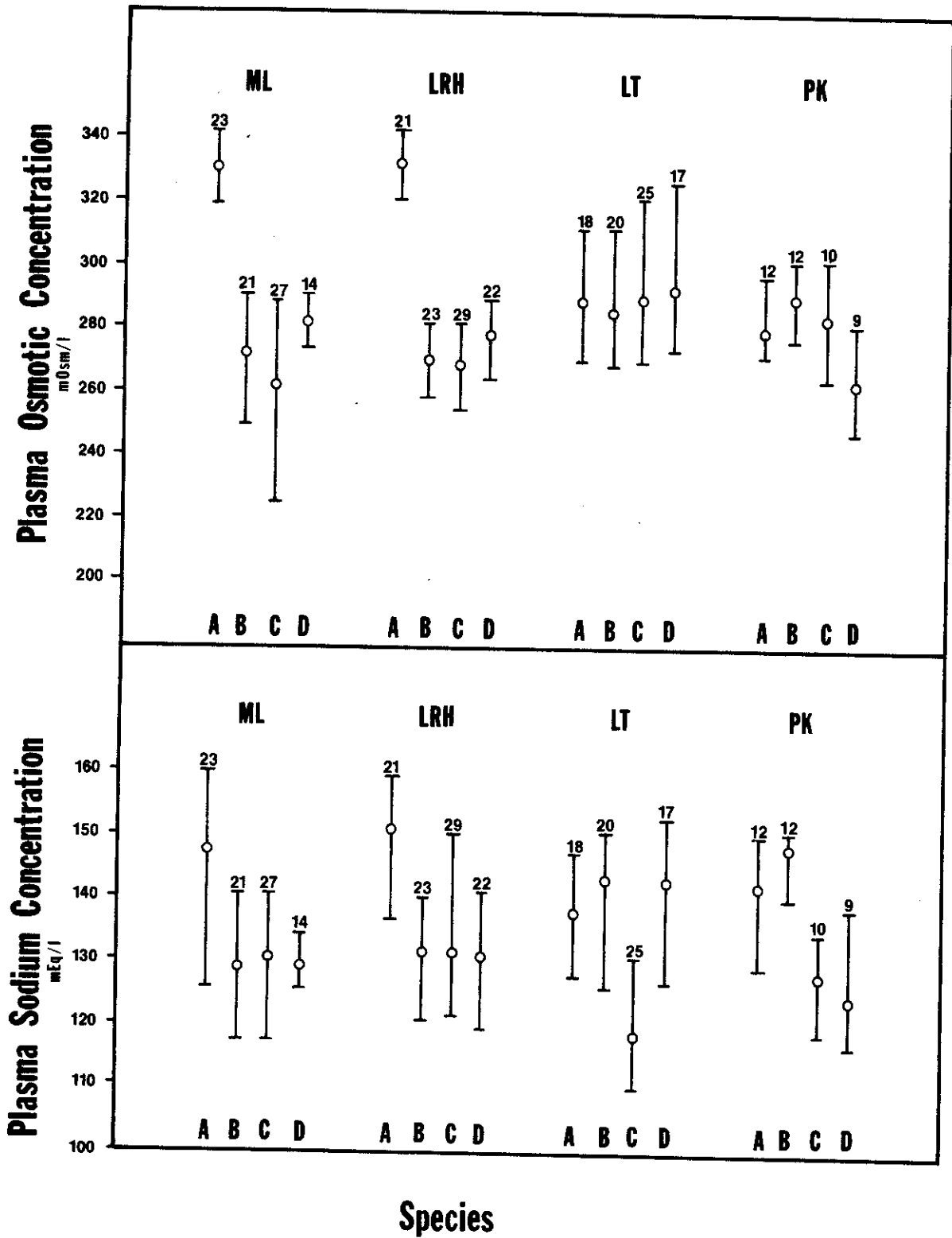
Mean hematocrits in flathead catfish were not significantly different ($p < 0.05$) among reservoirs. They ranged from 28.2 in Lake Ray Hubbard to 30.3 in Possum Kingdom Lake.

Plasma ionic and osmotic concentrations in flathead catfish were not related ($p < 0.05$) to environmental values. No variations in measured variables due to gender were noted.

Interspecific Comparisons Within Reservoirs

Moss Lake.--A total of 85 fishes from Moss Lake were examined. Plasma osmotic concentrations varied significantly ($p < 0.05$) among species (Figure 16). Channel catfish, carp, flathead catfish and largemouth bass had mean values of 263.1, 272.8, 281.8 and 332 mOsm, respectively. Osmotic concentrations in largemouth bass were significantly higher than the other three species. Largemouth bass had the highest plasma sodium concentrations (mean = 147.7 meq l^{-1} , Figure 16) and

Figure 16. Interspecific comparisons of mean plasma osmotic and sodium concentrations (\pm ranges) within reservoirs. A = largemouth bass, B = carp, C = channel catfish, D = flathead catfish, ML = Moss Lake, LRH = Lake Ray Hubbard, LT = Lake Texoma and PK = Possum Kingdom Lake. Numbers above the bars = n for each species.



differed significantly ($p < 0.05$) from the other three species. Mean plasma sodium levels among channel catfish, flathead catfish and carp ranged from 129.1 to 130.1 meq l^{-1} and were not significantly different.

Mean plasma chloride, potassium and calcium levels were not significantly different ($p < 0.05$) among the four species in Moss Lake. Mean plasma chloride concentrations ranged from 105.4 meq l^{-1} for flathead catfish to 108.1 meq l^{-1} for carp (Figure 17). Mean plasma potassium values ranged from 3.4 meq l^{-1} for carp to 4.48 meq l^{-1} for flathead catfish (Figure 17). Mean plasma calcium levels (Figure 18) were highest in channel catfish (4.8 meq l^{-1}) and lowest in largemouth bass (4.5 meq l^{-1}).

Hematocrits, ranging from 29.0 in flathead catfish to 30.8 in channel catfish (Figure 18), were significantly higher ($p < 0.05$) in largemouth bass (mean = 42.6) than in the other three species.

Lake Ray Hubbard.--The plasma constituents measured in the 95 fishes captured at Lake Ray Hubbard displayed similar patterns to those found at Moss Lake. Largemouth bass had a mean osmotic concentration (333.4 mOsm) significantly higher ($p < 0.05$) than the other three species (carp = 270.7, channel catfish = 269.3 and flathead catfish = 279.1 mOsm, Figure 16). There were corresponding differences ($p < 0.05$) in mean plasma sodium concentrations (Figure 16) between largemouth bass

Figure 17. Interspecific comparisons of mean plasma chloride and potassium concentrations (\pm ranges) within reservoirs. Symbols are the same as in Figure 16.

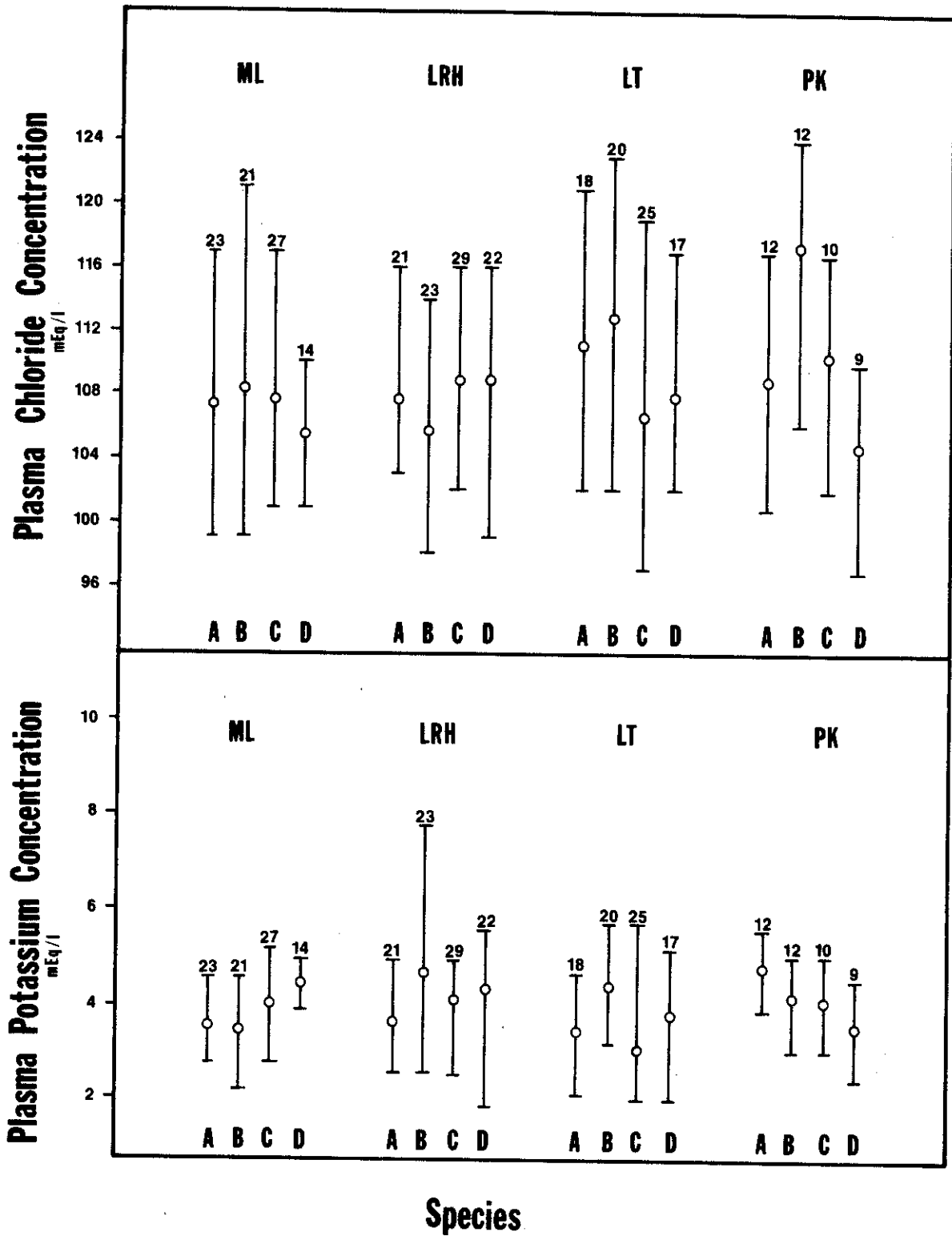
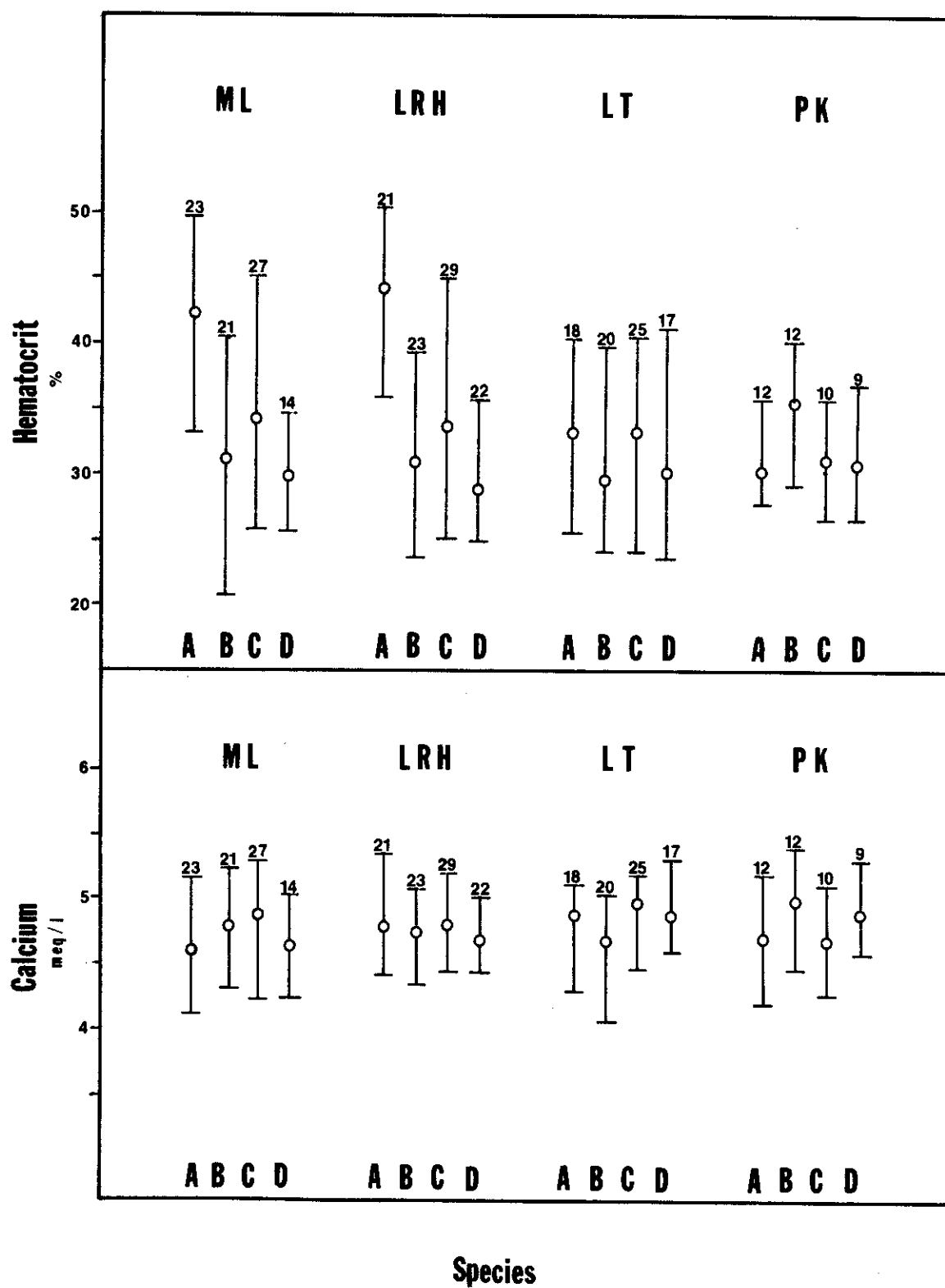


Figure 18. Interspecific comparisons of mean plasma calcium concentrations and hematocrits (\pm range) within reservoirs. Symbols are the same as in Figure 16.



(151 meq l^{-1}) and the other three species. Mean plasma chloride, potassium and calcium concentrations (Figures 17 and 18) were not significantly different ($p < 0.05$) among species in Lake Ray Hubbard.

Hematocrits (Figure 18) were significantly higher ($p < 0.05$) in largemouth bass (mean 44.1) than those of the other species (carp = 31.1, channel catfish = 34.8 and flathead catfish = 28.6).

Lake Texoma.--A total of 80 fishes from Lake Texoma were examined. Although mean osmotic concentrations among species (Figure 16) were not significantly different ($p < 0.05$), significant variation was observed in three of the major osmoregulatory ions. Plasma sodium levels in channel catfish (mean = 143.5 meq l^{-1} , range 126 to 152) were significantly lower than the other three species (Figure 16). Plasma chloride levels (Figure 17) were significantly higher in largemouth bass (mean = 113.3 meq l^{-1}) and carp (112.8 meq l^{-1}) than in the catfishes (channel catfish = 106.6 meq l^{-1} and flathead catfish = 108.4 meq l^{-1}). Largemouth bass and channel catfish were not significantly different in mean plasma potassium values but were significantly lower than carp and flathead catfish (Figure 17).

Plasma calcium levels (Figure 18) varied only slightly among species. Means ranged from 4.6 meq l^{-1} in carp to 5.0 meq l^{-1} in channel catfish. Mean hematocrit values in

largemouth bass (32.8) and channel catfish (33.0) were significantly higher than those in carp (29.6) and flathead catfish (30.0, Figure 18).

Possum Kingdom Lake.--Plasma osmotic concentrations (Figure 16) in the 43 fishes collected at Possum Kingdom Lake ranged from a mean of 263.4 mOsm in flathead catfish to 291.5 mOsm for carp. Flathead catfish were significantly lower than the other three species. Mean plasma sodium concentrations (Figure 16) followed a similar pattern: 124.7 meq l⁻¹ in channel catfish, 142.8 meq l⁻¹ in largemouth bass and 147.7 meq l⁻¹ in carp. Carp did not differ significantly ($p < 0.05$) from largemouth bass; however, these two species were significantly higher than channel catfish and flathead catfish.

Carp were significantly higher ($p < 0.05$) in plasma chloride concentration than the other three species (Figure 17). Flathead catfish were significantly lower than the other three species and largemouth bass and channel catfish were intermediate in mean plasma chloride levels and not significantly different from each other. Plasma potassium levels were not significantly different among species (Figure 17). They ranged from means of 3.6 meq l⁻¹ in flathead catfish to 4.77 meq l⁻¹ in largemouth bass.

Mean plasma calcium values were not significantly different among species in Possum Kingdom Lake (Figure 18). They

ranged from 4.6 meq l⁻¹ in channel catfish to 5.0 meq l⁻¹ in carp. Hematocrits in carp (Figure 18) were significantly elevated over those found in the other three species.

Key Results

The key results obtained in this research were:

1. An electroshock of 250 volts and 5 amps (60 cycles sec⁻¹) for 15 sec did not significantly affect ($p < 0.05$) plasma levels of sodium, chloride, calcium, potassium and total osmotic concentration in channel catfish.
2. Plasma osmotic and sodium concentrations in carp were correlated to environmental values.
3. Plasma chloride, potassium and calcium concentrations were not related to environmental concentrations in any of the four species in this study.
4. There was a large variation among species in plasma ionic and osmotic concentrations within individual reservoirs.
5. The salinities encountered in the four reservoirs did not induce any apparent stress in the species studied although sodium and chloride concentrations in Lake Texoma and Possum Kingdom Lake were two to four times higher than plasma levels in all four species.

CHAPTER IV

DISCUSSION

Electroshock Experiment

The magnitude of effects of handling and capture in fishes is dependent on a host of factors. These effects are unavoidable aspects of collecting fishes and unfortunately can affect their physiological states. Physiological effects reported include elevations in plasma glucose, lactate, cortisol, osmotic concentration and calcium, and reductions in blood pH, hematocrit, red and white cell counts, potassium, chloride and plasma proteins (Wedemeyer 1972; Hattingh and Van Pletzen 1974; Davis and Simco 1976; Madden and Houston 1976; Soivio and Oikari 1976). The effects observed in the blood chemistry of fishes have been attributed to activation of the pituitary-interrenal axis in conjunction with osmoregulatory dysfunction (Lewis 1971). Fagerlund (1967) has noted capture-induced interrenal stimulation of Salmonid fishes.

The plasma ions of channel catfish principally involved in osmoregulation were not affected by the experimental electroshock exposure. This finding contrasts with research on Salmonids (Fagerlund 1967; Madden and Houston 1976; Schreck et al. 1976) but corroborates results for fishes of Ictaluridae, Esocidae and Centrarchidae families (Davis and Simco 1976;

Soivio and Oikari 1976; Burns and Lantz 1978). Wedemeyer (1972) found that netting caused hypochloremia and hypercalcemia in juvenile coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri). These ion concentrations returned to control levels in 24 hours. Madden and Houston (1976) found exposure to A.C. electroshocking increased plasma chloride and decreased potassium in rainbow trout. Again return to control levels was completed by 24 hr. No effect of netting stress was noted by Soivio and Oikari (1976) on plasma sodium and potassium in northern pike (Esox lucius). Plasma chloride was not affected by handling or A.C. electroshock in another Esocid, the muskellunge, Esox masquinongy by Miles *et al.* (1974).

In experiments with channel catfish, Stickney (1971) and Davis and Simco (1976) found no changes in plasma chloride owing to handling or laboratory crowding. The little or no change in plasma ionic concentrations of channel catfish, muskellunge and northern pike indicate that these species are less susceptible to handling and capture than Salmonids.

The "hardiness" of a fish species is dependent upon its tolerance of such abiotic entities as water quality, temperature, low dissolved oxygen, other physical-chemical entities in their environment and biotic factors such as diseases. I had initially intended to include the euryhaline gizzard shad (Dorosoma cepedianum) in this study. However, capture caused

gross erythrocytic hemolysis in each shad examined. The plasma samples actually clogged the membranes of the SMAC autoanalyzer. Hemolysis was only a minor problem in the other species studied.

A key question implicit in electroshock exposure is the presence of tissue damage. I believe no appreciable tissue damage occurred in the channel catfish. The lack of significant glucose oxylate transaminase elevations in its plasma (Figure 8) implies that no increases occurred in the substrate, amino acids, which primarily come from tissue proteins. Transaminases involve the enzymatic removal of the alpha-amino group of at least twelve amino acids (Lehninger 1975). Steady levels of plasma proteins are further evidence that considerable tissue damage did not occur. In the presence of high amino acid levels from tissue damage, the liver will manufacture plasma proteins within hours (Lehninger 1975).

In comparing the hematological effects of angling, hand netting, seining and electroshock, Bouck et al. (1978) found electroshock to be the least disruptive collection method.

The short duration (15 sec) of electroshock in this research, which was similar to that employed during field sampling, produced no deaths in the 60 test catfish. Whaley et al. (1978) demonstrated that nearly 100% of bluegills (Lepomis macrochirus) survived short duration shocks of

pulsed D.C. They showed further that lower pulse frequencies and exposure times were the most critical variables in preventing mortalities. Even in the sensitive rainbow trout, Bouck and Ball (1966) found D.C. electroshock of less than 20 sec to be non-lethal, while angling produced up to 85% mortality.

Electroshock may elicit the least amount of change because the fishes are immediately stunned and are unaware of further handling. In contrast, seining and angling usually entail prolonged struggling, increasing the time for associated physiological and enzymatic responses.

Experimental bias from ionic fluctuations following handling stress can be avoided by two methods: salt additions and allowing a recovery period. Wedemeyer (1972) reported that additions of sodium chloride and calcium carbonate which raised ambient water concentrations to 100 mOsm and 75 to 120 ppm, respectively, partially or completely alleviated ionic changes in Salmonids exposed to netting. The addition of sodium chloride prior to handling prevented hypochloremia and appeared to relieve symptoms of physiological stress in muskellunge (Miles et al. 1972). Burns and Lantz (1978) and Schreck et al. (1976) recommend a post-collection interval of 24 hr to reduce effects of capture stress. For salinity related hematological studies, a 24 hr recovery period eliminates all capture-related stresses for channel catfish.

One must be careful in using the results from the channel catfish experiment in the interpretation of field measurements of other species. Use of electroshock to capture channel catfish appears to be essentially non-stressful for most applications. I would assume electroshock had similar effects on plasma ionic concentrations of the closely related flathead catfish. Carp are probably hardier than either species of catfish. Burns and Lantz (1978) found little effect from electroshock on hemoglobin, hematocrit, plasma proteins and water content of tissues in largemouth bass. Although they did not measure plasma ionic concentrations, the lack of changes in other plasma constituents indicate that neither tissue damage nor changes in water compartment volumes occurred. Therefore I am confident that the plasma ionic and osmotic concentrations measured in the four species in the field were not biased by the use of electroshock during capture.

Field Collections

Water Parameters

The large differences in water solute concentrations among reservoirs, especially sodium and chloride levels, were ideal for this type of study (Table 5). In Moss Lake and Lake Ray Hubbard sodium and chloride concentrations were much lower than plasma concentrations of all four species studied. The

opposite occurred in Lake Texoma and Possum Kingdom Lake. This indicates that opposing osmoregulatory challenges were faced by fishes in these two groups of reservoirs.

The lack of any significant differences in ionic concentrations within each reservoir among sampling trips permitted pooling of data with a corresponding increase in sample sizes. These data confirmed my original hypothesis that Moss Lake and Lake Ray Hubbard have low ionic and osmotic concentrations and Lake Texoma and Possum Kingdom Lake have high concentrations of these constituents.

Unfortunately, the SMAC autoanalyzer did not measure magnesium, a contributor to total osmotic concentration in fish plasma and reservoirs. However, data from U.S.G.S. (1979) indicate that magnesium does not comprise more than one percent of the total dissolved solids in any of the four reservoirs. Bicarbonates and sulphates typically constitute about 18 percent of the solids in Lake Texoma and Possum Kingdom Lake but comprise up to 30 percent of the solutes in Moss Lake and Lake Ray Hubbard (U.S.G.S. 1979).

Intraspecific Comparisons Among Reservoirs

Largemouth bass.--The observed inverse relationship between water salinity and plasma osmotic concentration in largemouth bass is paradoxical. These variations may indicate that largemouth bass have less stringent tissue requirements

than other species from other families such as Clupeidae, Galaxidae and Atherinidae (Hubbs et al. 1971; Chittendon 1973; and Chessman and Williams 1975). A laboratory experiment exposing largemouth bass to different salinities ranging from hyposmotic to hyperosmotic with controlled temperature and water chemistries could help identify other factors which might be influencing osmotic concentration.

The significant correlation between hematocrits and temperature in my study has been previously described by Hazen et al. (1978), who also observed a seasonal correlation between hematocrits and K-factor in largemouth bass. According to Blaxhall (1972), changes in hematocrit may be attributable to several variables including method of collection, season and disease. Mulcahy (1970) found wide variability in hematocrit values from healthy adult northern pike, Esox lucius. Wide variability (24 to 51) occurred in my study. Salinity could have been a contributing factor in variability of hematocrits and osmotic concentrations but such speculation must be tempered until further studies are completed.

The high plasma sodium values in bass from Moss Lake and Lake Ray Hubbard indicate that sodium is the major contributor (about 47 percent) to osmotic concentration and is probably the most dynamic ion in bass.

Carp.--The direct relationship between plasma sodium concentration and ambient sodium found in carp corroborates

observations of Natchin and Lavrova (1974) and indicates that carp have a wide tolerance to sodium. Carp can tolerate salinities up to 17 ppt (96 hr LC-50) which is an order of magnitude higher than their plasma concentration (Black 1969). Concentrations of both tissue and urinary salts rise under hyperosmotic conditions and urine flow is reduced accordingly (Black 1969). The slower such conditions are imposed, the greater the tolerance of change. In Lake Texoma and Possum Kingdom Lake, chronic elevated salinities probably require only hormonal adjustments involved in control of glomerular filtration rates and urine production. This is supported by K-factors, which were not significantly different in carp from the four reservoirs.

Capacities of freshwater fishes to adjust to elevated concentrations of external salts also depend on species-specific factors such as gill to body surface ratio (high in carp), gill histology, neurosecretory and hormonal control of membrane permeability as well as ambient oxygen and temperature (Conte 1969).

Also Natchin and Lavrova (1974) observed no correlation between plasma potassium, calcium and magnesium in carp and the concentrations of these ions in the environment indicating that in carp these ions are highly regulated. Plasma chloride levels in carp, in addition to those previously mentioned, were also not related to ambient levels in each of

the four reservoirs studied. Apparently carp do not regulate sodium tightly and can possibly utilize this energy savings for other metabolic processes.

Van Vuren and Hattingh (1978) found no correlation between body size or season and hematological values in carp. However, some acclimation to seasonally high temperatures and associated low dissolved oxygen would be expected in oxygen carrying capacity (indicated by hematocrit). This was not observed in carp. In brown trout (Salmo trutta), Gordon (1959) observed no relationships among osmotic concentration, plasma sodium, chloride and potassium and season or sex. Also no correlation was found between plasma ions and external environmental concentrations. These types of responses to varying physical-chemical factors have led Liu (1942) and Parry (1961) to suggest that increased salinities induce development of chloride cells in Galaxids, Cyprinids and Salmonids. An increase in the number of chloride cells, although not demonstrated in carp, could be associated with an increased efficiency in osmoregulation. The lack of any obvious osmotic stress in carp from these studies is supported by the absence of correlation between water salinity and K-factor. Crivelli (1981) observed that K-factors of carp were not significantly different between carp collected in fresh water and waters with salinities as high as 14 ppt. Also no influence owing to season was noted by Crivelli.

Channel catfish.--Channel catfish occur naturally in brackish waters (Perry 1968). They tolerate up to 11.5 g l^{-1} (96 hr LC-50) total salt (Allen and Avault 1969) and sea-water diluted to 12 g l^{-1} (Stickney and Simco 1971). Acclimation from less than 1 g l^{-1} to 8 g l^{-1} increased upper salinity tolerance by only 0.5 g l^{-1} (Allen and Avault 1969). Exposure of channel catfish to moderately high salinities (4 and 8 g l^{-1}) did not result in plasma sodium and chloride levels significantly different from catfish held in fresh water (Davis and Simco 1976). These studies support my findings for channel catfish. Apparently the salinities of Lake Texoma and Possum Kingdom Lake (2.1 and 2.8 g l^{-1} , respectively) were not high enough to elicit changes in plasma electrolytes.

Norton and Davis (1977) demonstrated that from a range of fresh water to isosmotic salt water, channel catfish can regulate internal body fluids independent of the salinity of the medium. In hyperosmotic salinities up to the tolerance limit, body fluid osmotic pressure, plasma electrolyte concentrations and renal electrolyte excretion all increase while urine volume decreases (Stevens et al. 1975).

Flathead catfish.--I could find no information in the literature concerning the responses of flathead catfish to salinity. Plasma osmotic and ionic concentrations of flathead catfish in this study were not correlated to their

concentrations in the environment. The high plasma sodium concentrations in fish from Lake Texoma did not occur in Possum Kingdom Lake where sodium concentration was the highest of the four study sites. Plasma sodium levels in black bullheads (Ictalurus melas) were consistently elevated in isosmotic salt water compared to a similar group held in fresh water (Chidambaram 1972). The changes in sodium and chloride levels in the bullheads were not always in a 1:1 ratio suggesting independent control mechanisms. Independent sodium and chloride transport has been demonstrated in goldfish (Renzis and Maetz 1973; Maetz and Romeu 1964).

Perry (1968) saw no differences in the geographic distribution of blue catfish (Ictalurus furcatus) and channel catfish owing to salinity. Perry and Avault (1968) reported that these species and white catfish (Ictalurus catus) had similar salinity tolerances (up to 12 ppt salt - 96 hr LC-50). Schwartz (1968) measured a 60 hr salinity LC-50 in white catfish at 14 ppt.

Flathead catfish would be expected to make similar responses. Probably flathead catfish were not salinity stressed in either Lake Texoma and Possum Kingdom Lake. Obviously their salinity tolerance is much higher than those encountered in these reservoirs. Any adjustments necessary could be made by flathead catfish in the manner shown by channel catfish (Norton and Davis 1977) and goldfish (Lahlou

1969), i.e., by reductions in glomerular filtration rate, sodium and chloride retention in renal tubules and urine volume.

Interspecific Comparisons Within Reservoirs

The four species in this study, largemouth bass, carp, channel catfish and flathead catfish, are quite salinity tolerant. The highest salinity encountered in this study (2.01 ppt) was not osmotically difficult for these four species. However, sodium and chloride concentrations in Lake Texoma and Possum Kingdom Lake were two to four times higher than plasma levels in all four species.

The variation observed in plasma components among fish species within reservoirs reflects the degree to which various species adjust to different salinities. The differences observed among species in salinity tolerance and adjustments to salinity changes are due to anatomical variation, degree of physiological control of osmoregulatory mechanisms and physical factors in the environment (Conte 1969; Norton and Davis 1977). Anatomical differences include gill to body surface ratio, gill histology and number of chloride cells present in the branchial epithelium of the gills. Hormonal and neurosecretory control of membrane permeability, glomerular filtration rate and reabsorption of sodium and chloride by the renal tubules facilitate water and ion balance (Bentley 1971; Griffith 1974). Ambient temperature and dissolved

oxygen requirements also influence osmoregulation differently among species (Allen and Strawn 1971). Higher than isosmotic ambient salinities can elicit increases in the number and efficiency of chloride cells (Foskett and Sheffey 1982). These adjustments in response to ambient salinity are species-dependent as are the ambient concentrations which begin to elicit changes.

Some fish families, e.g. Petromyzontidae and Cyprinide, can actively transport chloride through the gills at external concentrations of less than $0.05 \text{ mmol Cl}^{-1} \text{ l}^{-1}$ (Kirschner 1967). Other freshwater fishes such as perch (Percidae) require higher chloride concentrations before active uptake can occur (Black 1951).

Salt losses vary according to species; the Atlantic salmon, Salmo salar, loses up to 17 percent of its body chlorides per day in fresh water whereas the goldfish, Carrassuis auratus, loses only 5 percent (Conte 1969).

In freshwater fishes there is also variation in the capacity to adjust to higher than isosmotic salinities. Carp and goldfish can tolerate salinities up to 17 ppt (96 hr LC-50) which is equivalent to a salt concentration considerably higher than that in their own bodies (Black 1969; Lahlou et al. 1969). The 96 hr LC-50 for channel catfish is 11.5 ppt salinity (Allen and Avault 1971; Stickney and Simco 1971; Perry 1973).

For the fishes in my research, adjustments in ion and water balance were necessary and osmotic control mechanisms had to be functioning in reverse among fishes in the low salinity and high salinity reservoirs.

The higher salinity reservoirs, while not osmotically stressful, apparently caused some adjustments beyond those employed by freshwater fishes in hyposmotic waters. Evidence is provided by the wider variability in plasma ion concentrations among species in the more saline reservoirs. In Moss Lake and Lake Ray Hubbard, where ionic and osmotic concentrations are much lower than plasma levels in the fishes, concentrations of chloride, potassium and calcium were not significantly different among species. If this is seen as a "normal" occurrence for these four species in fresh water, the high degree of variability, especially in chloride concentrations, noted in Lake Texoma and Possum Kingdom Lake can be attributed to species-dependent adjustments to the higher salinities. These adjustments are working in reverse in hyperosmotic waters: water retention instead of excretion, ion excretion and other physiological processes mentioned previously.

Sodium appears to be the most variable plasma ion among species (116 to 160 meq l^{-1}). This would indicate that it is the most dynamic ion in these species, accounting for a large portion of the variability in total osmotic concentration in

plasma. Plasma calcium was not significantly different among species in any of the four reservoirs. This implies that in these four species it is the most highly regulated ion and that the internal tolerance limits are probably more stringent than sodium.

Natochin and Lavrova (1974) and Van Vuren and Hattingh (1978) reported similar interspecific variation within lakes and attributed it to normal specific variation encountered within a water body. This most likely reflects the situation in the four Texas reservoirs studied. Factors influencing the osmotic and ionic concentrations in fishes are complex and specific conclusions as to causes from limited observations must be conservative. However, the maximum salinities encountered at Lake Texoma and Possum Kingdom Lake, while high for freshwater reservoirs, were not osmotically stressful.

The following list contains the major contributions of this research:

1. An electroshock of 250 volts and 5 amps (60 cycles sec^{-1}) for 15 sec did not significantly affect plasma concentrations of sodium, chloride, calcium, potassium and total osmotic concentration of channel catfish. The literature indicates that electroshock is the least osmotically stressful method of collecting most species. The degree to which capture methods affect fishes is species-dependent but should

be lower using electroshock with most species as long as duration and current are limited.

2. Plasma osmotic and sodium concentrations in carp were correlated to environmental values. Carp possibly have a wide range of internal tolerance to sodium and sodium is the major osmoregulatory ion (about 50%) in carp, comprising the most dynamic component of total osmotic concentration in plasma.

3. Plasma chloride, potassium and calcium concentrations were not correlated to environmental concentrations in any of the four species in this study. The internal tolerance limits to these ions might be narrower than for sodium and therefore more stringently regulated.

4. There was a large variation among species in plasma ionic and osmotic concentrations within individual reservoirs. This has been attributed to specific variations in anatomical features, physiological control of osmoregulatory mechanisms and responses to physical environmental factors.

5. The salinities encountered in the four reservoirs did not induce any apparent stress in the species studied. However, sodium and chloride concentrations in Lake Texoma and Possum Kingdom Lake were two to four times higher than plasma levels in all four species. Osmoregulatory mechanisms apparently operated successfully from hypotonic to strongly hypertonic waters.

In conclusion, use of pulsed direct current electro-shock is an excellent non-biasing collection method for comparative salinity studies of fish plasma. With the exception of sodium in carp, plasma ionic and osmotic concentrations in bass, carp, channel catfish and flathead catfish are not directly influenced by environmental concentrations up to 2.01 ppt salinity. The major contributor to total osmotic concentration in these species is sodium.

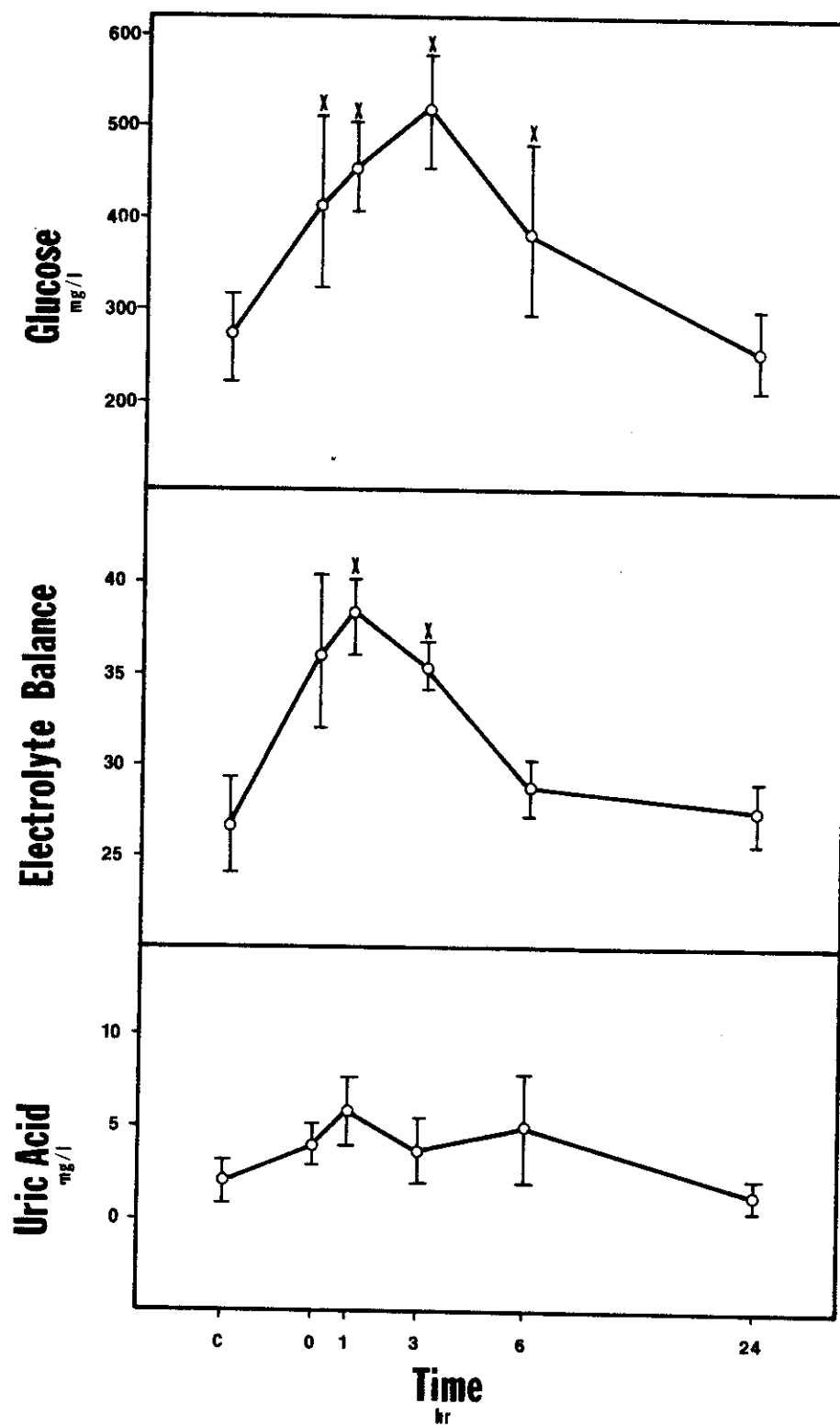
APPENDIX

Many plasma constituents not directly related to osmoregulation were measured by the SMAC autoanalyzer and the results are presented here. Plasma constituents that were significantly affected ($p < 0.05$) by electroshock were glucose, electrolyte balance, uric acid, alkaline phosphatase, phosphorus, albumin, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). Mean glucose concentrations (Figure 19) rose from control levels of 358 mg l^{-1} to a peak of 821 mg l^{-1} three hr postshock. Return to control levels took 24 hr. Electrolyte balance is a measure of ionic equilibrium calculated by the equation:

$$([\text{Na}] + [\text{K}] + \frac{[\text{Ca}]}{2} + 2) - ([\text{Cl}] + \text{CO}_2)$$

Following electroshock mean electrolyte balance quickly elevated from control levels of 26.57 (Figure 19) to a peak after one hr of 38.42. Return to control levels required about six hr. The SMAC autoanalyzer was designed for use on human plasma and this is reflected in the equation of balance. Therefore any changes in these values from fish plasma might not be valid. Mean uric acid concentrations (Figure 19) rose immediately after electroshock and peaked after one hr at 6.2 mg l^{-1} and returned to control levels (2.1 mg l^{-1}) within three hr of electroshocking. Mean alkaline phosphatase

Figure 19. Mean plasma glucose, electrolyte balance and uric acid concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . X denotes groups that were significantly different ($p < 0.05$) from the control group.



levels (Figure 20) rose initially from control values of 51.1 U l^{-1} to a peak of 59.2 U l^{-1} and gradually declined to below control values after 24 hr. Mean phosphorus concentrations (Figure 20) were elevated above control levels (66.4 meq l^{-1}) to a maximum of 105.2 meq l^{-1} one hr post shock. Phosphorus levels fell below control values after six hr. Mean albumin concentrations rose rapidly from control levels of 8.9 g l^{-1} to a peak after one hr of 12.5 g l^{-1} . Return to pre-shock levels took 24 hr (Figure 20). Mean CPK concentrations (Figure 21) rose initially from control values of 305 U L^{-1} to a maximum of 581 U l^{-1} after three hr. Return to control levels required three hr. Mean LDH concentrations (Figure 21) rose soon after electrochock to a maximum of 560 U l^{-1} after three hr. Return to pre-shock levels required six hr.

A second group of plasma components was not significantly affected ($p < 0.05$) by the electroshock treatment. These included low density lipoprotein (LDL) cholesterol, globulin and creatinine (Figure 22) and cholesterol, iron and triglycerides (Figure 23).

Consistent with studies of Racicot et al. (1976), Schreck et al. (1976), Bouck et al. (1978) and Burns and Lantz (1978) increases in LDH, glucose, alkaline phosphatase and CPK occurred in channel catfish subsequent to electroshock. Handling stress and the associated muscular activity have been

Figure 20. Mean plasma alkaline phosphatase, phosphorus and albumin concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . X denotes groups that were significantly different ($p < 0.05$) from the control group.

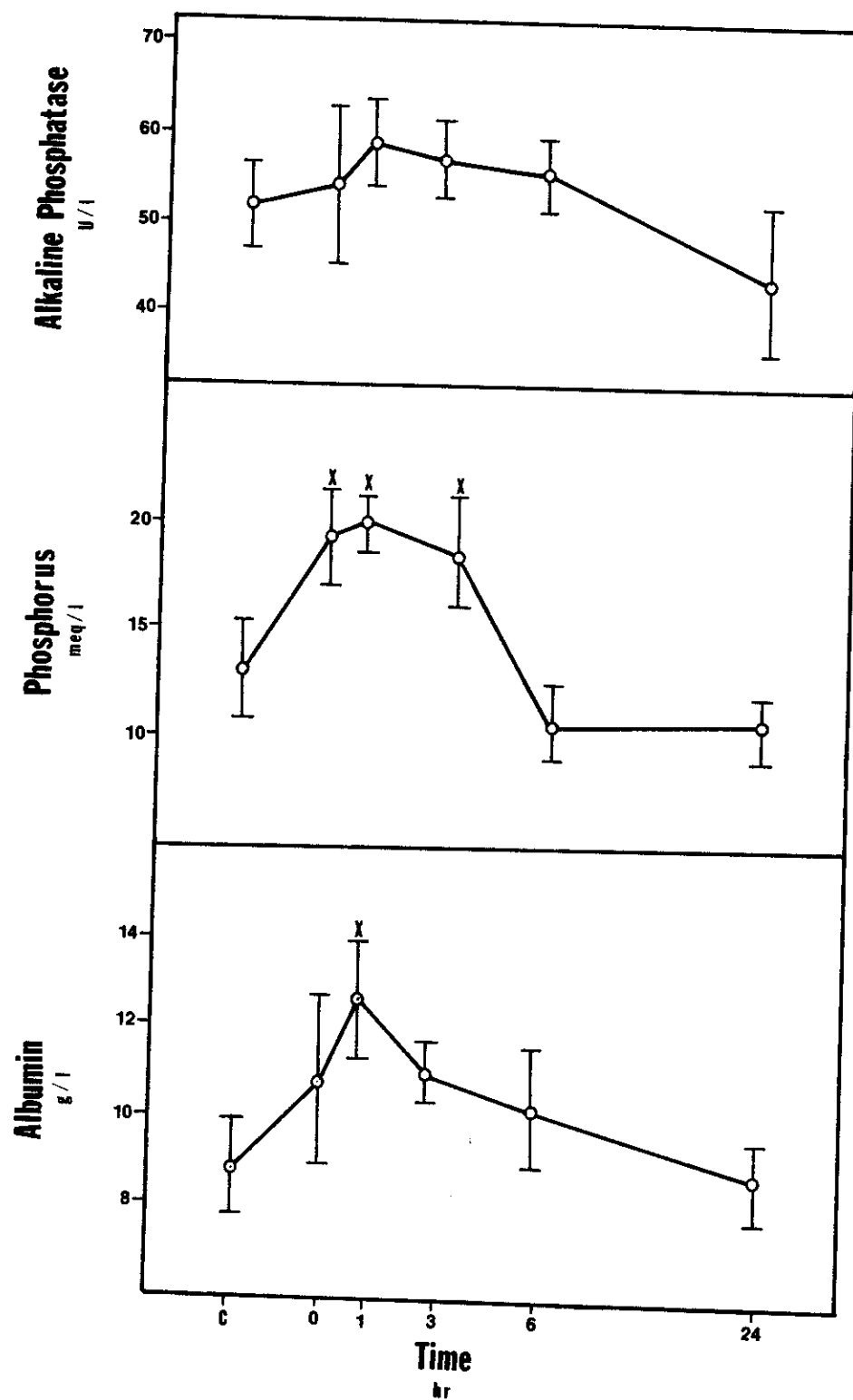


Figure 21. Mean creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . X denotes groups that were significantly different ($p < 0.05$) from the control group.

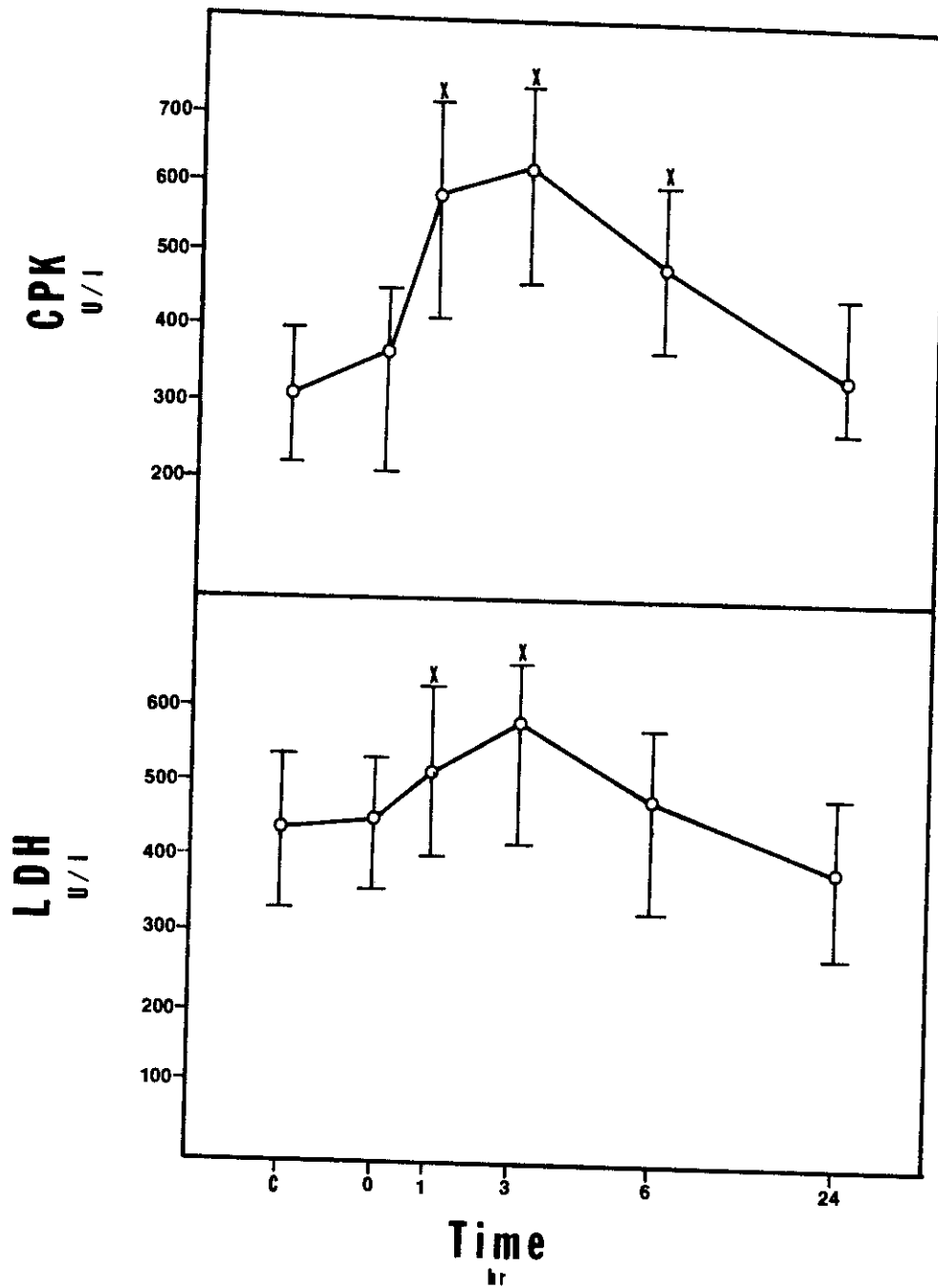


Figure 22. Mean low density lipoprotein (LDL) cholesterol, globulin and creatinine concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . X denotes groups that were significantly different ($p < 0.05$) from the control group.

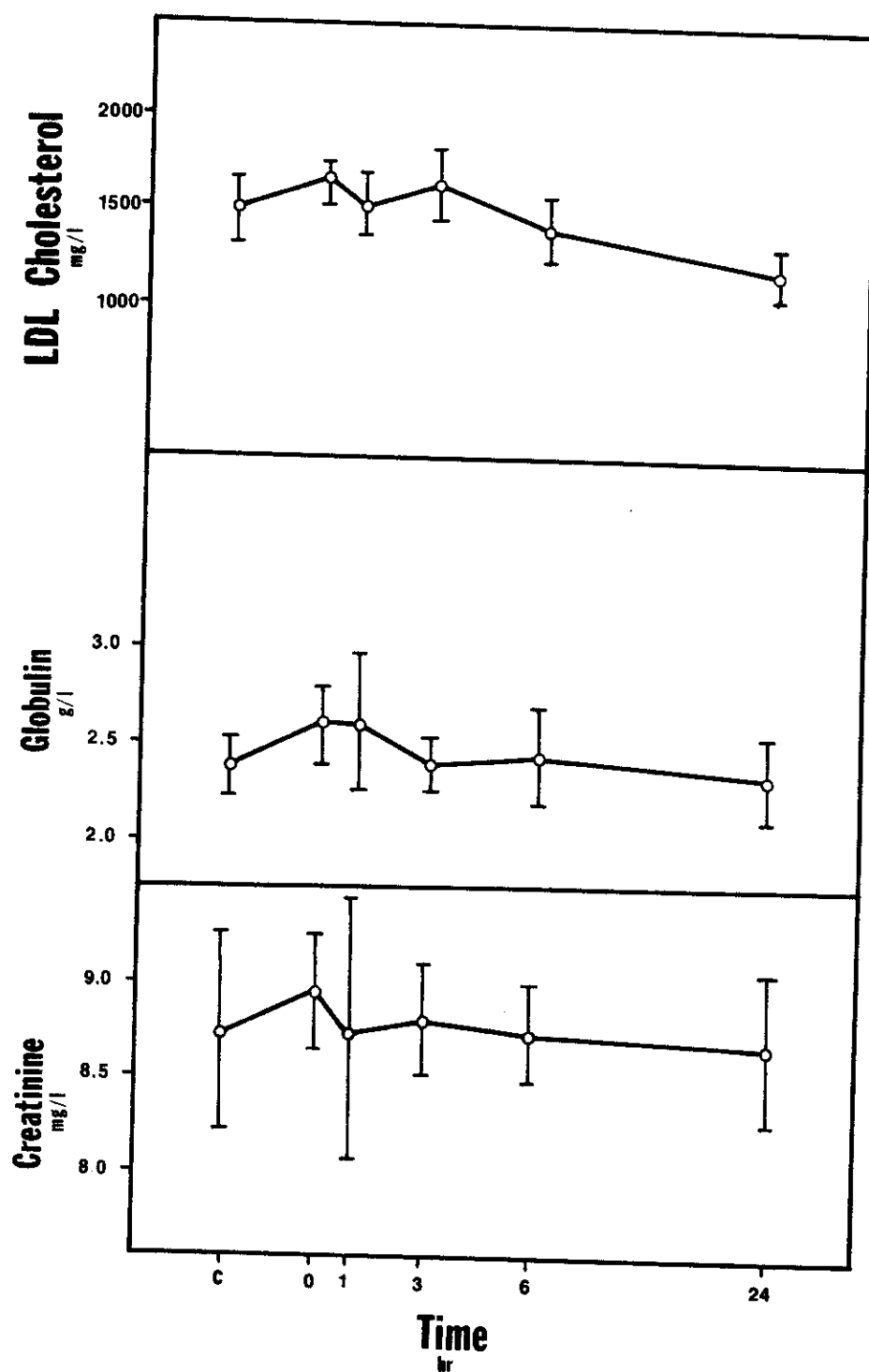
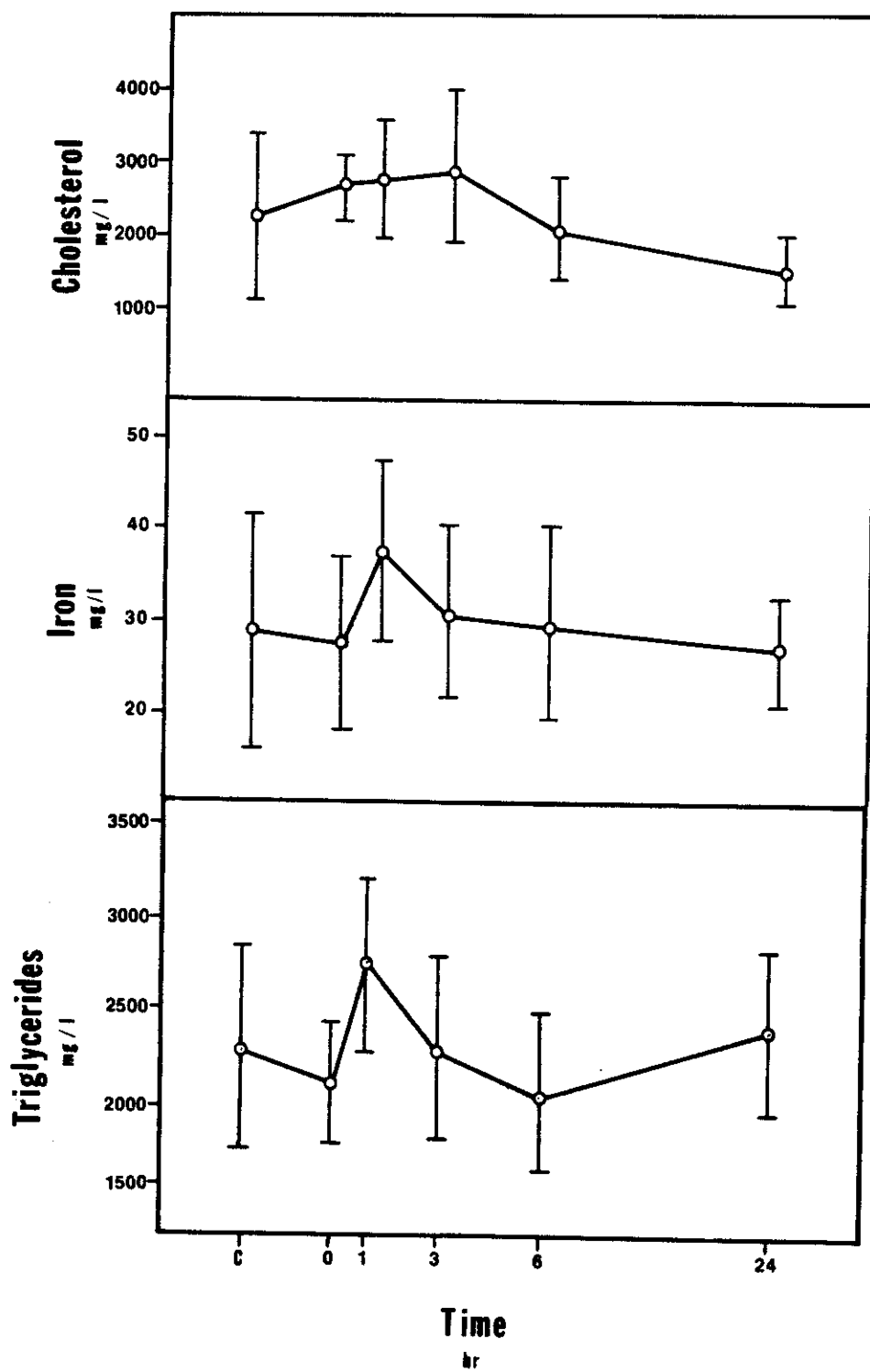


Figure 23. Mean plasma cholesterol, iron and triglyceride concentrations (\pm ranges) in five groups of channel catfish and one control group (n = 10) following exposure to a 15 sec direct current electroshock of 250 volts and 5 amps at 60 cycles sec^{-1} . X denotes groups that were significantly different ($p < 0.05$) from the control group.



reported by Burns and Lantz (1978), Perrier et al. (1978) and Soivio and Oikari (1976) to significantly elevate plasma glucose and lactate concentrations. The increases observed in channel catfish are most likely a consequence of anaerobic muscular activity induced by electroshock (Schreck et al. 1978). This activity generated large amounts of lactate and depleted glucose, which was ameliorated by conversion of glycogen to blood glucose in the liver. The data on alkaline phosphatase, LDH and CPK support this conclusion. Alkaline phosphatase has broad specificity and is capable of acting on a number of substrates, mainly esters of phosphoric acid. These esters are produced by muscular activity (Lehninger 1975).

During anaerobic muscular stimulation, phosphocreatine is depleted. This increases the substrate levels of creatine and ADP which are acted upon by CPK in the rephosphorylation of phosphocreatine.

An additional finding was the sex-dependent nature of plasma cholesterol levels in carp from the field studies. Males ranged from 612 to 2120 mg l⁻¹ (mean 1280) and females ranged from 1940 to 4860 mg l⁻¹ (mean 2860). These differences were significant ($p < 0.05$). This difference is most likely related to reproduction and the need for cholesterol in egg yolking in females. All collections were made after the peak spring reproduction stage. The plasma cholesterol concentrations of female carp captured during different collecting

trips were not significantly different. Similar results were obtained for males.

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