PRELIMINARY BIOCHEMICAL OBSERVATIONS OF FISHES INHABITING AN ACIDIFIED LAKE IN ONTARIO, CANADA

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ABSTRACT

Blood serum and other tissues of white suckers *(Catostomus commersoni)* and other species of fish have been examined in a preliminary effort to understand losses of fish from acid lakes. Maturing females of highly acid George Lake failed to show the normal degree of increase in serum calcium over males captured in the same location. Elevated serum calcium was always observed in females, relative to males, from non-acid locations when maturation stages were similar. A number of body tissues were examined to determine whether the distributions of calcium or other elements were altered and the only striking change evident in fish from George Lake was their high manganese content. Bone in particular was examined for N, Na, Mg, P, K, and Ca and no evidence of demineralization was found. Other tests such as hemoglobin indicated that an anticipated adaptive response to asphyxia was absent. Our results indicated that potential mechanisms of fish losses such as asphyxia and starvation are unlikely to have been significant. However, they do not firmly establish any alternate mechanism. As a working hypothesis we suggest that calcium dynamics may limit ovarian development and that absorption of this metal at exchange surfaces may be impaired.

INTRODUCTION

The effects of acid upon fishes have been subjects of scientific study for well over half a century, and they nonetheless remain essentially unknown. Early studies attempted to explain the presence or absence of certain fish species on the basis of water chemistry data including pH (Jewell and Brown, 1924; Powers, 1929; Creaser, 1930) with varying degrees of success. Several more recent studies have noted declines and losses of fish populations coincident with acidification (Harrison, 1958; Beamish and Harvey, 1972; Jensen and Snekvik, 1972). Field observations of fish in a number of lakes in the Sudbury area of Ontario indicated that some species of fish were more sensitive to acidification than others (Beamish and Harvey, 1972; Beamish, 1974a) and further that fish losses were not the result of prior elimination of food organisms (Beamish, 1974b). There seemed little doubt that acid, possibly in combination with other pollutants, acted upon fish to bring about the collapse of populations as a result of failure of mature individuals to spawn (Beamish and Harvey, 1972). Our fundamental assumptions were simply that some abnormality must be present in fish in acidified lakes - particularly those fish of reproductive age - or spawning would not cease and further that it should be possible to identify and measure any such abnormalities.

This report will include some preliminary screening analyses made in our efforts to apply biochemical pathology to fish in George Lake, a lake undergoing acidification some 65 km southwest of Sudbury, Ontario. The analyses are compared with those from fish taken from other locations, and with those in literature reports. The types of analyses to be made were selected partly because previous studies had suggested they might be instructive and partly because we had some experience in their performance. Certain highly desirable analyses such as blood respiratory gases were excluded on the grounds of difficult sampling or storage requirements. Considering the widespread nature of the acidification problem, and the long history of investigation, there is a striking paucity of literature on the mechanisms involved in acid toxicology. An equally striking paucity of literature exists to help the pathologist recognize those values which may be abnormal. With the exceptions of those few species of fish which have enjoyed intensive culture for economic or research purposes little work has been done to understand the biochemistry of freshwater fish. It is hoped that the data presented here will prove useful for comparison with future studies of fish in acidified lakes, and that future work will enable us to interpret these data more rigorously.

MATERIALS AND METHODS

Fish were captured form several sources by various means including trap nets, gill nets, and hand dip nets. In some cases we wished to observe the effect of forced exercise on suckers *(Catostomus commersoni)* and a period of one half hour was devoted to "chasing" the fish with a dip net either in a mesh enclosure in the acid lake or in an aerated laboratory tank.

Blood samples were taken by syringe from caudal vessels except in the case of white suckers for which heart puncture was found to be more convenient. Blood was allowed to clot at 0°C and serum was decanted either for immediate analyses or more commonly for storage frozen until laboratory examination could be made. After blood was obtained, fish were examined internally to determine sex and maturity and they too were frozen until analyzed. The methods of chemical analyses were, in general, standard procedures of microanalyses (Mattenheimer, 1970) adapted to the small quantities of sample available and they are tabulated below.

Analysis	Procedure
serum sodium	flame emission
serum chloride	microtitration (Schales and Schales, 1941)
serum protein	biuret
serum inorganic phosphate	molybdate
serum glucose	glucose oxidase
serum calcium	atomic absorption
serum cortisol	fluorometric (Mattingly, 1962)
total serum phosphorus	ultraviolet irradiation, then molybdate
whole blood lactate	lactate dehydrogenase
whole blood hemoglobin	cyanmethemoglobin
whole blood hematocrit	centrifugation
whole blood pH	Radiometer combination electrode
metals in bone, skin, gonads and scales	hydrochloric acid digestion and flame emission (Na, K) or atomic absorption (Ca, Mg, Mn)
metals in muscle	nitric acid digestion and flame emission (Na, K) or atomic absorption (Ca, Mg,Mn)
nitrogen in bone	sulfuric acid digestion and micro Kjeldahl procedure
Phosphorus in bone	hydrochloric acid digestion, ultraviolet irradiation, then molybdate

RESULTS AND DISCUSSION

Our earliest hypothesis that fish in an acid lake might suffer asphyxia was based upon several literature reports and upon the simple reasoning that gill surfaces are large and in constant contact with the acid water, hence they may well be expected to function at a reduced capacity. There is no doubt that water pH effects the minimum levels of dissolved oxygen which can be tolerated by silver salmon (Townsend and Cheyne, 1944). A possible mechanism by which acid might limit oxygen transport is the precipitation of mucus on gill filaments with accompanying decreased permeability (Westfall, 1945). Decreased oxygen consumption has been shown to result from acid exposure of brook trout

(Packer and Dunson, 1972). If oxygen transport by fish has been reduced in the acid lake, we might reasonably expect to observe adaptive responses such as increases in hemoglobin and hematocrit, since these have been produced experimentally both by acid exposure (Vaala and Mitchell, 1970; Anthony et al. 1971) and by asphyxia (Phillips, 1947). Hemoglobin levels were determined for five groups of white suckers in 1972 and results are tabulated below.

Hemoglobin levels in white suckers (*Catostomus commersoni*) from an acid Lake (George) and two other lakes

Group and time	Condition	Hemoglobin concentration (g/dl) ±S.D	Range	No. of fish
George Lake Sept. 1972	trap net unexercised	8.67 ± 1.25	5.85-10.24	10
George Lake Sept. 1972	trap net exercised	9.53 ± 0.80	8.38-10.53	12
Georgian Bay Sept. 1972	gill net	8.28 ± 1.44	6.33-11.22	15
Lake Manitoba Dec. 1972	laboratory unexercised	8.84 ± 0.83	7.53-10.01	9
Lake Manitoba Dec. 1972	laboratory exercised	8.22 ± 0.83	6.89-9.32	7

These values are generally quite similar and they indicate that little if any adaptive increase has occurred in George Lake suckers. Black (1955) found mean hemoglobin levels from 8.8 to 11.5 g/dl in the sucker *Catostomus catostomus* from British Columbia and our values are comparable with Black's lower groups. The George Lake pre-exercise values are almost the same as the Lake Manitoba/laboratory pre-exercise group. Vaala and Mitchell (1970) noted a 29 percent increase in hemoglobin concentrations of acid-treated brook trout and certainly changes of that magnitude are absent from these acid lake (pH 5.1) suckers.

Hematocrit measurements were made on George Lake brown bullheads (*Ictalurus nebulosus*) trap netted in August, 1973 and the mean hematocrit (± S.D.) was 28.9% ± 4.6 with a range of 21% to 36%. For comparison, Haws and Goodnight (1962) reported a mean hematocrit of 27.9% ± 6.5 with a range of 15.0% to 40%. Black (1940) found a slightly higher mean value of 32.2%, the range being 20.5% to 39.7%. By comparison with literature values then, brown bullheads in the acid lake exhibited no obvious adaptive increase in hematocrit. Some however, she exceeded males by only a factor of 1.3, consistent with other failing species. A ratio of serum calcium in females to that in males of about 1.4, then has separated species failing to reproduce in George Lake from species still reproducing in that lake and also from similar species reproducing in other lakes. Assuming the low calcium ratios are more than coincidence, and lacking data from fish inhabiting George Lake prior to acidification, we cannot be certain whether low calcium excesses in females have resulted from acidification, or whether acidification has erected a selective barrier which can be met only by species maintaining higher female:male calcium ratios during maturation.

Sites of uptake of calcium from water have been reviewed by Simmons (1971) and it seems that calcium is exchanged at gills and other body surfaces, particularly those around certain fins. Dietary calcium has been reported to accumulate most readily in skeleton and skin in brown trout (Podoliak and Holden, 1964). As shown earlier, we have found no indication of reductions in calcium content of bone, muscle, or scales and similarily we found no shortage of calcium from skin as shown below.

Skin calcium concentrations in white suckers
from George Lake and Lake Manitoba

Source and time	Skin calcium (g/100 g wet skin) ±S.D	Number of Samples	Range
George Lake, Sept. 1972	8.44 ± 0.80	5	7.1 - 9.0
George Lake, Dec. 1972	7.73 ± 1.02	7	6.5 - 9.6
Lake Manitoba Jan. 1973	8.54 ± 1.38	5	6.9 - 10.3

Ovaries developing in two white suckers taken in December, 1972 contained calcium at concentrations of 215 and 193 mg/kg (wet weight) concentrations above muscle concentrations for the same fish (100 and 90 mg/kg respectively). For comparison, suckers maturing in Roddy Lake in March, 1974, had ovarian calcium levels averaging 272 mg/kg (Roddy Lake has an even lower calcium content than George Lake). It is obvious that substantial quantities of calcium are required for ovarian development or such high concentrations could not be achieved in such large organs. Our analyses suggest that calcium was not obtained from internal sources such as muscle, skin, scales, or bone and we agree with the suggestion by Fleming et al. (1964) that at least part of the extra calcium needed by maturing females must be obtained from the environment. It seems probable that the explanation of a calcium matabolism problem in fishes inhabiting an acid lake is more likely to be found in caution must be exercised with these hematocrit measurements, however, since they and several other measurements tended to change in an orderly manner throughout the sampling sequence. In fact, when hematocrit was plotted against the sequence number of a fish in the sampling order (Fig. 1), a significant ($p \leq 0.01$) correlation ($r = -0.83$) was found to exist. Other authors have not reported this effect and it is presumably some reaction of the fish to the sampling experience or possibly a segment in a diurnal rhythm.

Inorganic phosphate has also been found to increase in fish during asphyxia (Bange, 1962). We obtained a mean inorganic phosphate P level of 8.18 ± 1.65 mg P/dl in brown bullhead serum in August 1973. McCay (1931) reported a value of 7 mg P/dl in this species and Hunn (1972) found 18.77 mg PO_4/dl (6.12 mg P/dl) in the yellow bullhead (*Ictalurus natalis*) from the upper Mississippi river. The values we obtained then are somewhat higher than expected but once again some artifact may be suspected since phosphate decreased ($p \leq 0.01$; $r = -0.73$) throughout the sampling order (Fig. 1). Total serum phosphorus values were not related to sampling sequence (25.2 ± 6.77 mg P/dl) and it seems likely that high early phosphate values represent storage time. That is, the inorganic phosphate probably increased during blood and serum storage at 0°C at the expense of labile organic phosphorus compounds. The analyses were performed in the field on the evening of collection, and the times between sample collection and phosphate analyses decreased from about 9 hours for the first sample to about 5 hours for the last sample.

Serum glucose has been reported to increase during asphyxia in several fish species (Menton, 1927; Hall et al. 1926; Heath and Pritchard, 1965) including bullheads (Simpson, 1926). The review by Chavin and Young (1970) indicates that mean glucose levels from 22 mg/dl to 59 mg/dl have been reported for brown bullheads. Our values were almost double the previous maximum. For 15 fish from the acid lake in August 1973 (water temperature, 21°C) the mean glucose concentration (± S.D.) was 109.7 ± 46.9 mg/dl and the range was 32.8 to 196 mg/dl. There was a tendency to increase ($p \leq 0.05$; $r = +0.57$) throughout the sampling order (Fig. 1), which may indicate asphyxia or other stress during sampling, however, even initial values were high for bullheads. The first five values, for example, were 80.5, 74.3, 32.8, 102 and 64.5. Silbergeld (1974) has recently indicated the value of blood glucose assays as indicators of stress in fish, and these glucose levels suggest some sub-lethal stress related to life in the acid lake or possibly to the capture experience.

Blood pH also increased systematically as fish were sampled sequentially throughout the afternoon (Fig. 1). We wished to obtain measurements of blood pH because both Lloyd and Jordan (1964) as well as Packer and Dunson (1970) found a fall in blood pH of salmonids exposed to acid, and it has been established that acidosis induced by injection of acids can kill fish (Jonas et al. 1962). We measured blood pH in whole blood (lithium heparin anticoagulant) from 15 brown bullheads in the acid lake at 21°C in August, 1973, and we obtained a very wide range from 7.112 to 7.816. Two standard buffers recommended by Bates (1973) were used before and after each blood sample, and it seems unlikely that measuring errors would extend beyond the last two decimal points. The upward trend throughout the afternoon of sampling was striking ($p \leq 0.01$; $r = +0.89$), and this direction is contrary to that expected during asphyxia (Caillouet, 1968). The average pH obtained simply as the sum of all readings divided by 15 (the number of samples) was 7.562, and the average obtained by first converting each pH to hydrogen ion concentration was 7.489. It seems that the first averaging procedure is in more common use in fish studies. Fish blood pH measurements have been reviewed by Houston (1971) and a range of means from 7.49 to 7.99 has been reported for *Ictalurus* species; in spite of the relation to sample sequence, then, our mean of 7.56 is within the range of previous means. Our early values are, however, the most acid reported. For example, our first three values were 7.198, 7.112, and 7.332 and our last three were 7.816, 7.806, and 7.763. In spite of low early values the fish showed no indication of distress, and evidently critically acid blood pH is below 7.112 in bullheads. Our early values suggest that acidosis is in fact a problem for bullheads in George Lake, however, the mean and later values argue against this. Garey (1972) found that several captured fish had much lower blood pH values than did the same species catheterized and swimming freely, the respective values for pink salmon (*Oncorhynchus gorbuscha*) being 7.54 and 8.10 at 14°C.

Animals generally turn to anaerobic metabolism when oxygen does not reach tissues rapidly enough to meet the needs of cellular respiration. Under these conditions we might expect an increase in some product of anaerobic metabolism, namely lactic acid. Lactate levels were determined in blood of white suckers captured in the acid lake in September 1972 with and without a period of forced exercise defined by "chasing" the caged fish with a dip net for half an hour. The results are shown below.

Lactate levels in white suckers (*Catostomus commersoni*) from an acid lake (George) and two other lakes

Group and time	Condition	Water temp. °C	Lactate concentration (mg/dl) ±S.D	Range	No. of fish
George Lake Sept. 1972	trap net unexercised	17	44.7 ± 16.5	22.2-69.8	10
George Lake Sept. 1972	trap net exercised	17	96.1 ± 8.63	82.9-110.0	12

Lactate levels in white suckers (*Catostomus commersoni*) from an acid lake (George) and two other lakes

Group and time	Condition	Water temp. °C	Lactate concentration (mg/dl) $^{\pm S.D}$	Range	No. of fish
Georgian Bay Sept. 1972	gill net	-	67.4 ± 24.3	30.7-112	15
Lake Manitoba Sept. 1972	laboratory unexercised	3.5	19.8 ± 3.30	14.9-26.3	15
Lake Manitoba Dec. 1972	laboratory exercised	3.5	35.1 ± 16.5	14.0-68.0	14

Generally the September samples are well above those reported by Black (1955) for *Catostomus catostomus*; Black obtained mean values from 15.4 to 19.1 mg/dl without exercise and from 54.4 to 56.4 mg/dl after 15 minutes exercise at 11.5°C. There is some disagreement in the literature concerning the relation between water temperature and blood lactate in fish. For example, Wendt (1964) observed a large lactate difference between groups of speckled trout (*Salvelinus fontinalis*) exercised at 5°C and 15°C; however, Black (1957) found no difference between groups of Kamloops trout (*Salmo gairdneri*) exercised at 11.5°C and 20°C. The total range for George Lake specimens was 22.2 to 110 mg/dl, almost the same as that for gill netted fish from Georgian Bay (30.7 - 112 mg/dl); however, the effect of gill netting upon lactate levels in the Georgian Bay group may have been large. The important question is whether lactate concentrations before exercise in George Lake suckers were pathologically high, and a definitive answer will have to await comparison with future data. Striking seasonal variations in lactate levels have been observed in exercised rainbow trout (*Salmo gairdneri*) (Lockhart et al. 1975); increases in our values over those reported by Black (1955) may have related to temperature or some other natural variable. The George Lake suckers and those from Lake Manitoba approximately doubled their blood lactate levels as a result of the forced exercise, indicating that life in the acid lake had left the mechanism of glycolysis intact at least qualitatively.

In general, the measurements carried out to detect the presence of asphyxia do not answer the question unequivocally. Adaptive responses such as increases in hematocrit and hemoglobin seem to be absent, but relatively high levels of glucose and lactate in unexercised groups of George Lake fish leave the question in doubt. The four measurements which changed throughout the sampling sequence (Fig. 1)

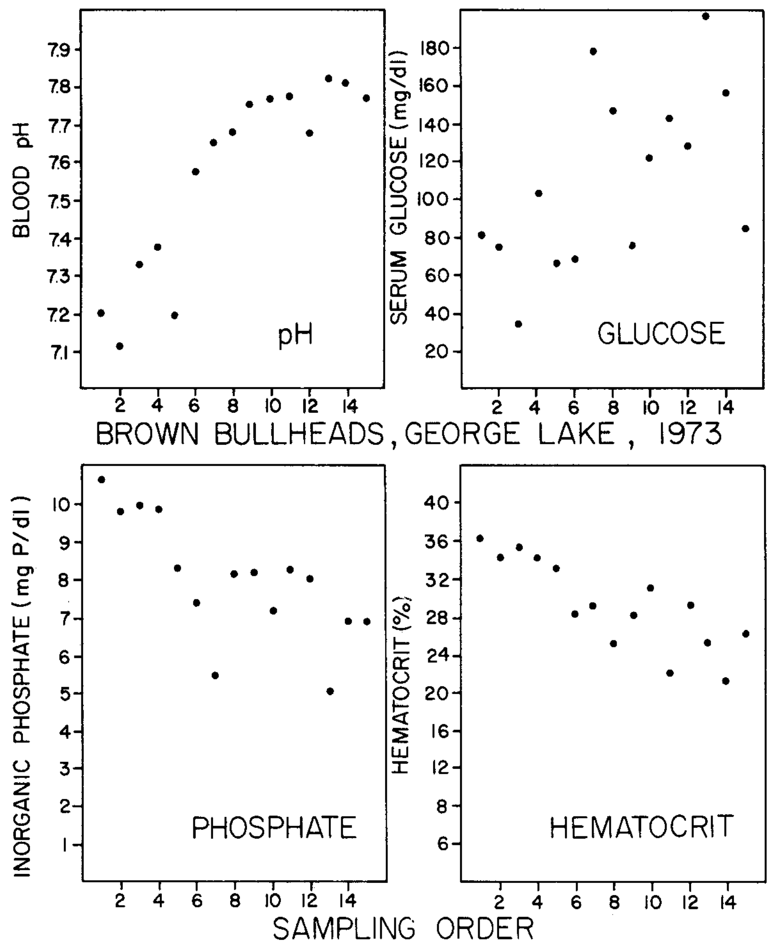

Figure 1. Apparent relationship between position in sampling sequence and four blood measurements for brown bullheads trapped in George Lake in August, 1973. Fish were removed from the water one at a time for collection of blood, thus the first fish removed was number 1 in the sampling order, and the last fish some 4 hours later, was number 15. Correlation co-efficients between these numbers in the sampling order and the four blood measurements shown were: $r = +0.89$ for pH ($p \leq 0.01$); $r = +0.57$ for glucose ($p \leq 0.05$); $r = -0.73$ for phosphate ($p \leq 0.01$); $r = -0.83$ for hematocrit ($p \leq 0.01$).

do not support the hypothesis that asphyxia was developing progressively during the sampling period since three of the four (hematocrit, inorganic phosphate, and pH) changed in the unexpected direction.

An unusually high frequency of deformities was noted in the white sucker population of George Lake (Beamish et al. 1975). In view of the fall in blood pH demonstrated in acid-treated rainbow trout (Lloyd and Jordan, 1964) and brook trout (Packer and Dunson, 1970), we wished to test the attractive hypothesis that deformities were the result of demineralization of bone as salts were extracted to combat a condition of chronic acidosis. Experimental exposures of fish to acid solutions have resulted in deformities (Beamish, 1972; Mount, 1973); the stoichiometry of skeletal demineralization has been described by White et al. (1968). Unfortunately, suckers were not caught in trap nets during our 1973 sampling efforts and we used brown bullheads instead as the only species available. Measurements of blood pH seemed unlikely to reveal acidosis in this species since early work by Jobes and Jewell (1927) indicated no depletion of the alkali reserve of blood from bullheads in an acid lake in Michigan, and furthermore, deformities have not been observed in this species. The results of pH measurements in bullheads were presented above and they are difficult to interpret in view of the progressive increase throughout the afternoon while samples were collected. It seems that some but certainly not all the bullheads were in a condition of acidosis at the time of sample collection. The second part of a demineralization process seemed more readily detected simply by analyses of bone. We analyzed anterior ribs from suckers captured in the acid lake in 1972 for nitrogen, sodium, magnesium, phosphorus, potassium and calcium (Table 1) for comparison with suckers from Lake Manitoba. The greatest difference between groups of suckers was in rib magnesium content and acid suckers were 107% of non-acid suckers. Biltz and Pellegrino (1969) included one species of fish (*Lutjanus aya*) in their survey of cortical bone from the femora and tibiae of vertebrates and they found Ca, 23.26%; P, 10.85%; Mg, 0.3%; and Na, 0.32%. Komárková and Bilyk (1973) have reported calcium and phosphorus values for several different bones of various fish species, and their rib values were 22.9 to 26.0% for Ca and 10.2 to 11.2% for P. Based on rib analyses then, suckers in the acid lake had not experienced severe demineralization of bone, and other causes of deformities will need consideration. Deformities are easily produced in fish; Rasquin and Rosenbloom (1954) induced severe spinal deformities by simply maintaining fish in darkness and Mehrle and Meyer (1975) induced them by exposing fish to toxaphene.

Very severe loss of one mineral element, namely sodium, has been found to result from experimental acid exposure (Packer and Dunson, 1970). Sera of brown bullheads collected in August 1973 were analysed for both major ions, sodium and chloride, and the results are shown on the next page.

Source		Sodium (mg/dl)	Chloride (mg/dl)
George Lake (trap net)	$\bar{X}$	272.4	377.7
	SD	29.3	16.4
	range	223 - 323	352 - 412
	n	15	15
Georgian Bay (gill net)	$\bar{X}$	295.8	353.8
	SD	43.0	25.3
	range	180 - 380	302 - 387
	n	19	19

Table I

Bone (Rib) Analyses : White Suckers, December 1972

Nitrogen, Sodium, Magnesium, Phosphorus, Potassium, Calcium
as Percent Dry Weight of Bone

		N	Na	Mg	P	K	Ca
George Lake		5.1	0.35	0.40	10.0	0.44	22.9
		5.1	0.33	0.39	9.8	0.42	22.3
		4.8	0.37	0.56	10.4	0.46	22.7
		5.0	0.47	0.46	10.6	0.44	22.5
		5.1	0.44	0.47	9.4	0.46	20.8
		5.1	0.35	0.52	10.0	0.41	21.7
		5.1	0.41	0.57	10.4	0.39	22.4
	$\bar{X}$	5.04	0.39	0.48	10.1	0.43	22.2
Lake Manitoba		5.5	0.34	0.37	10.2	0.46	21.5
		5.1	0.39	0.47	10.4	0.45	22.6
		5.1	0.37	0.49	10.8	0.42	24.0
		5.0	0.36	0.45	10.7	0.41	24.4
	$\bar{X}$	5.2	0.37	0.45	10.5	0.44	23.1

Hunn (1972) obtained sodium and chloride values of 134.3 ± 1.4 meq/l (308.9 mg/dl) and 113.3 ± 3.9 meq/l (401.6 mg/dl) respectively. Comparison of mean sodium concentrations (using a transformation to natural logarithms) indicated that bullheads did not differ between George Lake and Georgian Bay ($t = 1.624$, $df = 32$). To the extent that Georgian Bay values are representative of bullheads, the acid-exposed fish have not suffered depletion of major serum ions sodium and chloride. The same may be said of bone sodium in suckers from the data in Table 1. Since the major portion of body weight is composed of muscle tissue, that too was examined in the 1972 white sucker samples, and the results (Table 2) suggest that neither sodium nor other major metals

Table II

White Muscle Metals, White Suckers. 1972

		Metal Concentrations (mg/kg wet tissue)			
		Sodium	Potassium	Calcium	Magnesium
George Lake					
Sept. 1972	$\overline{X}$	277	4440	98.1	348
Unexercised From Trap	SD	18.9	172	6.84	28.9
	Range	250-300	4200-4700	92-111	296-376
	n	7	7	7	7
George Lake					
Sept. 1972	$\overline{X}$	250	4110	95.3	315
Exercised From Trap	SD	28.7	176	7.47	12.6
	Range	200-300	3900-4300	86-107	292-334
	n	9	9	9	9
George Lake					
Dec 1972	$\overline{X}$	400	3750	130	290
Gill Netted	SD	67.3	206	70.9	17.6
	Range	290-500	3430-3930	90-287	262-309
	n	7	7	7	7
Georgian Bay					
Sept 1972	$\overline{X}$	250	4190	92.1	321
Gill Netted	SD	57.4	183	6.26	21.0
	Range	180-340	3800-4400	79-104	275-349
	n	12	12	12	12

were depleted below Georgian Bay values in September 1972. Thurston et al. (1959) reported sodium and potassium concentrations for fillets of a number of freshwater species and for suckers (*Catostomidae* sp) the mean sodium concentration was 590 mg/kg (range, 460 - 860 mg/kg); for potassium the mean concentration was 3280 mg/kg (range, 3260 - 3330 mg/kg). In comparison with fillets then, all the muscle analyses here are low in sodium and high in potassium. It is of interest that Packer and Dunson (1970) did not observe sodium differences among wild trout inhabiting streams ranging in pH from 6.05 to 7.10.

Serum cortisol has been measured in fish as a response to non-specific stress (Wedemeyer, 1969) although the value of this measurement as an indicator of chronic low-level stress is in doubt (Hill and Fromm, 1968). Little attention has been given to suckers, however, Phillips (1959) reported plasma cortisol concentrations of 44 µg/dl for male suckers and 20 µg/dl for females. Cortisol concentrations were measured in sera of suckers captured in 1972 and the results are tabulated on the next page.

Serum cortisol levels for white suckers (*Catostomus commersoni*) from an acid lake (George) and a non-acid lake (Georgian Bay)

Group and time	Condition	Water Temp. °C	Concentration (μg/dl) ± S.D	Range	No. of fish
George Lake Sept. 1972	trap net unexercised	17	50.0 ± 11.1	28.0-62.0	10
George Lake Sept. 1972	trap net exercised	17	109. ± 34.6	58.0-190.	12
Georgian Bay Sept. 1972	gill net	-	88.6 ± 28.0	35.2-119.	15

Prior to exercise, the George Lake males and females were indistinguishable on the basis of cortisol, but females appeared to respond slightly more to the forced exercise than did males; for the George Lake exercised group males had mean cortisol levels of 92.3 (Range 58 - 120) μg/dl and females 126.5 (Range 95 - 190) μg/dl. Fagerlund (1967) noted that female sockeye salmon also showed a greater response to stress than males. The response to forced exercise in these fish has been to approximately double the pre-exercise cortisol concentrations, and this might be considered a relatively low increase as compared with salmonids, however, the pre-exercise values may not be true resting levels since these fish were trapped in a net and since simple stresses such as holding a handnet in a tank without forcing exercise have caused increases in cortisol (Fagerlund, 1967). The pre-exercise values agree well with male values given by Phillips (1959) but females reported here are more than double those in the earlier report. Gill netted fish from Georgian Bay showed a wide range of values, presumably as a result of the netting. Cortisol increases in George Lake suckers following exercise indicate that the necessary mechanisms of pituitary and interrenal glands were qualitatively intact. Wedemeyer (1969) has suggested that production of 17-hydroxycorticosteroids in response to stress may be a measure of vigor useful in applied genetic studies. A low cortisol response to stress then may be a measure of reduced vigor, however, future data will be needed to define a normal response for this species before we can be certain that the response shown was in fact low.

White suckers in George Lake and acidified lakes in the same region have been shown to grow at an unusually slow annual rate (Beamish, 1974b; Beamish et al. 1975). Acidification has also altered zooplankton communities (Sprules, 1975) giving rise to the hypothesis that reduced productivity at low trophic levels has been a cause of fish losses. Some of the measurements reported here are relevant to this hypothesis and generally they do not support an argument that fish were grossly starved. For example, anemia has been shown to result from starvation in fish (Kawatsu, 1966) and neither suckers nor bullheads from George Lake were anemic as judged by hemoglobin and hematocrit measurements. Similarly blood sugar has been shown to fall in some chronic starvation experiments (Larsson and Lewander, 1973) and bullheads from George Lake showed elevated rather than reduced sugar values. A number of biochemical changes have been associated with starvation in fish (Love, 1970) including reductions in serum protein in some species (Creach and Bouche, 1969). Serum protein concentrations were measured in white suckers from George Lake and the results below show no apparent reduction.

Serum protein concentrations in white suckers (*Catostomus commersoni*) from George Lake and other sources

Species	Source and time	Sample Number	Condition	Serum Protein (g/dl) ± S.D	Range
White Sucker	George Lake Sept. 1972	10	trap net unexercised	4.61 ± 0.54	3.90-5.40
White Sucker	George Lake Sept. 1972	12	trap net exercised	4.33 ± 0.79	2.90-6.00
White Sucker	Georgian Bay Sept. 1972	11	gill net	4.98 ± 0.85	3.80-6.90
White Sucker	Lake Manitoba Dec. 1972	15	laboratory unexercised	4.43 ± 0.79	3.65-5.70
White Sucker	Lake Manitoba Dec. 1972	13	laboratory exercised	4.36 ± 0.70	3.22-5.43

Another interesting change associated with starvation was noted by Ichikawa (1953) and that change was reabsorption of scales. Scales of white suckers from George Lake were analyzed for calcium content and compared with scales from the same species in Roddy Lake. Calcium content of scales of George Lake suckers ranged from 10.8 to 13.5 percent with a mean of 11.9%; in suckers from Roddy Lake, it ranged from 8.9 to 13.9% with a mean value of 11.7%. These biochemical data do not indicate starvation and they support the argument advanced by Beamish (1974b) that suckers are disappearing from acid lakes "despite the presence of adequate food reserves".

Failure of a number of species in George Lake has been associated with failure of females to release ova (Beamish et al. 1975). Striking elevations in serum calcium concentrations have been observed during sexual maturation of female fishes (Hess et al. 1928; Fleming et al. 1964; Woodhead and Plack, 1968). Increases in serum calcium have been induced in fish by treatment with estradiol (Bailey, 1957) and the extra calcium has been described as "complex calcium phosphoproteinate" being transported from liver to ovary for deposition of egg yolk (Urist and Schjeide, 1961). There is evidence that fish obtain most of their calcium supply directly from water rather than from food (McCay et al., 1936) and hence the same reasoning which has prompted several studies of oxygen consumption and our own interest in asphyxia can be applied to calcium metabolism - namely that it is an essential element which must be obtained at surfaces in contact with the acid water. The general subject of calcium metabolism in fish has been the subject of several recent reviews (Simmons, 1971; Simkiss, 1973; Fleming, 1967; Urist, 1966) and we have analyzed serum and several other tissues of fish from George Lake and other lakes for calcium.

Serum calcium concentrations for white suckers are shown in Table 3 and for several other species in Table 4. From the serum data there

Table III

Mean Serum Calcium Concentrations (mg/dl) for White Suckers from George Lake and other sources with number of samples.

Source	Date	Calcium in Females	Calcium in Males	Female/Male Ratio
George Lake	Sept '72	11.44(9)	12.47(13)	0.917
	May '73	14.36(16)	11.26(10)	1.275
	Nov '73	14.97(7)	11.07(7)	1.352
Georgian Bay	Sept '72	17.16(7)	11.13(3)	1.541
	May '73	15.65(2)	10.95(2)	1.429
Roddy Lake	Sept '73	12.94(18)	11.00(10)	1.177*
	Jan '74	14.71(10)	8.60(4)	1.710
	Mar '74	14.25(6)	8.43(3)	1.690
Beaver Glen	May '73	10.75(3)	10.54(3)	1.020**
Creek	May '74	11.43(4)	8.65(8)	1.321**
Kakakise	May '73	16.40(1)	12.00(3)	1.367
Lake	Nov '73	15.18(6)	10.00(4)	1.518
Lake Manitoba	Dec '72	22.98(11)	11.57(17)	1.986

* Very early in maturation

** From spawning run

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Table IV

Mean Serum Calcium Concentrations (mg/dl) for various species of fish from George Lake and other sources with numbers of samples.

Species	Source	Date	Calcium Females	Calcium Males	Female/Male Ratio
Brown Bullheads	George Lake	May '73	10.70(4)	8.95(6)	1.196
		Aug '73	12.33(4)	12.70(10)	0.970
		Nov '73	13.18(11)	10.20(7)	1.292
	Georgian Bay	May '73	22.95(2)	9.70(4)	2.366
		Aug '73	20.63(10)	14.38(9)	1.435
Yellow Perch	George Lake	May '73	22.63(10)	12.83(4)	1.765
	Georgian Bay	May '73	32.08(4)	9.90(1)	3.240
	Muriel Lake	May '73	30.34(8)	11.38(8)	2.667
Lake Herring	George Lake	Nov '73	20.64(5)	11.47(6)	1.800
	Kakakise Lake	Nov '73	19.67(12)	11.81(8)	1.665
Northern Pike	George Lake	May '73	25.5(1)	19.15(8)	1.332

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appears to be some depletion in females of failing species in acid lakes. When samples were taken very early in gonadal maturation (Table 3, Roddy Lake, Sept. 1973) or as late as the annual spawning run (Table 3, Beaver Glen Creek, May 1973 and May 1974) then females show little if any excess over males captured at the same time. During periods of ovarian development, however, female suckers exceeded males by a factor of about 1.4 or greater, except in George Lake. The only exception we have noted was based upon four fish from Kakakise Lake in May 1973 when females exceeded males by a factor of only 1.367. Kakakise Lake is very near George Lake, and it is also undergoing acidification, although at a reduced rate (Beamish and Harvey, 1972) and fish losses have not yet been observed. Considering other species (Table 4), a similar phenomenon is present; brown bullheads are failing in George Lake and females did not show a high excess over males. Yellow perch, (*Perca flavescens*) and lake herring (*Coregonus artedii*) are still reproducing successfully in George Lake and in both these species serum calcium in females exceeds that in males by factors greater than 1.4. Unfortunately, only one female northern pike (*Esox lucius*) was captured from George Lake, however, she exceeded males by only a factor of 1.3, consistent with other failing species. A ratio of serum calcium in females to that in males of about 1.4, then, has separated species failing to reproduce in George Lake from species still reproducing in that lake and also from similar species reproducing in other lakes. Assuming the low calcium ratios are more than coincidence, and lacking data from fish inhabiting George Lake prior to acidification, we cannot be certain whether low calcium excesses in females have resulted from acidification, or whether acidification has erected a selective barrier which can be met only by species maitaining higher female:male calcium ratios during maturation.

Sites of uptake of calcium from water have been reviewed by Simmons (1971) and it seems that calcium is exchanged at gills and other body surfaces, particularly those around certain fins. Dietary calcium has been reported to accumulate most readily in skeleton and skin in brown trout (Podoliak and Holden, 1964). As shown earlier, we have found no indication of reductions in calcium content of bone, muscle, or scales and similarly we found no shortage of calcium from skin as shown below.

Skin calcium concentrations in white suckers from George Lake and Lake Manitoba

Source and time	Skin calcium (g/100 g wet skin) $\pm$S.D	Number of Samples	Range
George Lake, Sept. 1972	8.44 ± 0.80	5	7.1 - 9.0
George Lake, Dec. 1972	7.73 ± 1.02	7	6.5 - 9.6
Lake Manitoba, Jan. 1973	8.54 ± 1.38	5	6.9 - 10.3

Ovaries developing in two white suckers taken in December, 1972 contained calcium at concentrations of 215 and 193 mg/kg (wet weight), concentrations above muscle concentrations for the same fish (100 and 90 mg/kg respectively). For comparison, suckers maturing in Roddy Lake in March, 1974, have ovarian calcium levels averaging 272 mg/kg (Roddy Lake has an even lower calcium content than George Lake). It is obvious that substantial quantities of calcium are required for ovarian development or such high concentration could not be achieved in such large organs. Our analyses suggest that calcium was not obtained from internal sources such as muscle, skin, scales, or bone and we agree with the suggestion by Fleming et al (1964) that at least part of the extra calcium needed by maturing females must be obtained from the environment. It seems probable that the explanation of a calcium metabolism problem in fishes inhabiting and acid lake is more likely to be found in studies that measure rates of calcium exchanges and neuroendocine mechanisms controlling those exchanges than in static concentrations reported here.

High levels of manganese were found in tissues of George Lake fish (below).

Manganese concentrations in tissues of white suckers captured from acid George Lake and other sources

Source and time	Tissue	Manganese concentration (mg/kg) wet tissue ± S.D	Number of Samples	Range
George Lake Dec. 1972	scales	551 ± 96.9	7	414 - 654
Roddy Lake March 1974	scales	160 ± 39.8	8	107 - 221
George Lake Sept. 1972	skin	356 ± 103.	5	244 - 464
George Lake Dec. 1972	skin	305 ± 53.9	7	236 - 396
Lake Manitoba Apr. 1973	skin	7.20 ± 1.50	5	5.7 - 9.1
George Lake Sept. 1972	muscle	1.00 ± 0.52	7	0.24 - 1.96

Comparing scale analyses between George Lake and Roddy Lake suckers, we find a three-fold increase in George Lake; comparing skin levels we find a striking increase of some 40-fold over skin concentrations from Lake Manitoba. When calcium is accumulated from water by areas near fins it must pass through skin and we may speculate that high fluxes of manganese in skin, or even high static concentrations here, could compete with or otherwise impair calcium uptake. This situation appears to apply to uptake of magnesium by pine seedlings (Safford, 1975). The mechanism of manganese poisoning in humans includes disturbances in calcium metabolism since manganese poisoning is accompanied by abnormally high serum calcium concentrations (Chandra et al., 1974). The behavior of manganese in acidified lakes seems to merit additional research effort.

SUMMARY

Several biochemical properties of fish inhabiting an acidified lake near Sudbury, Ontario, are reported. Values for hemoglobin indicated that an anticipated adaptive response to asphyxia was absent. Apparent sampling artifacts were noted in brown bullheads with respect to hematocrit, blood pH, sugar and inorganic phosphate analyses. It was thought that deformities observed in white suckers in the acid lake might be explained by demineralization of the skeleton, however, no biochemical deficiencies were found in rib analyses. No indication of starvation was found when several measurements reported to change during starvation were made, and it appears that reduced growth shown by acid lake suckers was not caused by starvation. Female white suckers and other species undergoing reproductive failure did not achieve as great an excess in serum calcium values over males in the acid lake as they did in non-acid locations. Females of species still reproducing successfully in the acid lake did achieve the expected calcium excesses over males. It is suggested that calcium dynamics are altered in fish from the acid lake. High levels of manganese, particularly in skin, were found in acid lake suckers.

The subject of calcium metabolism in fish seems to deserve in creased attention in attempts to understand the pathology of acid pollution.

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