CHLORINE TOXICITY AND ITS EFFECT ON GILL TISSUE RESPIRATION OF THE WHITE SUCKER Catostomus commersoni (Lacepede)

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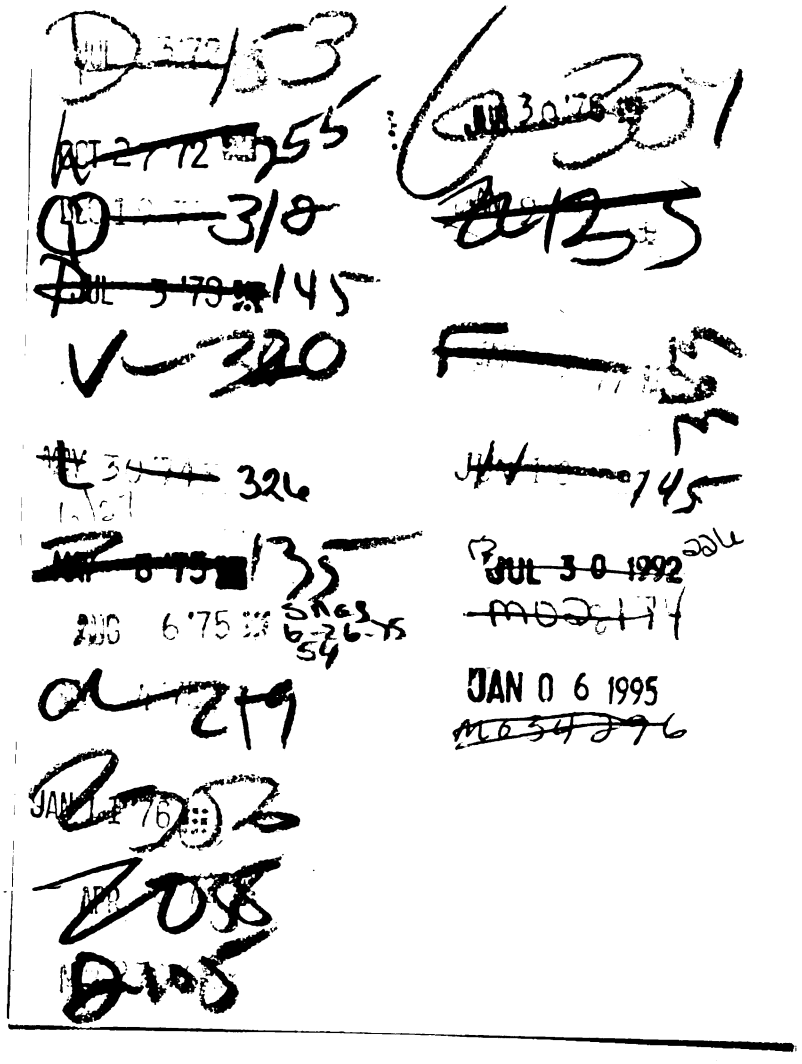
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ABSTRACT

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By

Ronald L. Fobes

The purpose of this investigation was to help determine the mechanism of chlorine toxicity to freshwater teleosts. White suckers of a relatively large size range were exposed to a lethal concentration of chlorine (one ppm total residual chlorine) for 30 and 60-minute periods. Following the assumption that normal filamental and lamellar gill tissues actively use oxygen while metabolizing, it was hypothesized that any damage to such tissue would alter its respiration rate. Subsequent to chlorine exposure, complete gills (arch and filaments) were excised from the fish and their respiration rate ($Q0_2$) determined with a Gilson differential respirometer.

An estimate of "normal" $Q0_2$ for white sucker gill tissue ranged from 1.5 to 1.7 μl 0_2/mg dry gill weight/hr. Statistical analysis indicated no significant difference between $Q0_2$ means of the control gills and those exposed to chlorine.

It was concluded that death resulting from relatively short exposures to lethal chlorine concentrations was not caused by gill damage and that gills were not the primary site of chlorine toxicity.

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Ronald L. Fobes

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INTRODUCTION

Need for Study

Beneficial facets of chlorination have been explored and expounded in previous studies. Chlorination has helped control or eliminate odors and noxious tastes, improved operation of sedimentation tanks, abolished psychoda flies, decreased pooling on trickling filters, reduced BOD and killed harmful bacteria (Scott and Van Kleeck, 1934). Chlorine also destroys or modifies decomposable organic wastes and reduces chemical oxygen demand (COD) (Moore, 1951). BOD reductions of 62%, bactericidal efficiences from 90-95%, and dissolved oxygen (DO) increases of 147% have been reported for sewage chlorinated to an average residual of 2.0 ppm (Baity et al., 1933; Eddy, 1934; Faber, 1944).

Nearly every major industry, domestic waste treatment facility and water treatment plant throughout the United States incorporates some aspect of chlorination in their processes. The extent and scope of research concerned with possible deleterious effects of chlorination is in no way

proportionate to the quantity of work expended on its beneficial oxidative and bacteriocidal properties.

Reports on the toxicity of chlorine and its derivatives to stream biota include those by Enslow, 1932; Doudoroff and Katz, 1950; Merkens, 1958. Enslow discovered that chlorinated organic waste products were not as assimilable to stream biota as the original material. In fact, at times the chlorinated products were toxic, even when highly diluted.

Representative early toxicity work was reported by Allen et al (1946, 1948). They determined that sewage plant effluents chlorinated with quantities much smaller than those required to give residual chlorine detectable by the ortho-tolidine test were highly toxic to stream fish. It was later discovered that the toxicity was caused by formation of cyanogen chloride from the reaction between chlorine and cyanates in the effluent.

More recently, chlorine concentrations within permissable limits for municipal water systems were found to be toxic to fingerling brook trout and fingerling smallmouth bass (Pyle, 1960).

Merkens (1958) investigated toxicity of chlorine and chloramines to rainbow trout and could only theorize that a safe concentration might be very low -- less than 0.08 ppm.

Tsai (1968) and Hynes (1960) agreed that chlorinated

sewage acts toxically on aquatic organisms. Tsai found chloramines to be more toxic to fish and they retained their toxicity longer than the free chlorine fraction of residual chlorine. He also theorized that DO and pH values, which are employed as primary water quality parameters for stream pollution assessment, actually are not decisive factors for fish mortality in areas immediately below chlorinated sewage outfalls.

Although chlorine toxicity studies on stream biota have increased, very few deal with the relative toxicity of chlorine and chloramines. In addition, there is a real lack of quantitative and qualitative measurements of chlorine and chloramine concentrations used in experiments. Lastly, and most importantly, there has been no investigation into physiological mechanisms of chlorine toxicity to freshwater teleosts.

Purpose and Scope of Study

The purpose of this investigation was to develop on a macroscopic level some understanding of the mechanism of chlorine toxicity to freshwater teleosts. Gill tissue was chosen for this study because of the sensitivity of this tissue to toxicants and its close proximity to water born

pollutants. Also, even though toxicants may effect a fish through gut or skin, it is more probable that they act on or through the gill and, finally, the physiological aspects of gill tissue are well documented.

Five major objectives comprise the basis of this research:

1. Establish an estimate of the "normal" tissue respiration rate for a complete gill.
2. Determine effects of a lethal concentration of residual chlorine on the respiration rate of a complete gill.
3. Help reveal whether death by chlorine toxicity is attributable to gill failure.
4. Assist in resolving the location of the primary site of chlorine toxicity.
5. Observe behavioral and physical changes in the test animal.

It is aspired that correlation of the five preceding objectives and their results will establish a base from which more in-depth studies into the exact mechanism of chlorine toxicity may be carried out.

METHODS

Fish Holding and Feeding

Advantages in choosing the white sucker Catostomus commersoni (Lacepede) follow:

1. Available from local private ponds.
2. Easily maintained under laboratory conditions.
3. A good test fish: not as sensitive as trout or salmon and not as resistant as carp or catfish.
4. Easy to work with: little fish smell, no spines or pointed fins, lack of teeth, and not excessively slimy.

Capture was effected by both glass and wire minnow traps from January to June, 1971. A total of 134 fish were collected and held in a 190-gallon metal tank interiorly coated with a non-toxic grey, epoxy paint. One-third of the tank was covered to afford a place of fish concealment. A single standpipe and one siphon hose provided drainage. Flow rate was about 2 gal per min of filtered water. East Lansing municipal water was passed through a 50-gal charcoal and gravel filter and then through a one-gal Nalgene container packed with polyethelene filter floss. The latter became necessary because forceful back flushing of the 50-gal filter tended to disintegrate the charcoal. Two air pumps oxygenated

the water through one 11-inch air stone and seven smaller 1-inch stones.

Photoperiod was not a factor because lighting was continually on.

The fish received daily feedings of salmon starter food produced by Aktiebolaget Ewos Co. of Sodertaljie, Sweden. The preceding diet was occasionally augmented by shredded frozen horse heart.

Toxicant Dilution System

The dosing apparatus employed during this study was developed for earlier studies at Michigan State University (Rosenberger, 1971). Rosenberger modified the basic design of Alabaster and Abram (1965) by incorporating a three-way electrical timer, solenoid valves, and various other building materials such as plastics, vinyls, and glass. Filtered tap water piped into an elevated head tank was gravity fed to the constant head vessels. Chronologically, the first valve would open and allow the filtered water to fill the 1-liter mixing flask to a level even with the constant head standpipe. Valve two released the toxicant, which finished filling the flask up to 1-liter as determined by the height of the toxicant filled Marriotte bottle.

Valve three then permitted the 1-liter of diluted toxicant to flow into the 5-gal test aquarium.

The previously described system recycled every six minutes giving a fill time of 2 hr. and a 90% replacement time of 4.5 hr. The latter was more rapid than Sprague's (1969) suggested replacement time of 8-12 hr. The aforementioned fill time was well below APHA's (1971) recommended time of 6.5 hr.

Three aquaria were utilized in this study. The first aquaria served as an acclimation chamber for the four test fish of any given run. Test fish were acclimated overnight. The following day two fish were placed in the control tank and two into the toxicant tank. Duration of exposure to approximately 1 ppm total residual chlorine was 30 or 60 minutes.

Toxicant was made from approximately 10 g of technical grade calcium hypochlorite ($Ca(OCl)_2$) dissolved in 20 liters of deionized, distilled water, which gave a concentration of about 200 ppm. Sulfuric acid helped bring the $Ca(OCl)_2$ into solution. The final solution had a pH of about 7.0, was filtered and placed in a 20-liter Marriotte bottle which, along with the toxicant reservoir, was covered with black plastic to help prevent chlorine breakdown due to light exposure.

Dissection Procedures

Following exposure to chlorine, each fish was pithed through the brain and anterior portion of the spinal column. Both gill membranes were severed anteriorly to a point just forward of the isthmus, which was transversely cut. The isthmus was separated from the underlying gills and pulled posteriorly. Each opercle and cheek was torn and pulled anteriorly and the gills, now exposed laterally and ventrally, were deftly excised taking care not to injure individual filaments. Esophageal tissue attached to the excised gill was carefully removed. The isolated gills (arch and filaments) were rinsed with distilled water and placed in the respirometer reaction flasks.

Gill Tissue Respiration Measurements

A Gilson Differential Respirometer employing the constant pressure method of measurement was used to monitor oxygen consumption of gill tissue. Each of the 14 reaction flasks had a capacity of approximately 16-ml. The reference flask, or thermobarometer, was 235 ml.

All flasks were cleaned by a modification of the nitric acid method described by Umbreit et al (1964) as follows:

1. Soak flasks in gasoline. Remove remaining grease with gasoline on a cotton swab.

2. Wash in a mild Alconox detergent solution; about one tablespoon Alconox per 2 gal water.

3. Rinse well with tap water.

4. Soak in a solution of equal parts H_2SO_4 and HNO_3 for at least 30 min.

5. Wash several times with tap water. Rinse twice using distilled water.

All fittings were then sealed with a high vacuum grease.

After randomly choosing four flasks for the test tissue, the remaining 10 flasks and reference flask were prepared. Four ml of distilled water and 6N NaOH-saturated filter paper (displacing 0.5 ml) were placed in each of the remaining 10 flasks. By adding distilled water, the reference flask gas volume was adjusted to approximate the cumulative gas volume of the reaction flasks. All 10 flasks and the reference vessel were then connected to the respirometer.

Readying the respirometer consisted of activating the stirring motor, shaking motor and setting the water bath at 23 C. Temperature equilibration was achieved while the test fish were exposed to the toxicant and dissected.

Immediately prior to dissection, 4 ml of Ringer solution (Stokes and Fromm, 1964) was added to each randomly chosen flask. After dissection, prepared gills of each fish were

placed in one of the four test flasks. Next, NaOH-soaked filter paper was lodged inside the inner well of each flask. The four vessels were connected to the respirometer. While the entire system equalized for 15 min. prior to the recording of oxygen consumption, manometer index lines were aligned and initial micrometer readings set at convenient, uniform values.

Data Collection

Water Chemistry

Approximately once a week pH, temperature, DO, alkalinity, and hardness were quantified for holding, acclimation, control and test tanks. The pH was measured to the nearest 0.1 and temperature recorded to the nearest 0.5 C. Alkalinity, DO and hardness were all measured in accordance with APHA (1965) standards.

Chlorine Determination

The APHA (1965) method for differentiation of monochloramine and dichloramine by amperometric titration was employed for all chlorine determinations. Free chlorine, monochloramine and dichloramine were determined twice for each run, midway through and immediately after exposure.

Concentrations were recorded to nearest 0.01 ppm.

The amperometric titration apparatus consisted of the following parts. The silver-silver chloride billet type reference electrode was immersed in a saturated NaCl solution, which was attached to the sample cell by a 10% NaCl agar bridge. A readily polarizable platinum electrode was spun in the sample cell. The electrodes were connected to a recorder sensitive to 0.01 milliamps.

Fish

Data on length and weight were collected subsequent to dissection. Total length was determined to the nearest millimeter. Fish wet weight without gills was measured on a top loading balance sensitive to 0.01 g. After monitoring tissue respiration, wet gill weight was determined to the nearest 0.0001 g on an analytical balance. Total fish wet weight was calculated by adding gill wet weight to wet weight of fish without gills. After drying for 48 hr at 100 C, dry gill weight was determined in the same manner as wet weight.

Respiration Rate

The QO_2 rate is expressed as µl of O_2 uptake per mg of dry gill tissue per hour. For six hours, each half-hour cumulative and incremental amount of O_2 consumed was recorded to the nearest 0.1 µl. A correction factor (CF) was applied to each half-hour increment of O_2 consumption. This factor was obtained by averaging the fluctuations in the 10 "normal" reaction flasks for each half hour. For example, if average fluctuations of the 10 flasks over a 30-min span was +1.5 µl, this indicated outside factors were increasing all 14 readings to that degree. Thus, 1.5 µl was subtracted from each of the four half-hour tissue readings. If CF were negative, it was added to the 30-min tissue readings. Each corrected half-hour tissue O_2 uptake reading was divided by its corresponding gill tissue dry weight and doubled to give the final QO_2 hourly rate.

Statistical Analysis

Basic statistics such as means, standard deviation and standard error are presented with the corresponding data in the Appendix Tables.

A model I, or fixed effects model, randomized complete-block design with 12 observations per experimental unit was used in this investigation. Covariance analysis was chosen for interpretation of results primarily because the independent variable (total fish weight) fluctuated widely and influenced the dependent variable (QO_2). This analysis was also chosen because it combines the concepts of analysis of variance and regression to furnish a more discriminating analysis than that afforded by either componet (Ostle, 1954). Ostle (1954) and Steel and Torrie (1960) discuss in detail the assumptions, models and mathematical procedures used in covariance analysis.

RESULTS

Water Chemistry

Data and statistical description concerning the five water parameters monitored are presented in Appendix Table A-1. A summary of means and standard errors for determinations of pH, temperature, dissolved oxygen, alkalinity and hardness in holding (H), acclimation (A), control (C) and test (T) tanks is found in Table 1.

There were no differences between control and test tanks in parameters quantified ($T=0.064$, $P>.9$). Therefore, it was assumed that water quality was constant and not an error factor in the experiment.

Chlorine Determination

Chlorine and chloramine determinations along with their complete statistical description are in Appendix Tables A-2, A-3. Free chlorine residual usually includes free chlorine, hypochlorous acid and hypochlorite ion whereas combined chlorine residual refers to chloramines (Moore, 1951; Sawyer and McCarty, 1967). In the present study total residual

TABLE 1. Range of pH and means and standard errors for determinations of temperature, dissolved oxygen, alkalinity, and hardness in holding (H), acclimation (A), control (C), and test (T) tanks.

Tank Type	pH	Temperature (C°)	D.O. (ppm)	Alkalinity (ppm $CaCO_3$)	Hardness (ppm $CaCO_3$)
H	7.5-7.6	13.40±0.10	6.78±0.36	306.3±5.5	318.7±1.0
A	7.5-7.8	15.20±0.12	7.67±0.14	306.7±3.5	322.3±0.8
C	7.8	17.25±0.14	8.03±0.03	312.0±2.9	321.5±1.0
T	7.7-7.8	17.20±0.12	8.15±0.03	319.0±1.3	323.0±1.3

chlorine is the sum of free chlorine and combined chlorine residuals.

Mean total residual chlorine (ppm) during the 30 and 60 min exposures were respectively 0.970 $\pm$ 0.024 (S.E.) and 1.008 $\pm$ 0.033 (S.E.). The two means did not significantly differ from 1.000 ppm ($T=0.094$, $P>.9$). Following pilot studies to determine a toxicant level lethal within one to two-hour exposure, the 1 ppm concentration was chosen. Total residual chlorine was chosen as the measure of toxicant because its concentration could be controlled. Combined chlorine and free chlorine residuals were in a constant state of flux as fish-excreted ammonia united with chlorine to form monochloramine and dichloramine. Full in-depth discussions of chlorine and its chemistry are presented by Moore (1951) and Sawyer and McCarty (1967).

By comparing means in Table 2 certain trends may be distinguished concerning the changing proportions of combined and free chlorine residuals with time. The following trends could not be proven significant and thus do lie in the realm of chance. During both exposure periods mean total residual chlorine decreased over time. This suggests a slight overall loss of chlorine, possibly due to an initial chlorine demand of the fish, loss to the atmosphere, or formation of trichloramine which could not be quantified.

TABLE 2. Means and standard errors(S.E.) for the different chlorine residuals during the 30 and 60-minute exposures.

Chlorine Form	30 Mean and S.E. (ppm)	60 Mean and S.E. (ppm)
Total Residual		
A[1]	0.995 ± 0.036	1.052 ± 0.036
B[2]	0.945 ± 0.030	0.965 ± 0.054
C[3]	0.970 ± 0.024	1.008 ± 0.033
Free Chlorine		
A	0.737 ± 0.082	0.640 ± 0.064
B	0.638 ± 0.062	0.582 ± 0.075
C	0.688 ± 0.051	0.612 ± 0.048
Mono-Chloramine		
A	0.148 ± 0.051	0.273 ± 0.090
B	0.182 ± 0.033	0.228 ± 0.044
C	0.165 ± 0.029	0.251 ± 0.048
Di-Chloramine		
A	0.110 ± 0.007	0.135 ± 0.020
B	0.125 ± 0.008	0.155 ± 0.012
C	0.118 ± 0.006	0.145 ± 0.011

[1]Determinations midway through exposure.
[2]Determinations immediately after exposure.
[3]Combined determinations.

Free chlorine also decreased over time during both exposures. This was expected as free chlorine would react with the excreted ammonia. Dichloramine increased during both exposures probably due to the continuing reaction between monochloramine and hypochlorous acid.

The fluctuations of combined chlorine residuals may be due to inability to coordinate chlorine determinations with the 6-min recycling of the toxicant diluter system. When a water sample was being taken for chlorine determination, the dosing apparatus may have been adding fresh toxicant, just finished or just ready to add, etc.

Fish

Data on total length, weight, wet gill weight and dry gill weight along with pertinent statistics are recorded in Appendix Tables A-4, A-5. Total length for all test fish ranged from 80 to 185 mm. Total weight ranged from 3.25 to 52.21 g.

The large variation in size of experimental fish was unavoidable due to the collecting method. The wire minnow traps selected against only very large and very small fish. Since fish size is closely related to metabolic rate (Fry, 1957; Muir and Hughes, 1969; Prosser et al, 1952; Winberg, 1960), the wide range in size dictated the choice of an

appropriate statistical analysis.

Inspection of Table 3 shows no differences ($T=0.08$, $P>.9$) between means for total length, weight and gill dry weight in the 30-min group and those in the 60-min exposure. In addition, both groups showed no differences ($T=0.03$, $P>.9$) between the means of test and control fish measurements.

Respiration Rate

Appendix Tables B (1-12) present data on correction factors (CF), fish weights, half-hour oxygen uptake readings and the corresponding calculated $Q0_2$'s. Inspection of these data reveals two main relationships. First, the total volume of oxygen consumed by gills varies directly with total fish weight. Secondly, there is an inverse relationship of $Q0_2$ to fish weight. These two observations agree with those of Winberg (1960).

In addition, it is also generally apparent that all gills tested remained viable over the six hours and that $Q0_2$ and oxygen uptake were fairly constant, decreasing less than 10 percent over time.

TABLE 3. Means and standard errors (S.E.) of total length, total weight, and dry gill weight for test (T) and control (C) fish exposed for 30 and 60 minutes.

Measurement	30 Mean and S.E.	60 Mean and S.E.
Total length (mm)		
T	119.6 ± 5.0	115.2 ± 8.0
C	116.5 ± 3.8	114.7 ± 7.2
Total weight (g)		
T	13.097 ± 1.806	13.469 ± 4.053
C	12.129 ± 1.124	11.713 ± 2.818
Gill dry weight (mg)		
T	33.17 ± 4.98	32.917 ± 5.397
C	33.08 ± 3.49	29.917 ± 3.736

Statistical Analysis

Individual fish QO_2 means, total weights and corresponding $logs_{10}$ appear along with pertinent statistics in Tables 4, 5. A preliminary two-way analysis of variance ignoring fish weight differences was performed to test the hypothesis that all four treatment means were equal; $H_0: \bar{Y}_1=\bar{Y}_2=\bar{Y}_3=\bar{Y}_4$ (Table 6.). The null hypothesis was accepted, inferring that all treatments were from the same population.

After initial examintion of scatter diagrams plotting QO_2 against fish weights and QO_2 against time, it was hypothesized that logarithmic transformation of QO_2 and weight would provide a better fit. With the transformation to log_{10} (Tables 4, 5), the correlation coefficient (R) was increased from .60 to .71 and the coefficient of determination (R^2) increased from .36 to .51.

The mathematical covariance model employed was:

$$Y_{ijk}=u+t_i+s_j+(ts)_{ij}+BX_{ijk}+e_{ijk}$$

where $Y_{ijk}=log_{10}$ of mean QO_2 reading for fish k, for fish type i (test or control) and strength j

TABLE 4. Log_{10} transformations of QO_2 means and fish weights (g) for two test (T) and two control (C) fish at each 30-minute exposure.

	T-30				C-30			
Date	QO_2 Mean	Log QO_2 Mean	Fish Weight	Log Fish Weight	QO_2 Mean	Log QO_2 Mean	Fish Weight	Log fish Weight
1971								
2 August	0.7021	-0.1536	18.19	1.2598	0.8284	-0.0818	17.52	1.2453
	0.7322	-0.1407	12.18	1.0856	0.7658	-0.1159	11.86	1.0741
27 August	1.7075	0.2324	12.77	1.1062	0.8427	-0.0743	14.71	1.1676
	1.1450	0.0588	14.26	1.1541	1.6315	0.2126	11.01	1.0418
29 August	1.3452	0.1288	11.72	1.0689	1.2869	0.1095	11.61	1.0648
	1.9725	0.2950	9.62	0.9832	1.2586	0.0999	13.36	1.1258
30 August	3.8636	0.5870	3.25	0.5119	2.4536	0.3898	6.11	0.7860
	2.4008	0.3804	5.70	0.7559	2.5821	0.4120	5.93	0.7731
1 September	1.3420	0.1278	26.20	1.4183	1.4322	0.1560	16.73	1.2235
	1.2197	0.0863	20.16	1.3045	1.2265	0.0887	16.83	1.2261
4 September	1.3230	0.1216	13.84	1.1411	1.5857	0.2002	10.43	1.0183
	1.8402	0.2649	9.27	0.9671	2.1110	0.3245	9.45	0.9754
Mean ($\bar{x}$)	1.6320	0.1657	13.0966	1.0630	1.5004	0.1434	12.1291	1.0601
S.D. (s_x)	0.7359	0.0429	39.1523	0.0593	0.3711	0.0313	15.1656	0.0246
S.E. ($s/\sqrt{n}$)	0.2476	0.0597	1.8062	0.0702	0.1758	0.0510	1.1241	0.0452

TABLE 5. Log_{10} transformations of QO_2 means and fish weights (g) for two test (T) and two control (C) fish at each 60-minute exposure.

Date	T-60 QO_2 Mean	T-60 Log QO_2 Mean	T-60 Fish Weight	T-60 Log fish Weight	C-60 QO_2 Mean	C-60 Log QO_2 Mean	C-60 Fish Weight	C-60 Log fish Weight
1971								
26 July	1.8876	0.2760	17.82	1.2509	1.1191	0.0489	20.10	1.3032
	2.1896	0.3404	8.54	0.9315	2.1951	0.3415	6.75	0.8293
10 August	2.0477	0.3113	6.34	0.8021	1.5211	0.1822	8.12	0.9096
	2.0143	0.3041	8.28	0.9180	1.6049	0.2054	9.30	0.9685
21 August	1.4355	0.1570	9.05	0.9566	1.5115	0.1794	8.06	0.9063
	1.3780	0.1392	10.05	1.0022	1.3785	0.1394	8.46	0.9274
22 August	1.6451	0.2162	13.88	1.1424	1.2942	0.1120	10.89	1.0370
	1.8139	0.2586	12.53	1.0980	1.5452	0.1890	11.00	1.0414
23 August	2.1140	0.3251	4.68	0.6702	2.1711	0.3367	4.26	0.6294
	2.3248	0.3664	4.78	0.6794	2.1229	0.3269	5.00	0.6990
24 August	0.9318	-0.0307	52.21*	1.7178	1.2933	0.1117	36.90*	1.5670
	0.6567	-0.1826		1.7178	0.9897	-0.0045		1.5670
Mean ($\bar{x}$)	1.7032	0.2067	13.4690	1.0739	1.5622	0.1806	11.7127	1.0320
S.D. (s_x)	0.2658	0.0272	180.6852	0.1201	0.1630	0.0122	87.3814	0.0913
S.E. ($s/\sqrt{n}$)	0.1488	0.0476	4.0529	0.1000	0.1165	0.0318	2.8184	0.0872

*Fish too large; gills cut in two, QO_2 determinations made on each portion.

TABLE 6. Two-way analysis of variance testing the effects of chlorine exposure (30 and 60 minutes) and fish type (test and control) upon gill tissue QO_2 without regard for fish weight.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	P[1]
Exposure	1	0.0530	0.0530	0.138	$.50 < P < .75$
Fish type	1	0.2230	0.2230	0.581	$.25 < P < .50$
Interaction	1	0.0004	0.0004	0.001	$P > .75$
Subtotal	3	0.2764	0.0921		
Error (within)	44	16.8952	0.3839		
Total	47	17.1716			

$F.05\ [1,44] = 4.08$

[1]Probabilities of obtaining larger F-values by drawing four samples from a normal univariate distribution.

(30 or 60-minute exposure). k=1,2...12; i=1, 2; j=1,2.

X_{ijk}=$\log_{10}$ of weight of fish ijk; covariate variable

u=general mean

t_i=variability component peculiar to fish type; $\sum_i t_i=0$

s_j=variability component peculiar to strength; $\sum_j s_j=0$

ts_{ij}=variablity component peucliar to fish type x strength interaction; $\sum_i (ts)_{ij}=0$, $\sum_j (ts)_{ij}=0$

e_{ijk}=variation contribution due to randomness

A summary of the covariance analysis for differences among the four treatment means is presented in Table 7. The null hypothesis $H_0:\bar{Y}_1=\bar{Y}_2=\bar{Y}_3=\bar{Y}_4$ is accepted.

Unrestricted regression coefficients and Y-intercepts of $\log_{10}$ $\overline{QO_2}$ vs. $\log_{10}$ fish weight were calculated for each of the treatments (Figures 1,2). An F-test for equality of slopes was performed on the four treatment regression lines yielding a value of F=3.14. The probability of obtaining a larger F-value by drawing four such samples from a normal univariate distribution is .025<P<.05. The null hypothesis $H_0:B_1=B_2=B_3=B_4$ is rejected, indicating that differences exist among these four regressions.

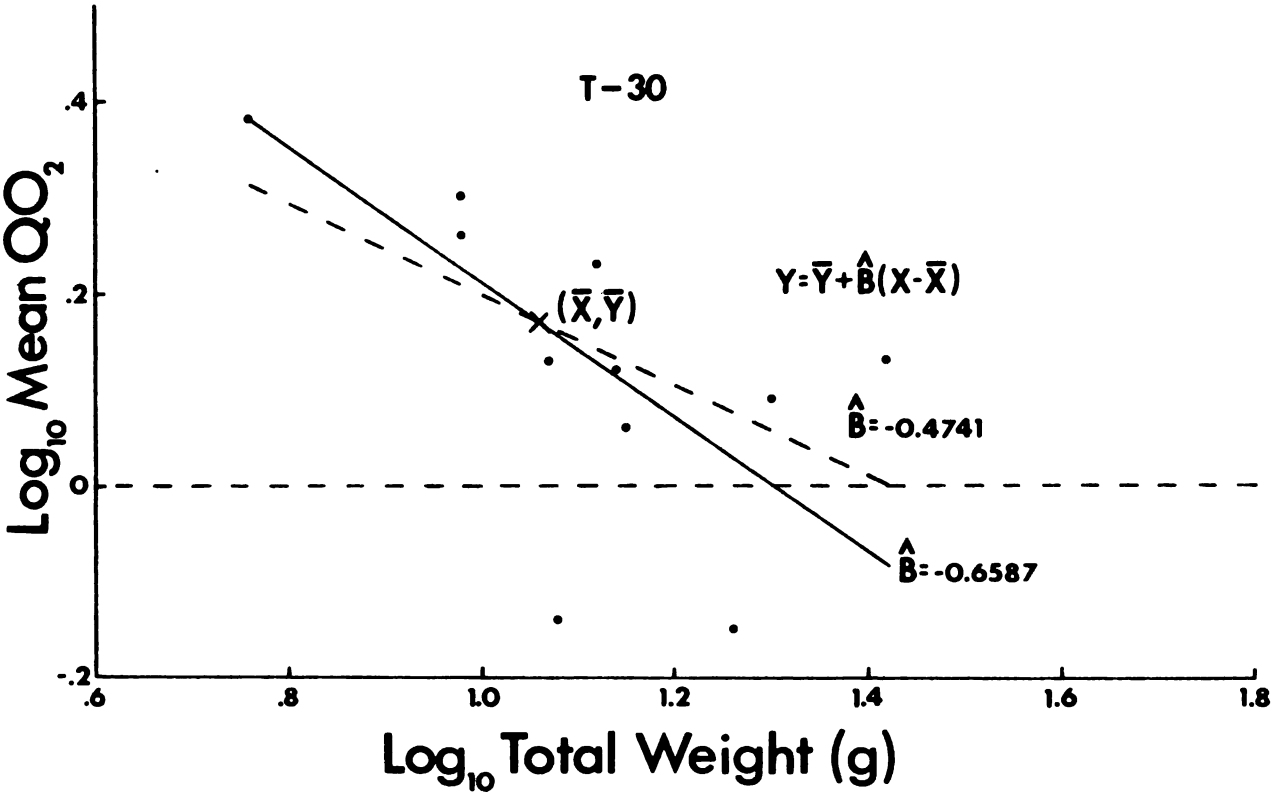
In order to separate slope inequalities, an F-test

TABLE 7. Analysis of covariance for data in Tables 4, 5.

Source of variance	df	Sum of products x^2	Sum of products xy	Sum of products y^2	df	Y adjusted for X SS	Y adjusted for X MS	Y adjusted for X F
Total	47	3.264209	-1.535447	1.278998				
Fish Type (T)	1	0.005999	0.006483	0.007006				
Strength (S)	1	0.000888	-0.004041	0.018396				
Inter. (TxS)	1	0.004544	0.000437	0.000042				
Error (E)	44	3.252779	-1.538326	1.253554	43	0.526039	0.012233	
T + E	45	3.258778	-1.531843	1.260560	44	0.540492		
Difference for testing adjusted treatment means					1	0.014453	0.014453	1.18 (.25< P[1] <.50)
S + E	45	3.253667	-1.542366	1.271950	44	0.540808		
Difference for testing adjusted strength means					1	0.014769	0.014769	1.21 (.25< P <.50)
TxS+E	45	3.257323	-1.537888	1.253596	44	0.527509		
Difference for testing adjusted TxS interaction					1	0.001470	0.001470	0.12 (.50< P <.75)
			F.05 [1,43] = 4.05					

[1]Probabilities of obtaining larger F- values by drawing four samples from a normal multivariate distribution.

Figure 1. Regression coefficients for test (t) and control (c) fish during the 30-minute exposure (solid lines). Dashed line represents estimated slope for all treatments, given: all regression coefficients equal.


T-30
Log_{10} Mean QO_2
.4
.2
0
-.2
.6
.8
1.0
1.2
1.4
1.6
1.8
$Y=\bar{Y}+\hat{B}(X-\bar{X})$
$(\bar{X},\bar{Y})$
$\hat{B}=-0.4741$
$\hat{B}=-0.6587$
Log_{10} Total Weight (g)

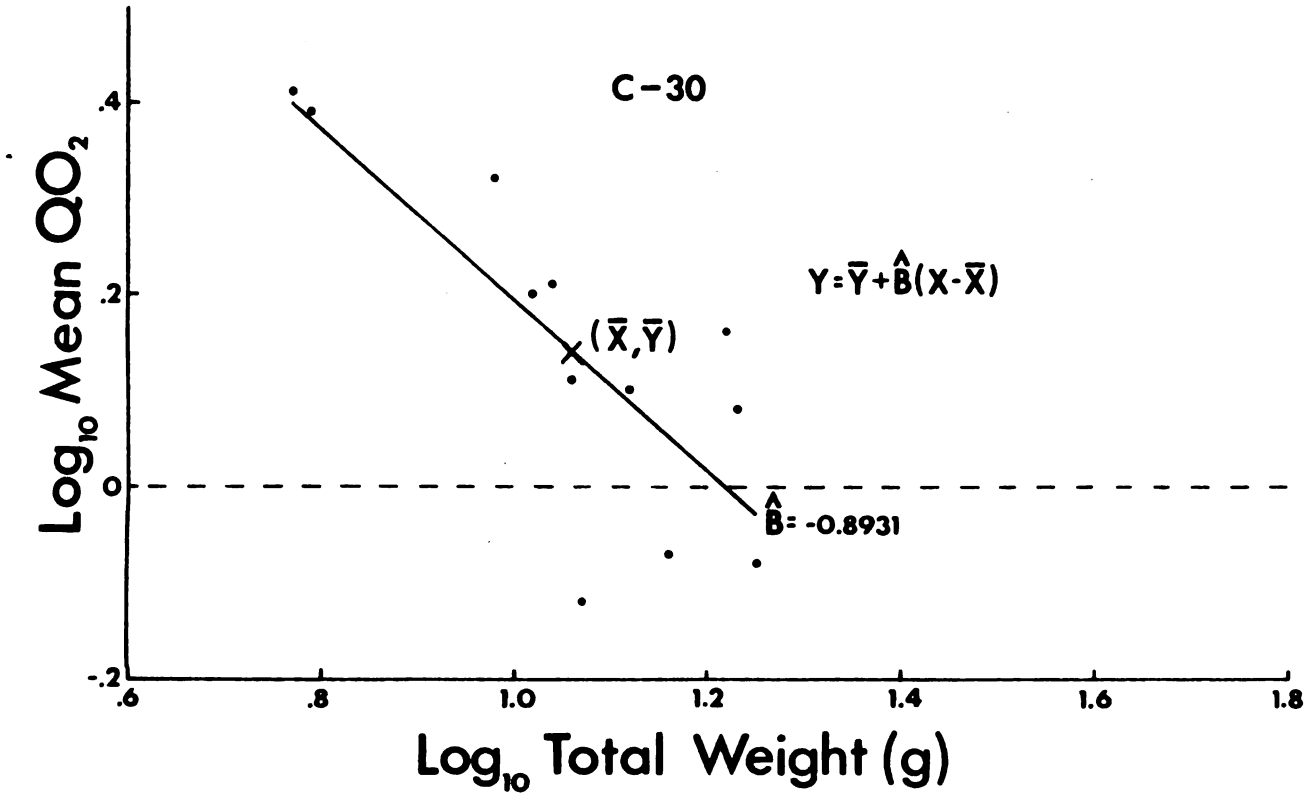
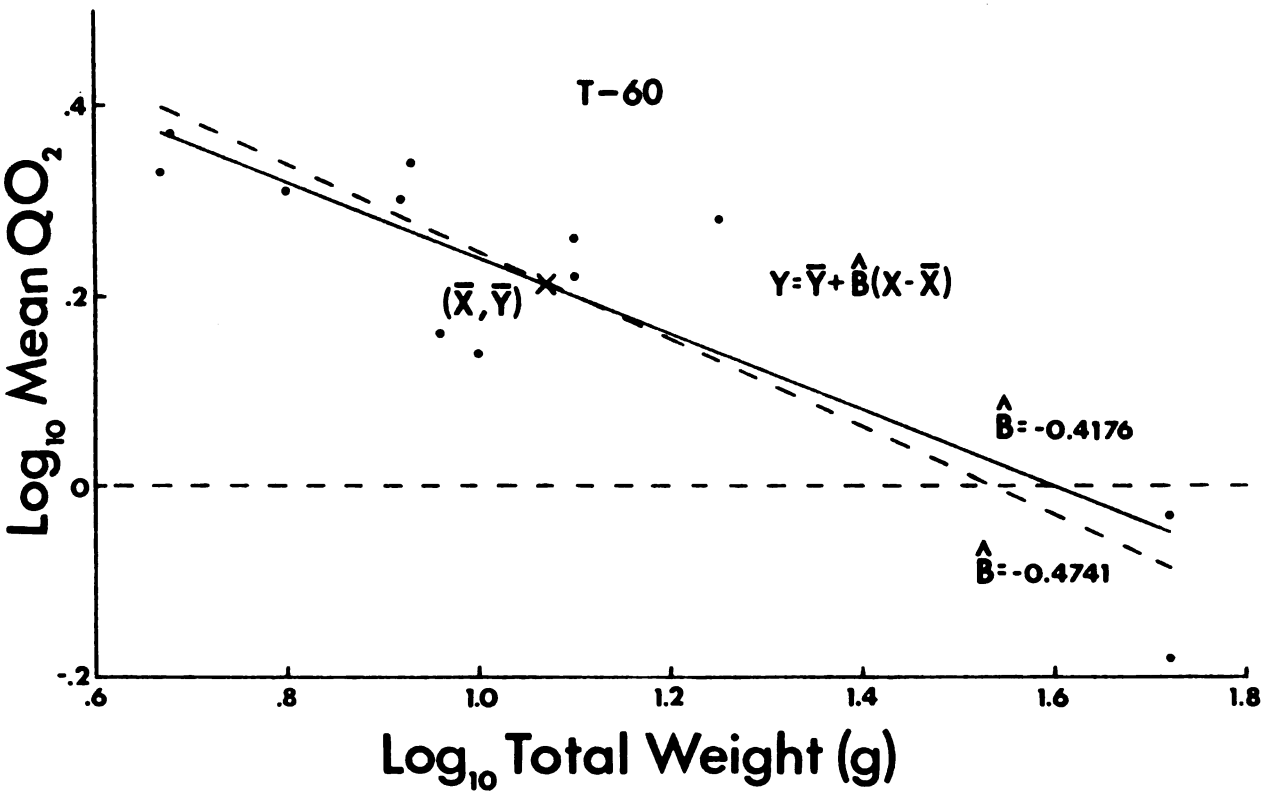

C-30
Log_{10} Mean QO_2
.4
.2
0
-.2
.6
.8
1.0
1.2
1.4
1.6
1.8
$Y=\bar{Y}+\hat{B}(X-\bar{X})$
$(\bar{X},\bar{Y})$
$\hat{B}=-0.8931$
Log_{10} Total Weight (g)

Figure 1

Figure 2. Regression coefficients for test (t) and control (c) fish during the 60-minute exposure (solid lines). Dashed line represents estimated slope for all treatments, given: all regression coefficients equal.


T-60
Log10 Mean QO2
.4
.2
0
-.2
.6
.8
1.0
1.2
1.4
1.6
1.8
(X̄, Ȳ)
Y=Ȳ+B̂(X-X̄)
B̂=-0.4176
B̂=-0.4741
Log10 Total Weight (g)

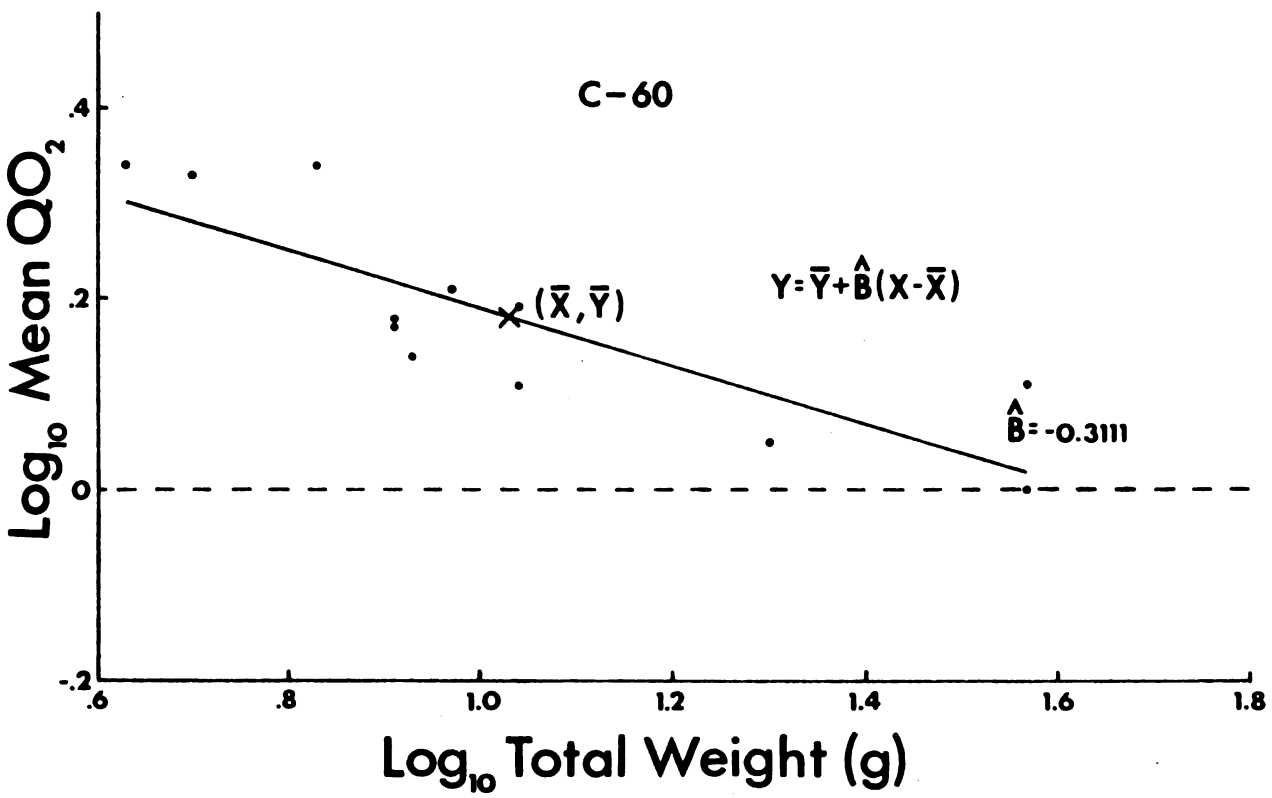

C-60
Log10 Mean QO2
.4
.2
0
-.2
.6
.8
1.0
1.2
1.4
1.6
1.8
(X̄, Ȳ)
Y=Ȳ+B̂(X-X̄)
B̂=-0.3111
Log10 Total Weight (g)

Figure 2

for difference between two regression coefficients (Sokal and Rohlf, 1969) was performed. Treatment comparisons, F-values and probabilities are given in Table 8 . For treatment comparisons C-30 vs. T-30, C-60 vs. T-60 and T-30 vs. T-60 we would accept the null hypothesis of equal slopes as a reasonable assumption. However, a slope difference between C-30 and C-60 is suggested, though questionable.

Normally we would not expect control group differences. It is possible that not all of the normality assumptions were met. Three observations in the C-60 group were determined on very large fish ($\log_{10}$ weight$\geq$1.3) which may have decreased its downward slope. It seems biologically plausible to combine all observations from both control groups into a single slope. A new regression coefficient (B_5) was calculated comprising all points of both control groups (Figure 3).

The F-test for equality of regression coefficients was repeated for the following three regression lines: B_2=T-30, B_4=T-60, B_5=(C-30)+(C-60). A value of F=0.04 was obtained; the chance of drawing a larger F-value from a normal univariate distribution is P>.75. This infers that acceptance of H_0:$B_2=B_4=B_5$ is reasonable.

Under the assumption that the three regression

TABLE 8. F-tests for difference between two regression coefficients; test fish = (T), control fish = (C), exposure time = 30 or 60 minutes.

Treatment comparison	Calculated F-value	P[1]
C-30 vs. T-30	0.59	.25< P <.50
C-60 vs. T-60	0.11	.50< P <.75
C-30 vs. C-60	3.03	.05< P <.10
T-30 vs. T-60	0.44	.50< P <.75
	F.10 [1,20] = 2.97	

[1]Probabilities of obtaining larger F-values by drawing four samples from a normal multivariate distribution.

Figure 3. Regression coefficient for combined 30 and 60-minute control fish (c), solid line. Dashed line represents estimated slope for all treatments, given: all regression coefficients equal.

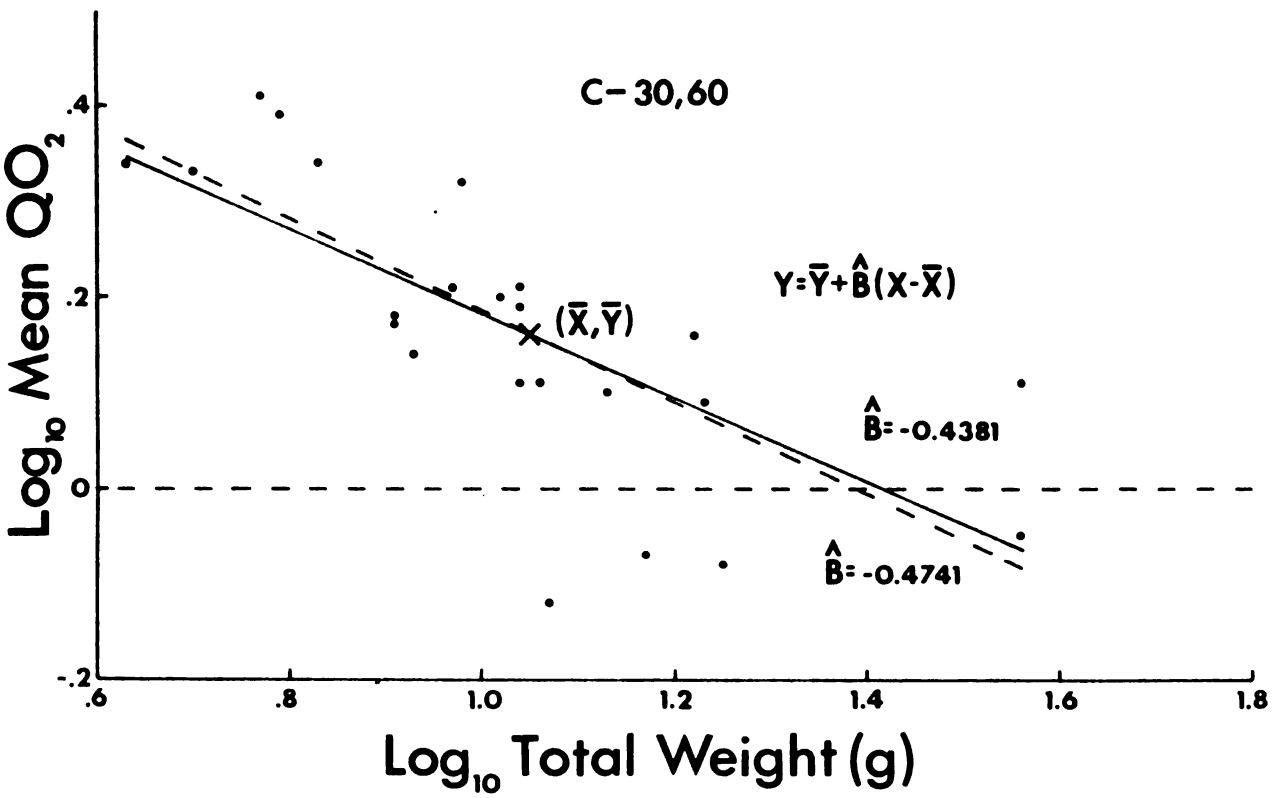

C-30,60
$Y=\bar{Y}+\hat{B}(X-\bar{X})$
$(\bar{X},\bar{Y})$
$\hat{B}=-0.4381$
$\hat{B}=-0.4741$
Log_{10} Mean QO_2
Log_{10} Total Weight (g)
.4
.2
0
-.2
.6
.8
1.0
1.2
1.4
1.6
1.8

Figure 3

coefficients are equal, a single slope was estimated for all three treatments and plotted as the dashed line in Figures 1, 2, 3.

A more sophisticated covariance analysis was performed by high speed computer utilizing each of 12 $Q0_2$ determinations per fish instead of $Q0_2$ means per fish. This program yielded results similar to the previously described covariance analysis; acceptance of null hypothesis of no difference between treatments. Computer analysis provided values of T=0.995 for fish type (t), T=1.166 for strength (s) and T=0.399 for fish type x strength interaction (ts). The probability of obtaining larger T-values by drawing four samples for a normal multivariate distribution is: $.2<P<.4$ for both (t) and (s), $.5<P<.9$ for (ts).

The following prediction equation was formulated from the computer covariance analysis:

$$Y=1.696 \pm 0.202 - 0.022 \pm .004\ (T) - 0.477 \pm 0.067(X) - 0.081 \pm 0.081(t) + 0.094 \pm 0.081(s) - 0.027 \pm 0.081(ts)$$

where $Y = \log_e Q0_2$

$X = \log_e$ fish weight

T = Time of $Q0_2$ determinations; 1=30 min, 2=60 min, 3=90 min. . . 12=360 min.

t = fish type; 0 = test fish, 1 = control fish

s=strength; 0=30 min exposure, 1=60 min exposure

ts=fish type x strength interaction; 0 if t and s values are different, 1 if t and s values are the same.

From the computer analysis we can conclude that differences between treatments were insignificant compared to variation between individual fish. This is supported by the fact that deletion of fish variables from the analysis resulted in a decreased sum of squares for regression (about mean) from 90 to 52, increased error sum of squares from 13 to 51 and decreased coefficient of determination (R^2) from .87 to .51.

DISCUSSION AND CONCLUSION

Both hand calculated and computerized covariance analysis yielded results similar to the preliminary analysis of variance disregarding fish weight (Table 6). As previously described, this was primarily due to individual fish variations. The covariance analysis could have been improved by using more fish of one size and taking fewer $Q0_2$ determinations.

An estimate of "normal" $Q0_2$ for complete gills (arch and filaments) over a relatively wide size range of white suckers was obtained. $Q0_2$ means for the four treatments ranged from 1.5 to 1.7 µl 0_2/mg dry gill/hr. Individual $\overline{Q0_2}$ determinations ranged from 0.7 to 3.9 (Tables 4 , 5).

Since these $Q0_2$'s include cartilage weight in their calculations, the question arises as to whether the proportion of cartilage to tissue (filamental and lamellar) is constant over the wide range of fish sizes used in the study. Since gill area per g of fish decreases with increasing fish size (Muir, 1969), so might the proportion of cartilage. The only factor to help compensate for any

possible change in cartilage proportion is the relatively large fish size ranges used in both control and test groups. For a more precise QO_2 based solely on tissue, the filaments could have been excised from the arches. However, fear of excessive physical damage and expediency in placing gills into Ringer solution were decisive factors in not excising the filaments.

We conclude there was no effect on respiration rate of gills exposed for a relatively short time to a lethal concentration of chlorine (one ppm total residual chlorine). If normal gill tissues use oxygen while metabolizing, we would expect any damage to such tissue to alter its QO_2. Since pilot studies showed that 1 ppm chlorine was lethal in one to two hours to the species used in this study, but gill QO_2 was unaffected, it is concluded that death was not attributable to gill tissue destruction and that gills are not the primary site for toxic action of chlorine. Since there are reports of gills apparently damaged by chlorine (Mann, 1950), the previous statements raise many points worth investigating:

1. What physiologically causes death?
2. How and where does chlorine enter a fish?
3. What site or system is affected by chlorine? How?
4. Is the mechanism of kill with high clorine

concentrations over short exposures the same as with very low concentrations over long exposures?

5. Do different forms of the combined chlorine residual affect different sites?

6. Is chlorine toxicity reversible?

From the following description of behavioral characteristics an hypothesis will be offered. While fish were acclimating, they rested with their ventral side touching the aquarium bottom and pectoral fins spread laterally. When the fish were put in control and test tanks, control fish rested as above. After 15 to 20 minutes test fish would rest on the tips of their pectoral, pelvic and anal fins. At times, resting test fish would apparently lose their balance and roll laterally.

Control fish were quiet, sedentary and showed moderate opercular movements. Test fish appeared nervous, more active, prone to darting and colliding with sidewalls, and occasionally swam upside down and on their side. Their rapid operculating became irregular near death and they occasionally gulped air at the surface. Pigmentation in test fish decreased to almost white; control fish retained their dark, mottled appearance. There was little if any build-up of mucus on the body or gills of test fish. They were easier to net after exposure than control fish and they offered little or no resistance to pithing. Control fish writhed

and twisted violently when netted and pithed. Lastly, some test fish displayed small points of hemorraging in the caudal and anal regions.

Hogan (1969) in his study of dieldrin toxicity to green sunfish reported that chlorinated hydocarbon pesticides affect the nervous system and that in green sunfish the brain is the primary target. Since the symptoms he describes are somewhat similar to those presented above, it is hypothesized that chlorine enters through the gills and somehow either directly or indirectly affects the nervous system.

In summary, this study has again pointed out the deleterious effects of chlorine upon freshwater teleosts and the lack of knowledge about the mechanism of its toxicity. A base has been established from which further investigation may be launched into previously posed questions and areas of interest.

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LITERATURE CITED

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APPENDIX

TABLE A-1. Water chemistry data: pH, temperature, dissolved oxygen, alkalinity, and hardness readings for holding (H), acclimation (A), control (C) and test (T) tanks.

DATE	pH				Temperature (C°)			
	H	A	C	T	H	A	C	T
1971								
25 July	7.5	7.5	---	---	13.5	15.0	---	17.0
31 July	7.5	7.7	7.8	7.7	13.5	15.0	17.0	17.0
10 August	7.6	7.8	7.8	7.8	13.5	15.5	17.5	17.5
27 August	7.6	7.8	7.8	7.8	13.0	15.0	17.0	17.0
29 August	7.6	7.8	7.8	7.8	13.5	15.5	17.5	17.5
Mean ($\bar{x}$)					13.40	15.20	17.25	17.20
S.D. (s_x)					0.22	0.27	0.29	0.27
S.E. ($s_x/\sqrt{n}$)					0.10	0.12	0.14	0.12

TABLE A-1.Continued

	Dissolved Oxygen (ppm)				Alkalinity (ppm $CaCo_3$)			
	H	A	C	T	H	A	C	T
1971								
25 July	7.1	7.8	---	---	298	294	---	---
	7.2	7.9	---	---	300	300	---	---
31 July	8.2	7.0	8.1	8.2	324	306	318	316
10 August	6.0	7.8	8.0	8.2	318	314	304	318
27 August	6.0	7.7	8.0	8.1	310	310	312	320
29 August	6.2	7.8	8.0	8.1	288	316	314	322
Mean ($\bar{x}$)	6.78	7.67	8.03	8.15	306.3	306.7	312.0	319.0
S.D. (s_x)	0.88	0.33	0.05	0.06	13.5	8.5	5.9	2.6
S.E. ($s_x/\sqrt{n}$)	0.36	0.14	0.03	0.03	5.5	3.5	2.9	1.3

TABLE A-1. Continued

DATE	Hardness H	A	C	T
1971				
25 July	318	324	---	---
	320	322	---	---
31 July	316	320	322	322
10 August	322	324	320	326
27 August	316	320	320	320
29 August	320	324	324	324
Mean ($\bar{x}$)	318.7	322.3	321.5	323.0
S.D. (s_x)	2.4	2.0	2.0	2.6
S.E. ($s_x/\sqrt{n}$)	1.0	0.8	1.0	1.3

TABLE A-2. Chlorine and chloramine concentrations (in ppm) measured midway through (A) and immediately after (B) 30-minute exposures.

Date of Test	Time of Determination	Total Residual Chlorine	Free Chlorine	Mono-Chloramine	Di-Chloramine
1971					
2 August	A	0.87	0.35	0.40	0.12
	B	0.85	0.41	0.29	0.15
27 August	A	0.97	0.73	0.13	0.11
	B	0.94	0.55	0.28	0.11
29 August	A	0.93	0.75	0.10	0.08
	B	0.88	0.61	0.12	0.15
30 August	A	1.08	0.87	0.09	0.12
	B	1.05	0.84	0.10	0.11
1 September	A	1.02	0.82	0.07	0.13
	B	0.95	0.67	0.15	0.13
4 September	A	1.10	0.90	0.10	0.10
	B	1.00	0.75	0.15	0.10
Mean ($\bar{x}$)	All Tests	0.970	0.688	0.165	0.118
	A	0.995	0.737	0.148	0.110
	B	0.945	0.638	0.182	0.125
S.D. (s_x)	All Tests	0.082	0.177	0.102	0.020
	A	0.088	0.200	0.124	0.017
	B	0.073	0.151	0.082	0.200
S.E. ($s_x/\sqrt{n}$)	All Tests	0.024	0.051	0.029	0.006
	A	0.036	0.082	0.051	0.007
	B	0.030	0.062	0.033	0.008

TABLE A-3. Chlorine and chloramine concentrations (in ppm) measured midway through (A) and immediately after (B) 60-minute exposures.

Date of Test	Time of Determination	Total Residual Chlorine	Free Chlorine	Mono-Chloramine	Di-Chloramine
1971					
26 July	A	1.12	0.83	0.05	0.23
	B	1.07	0.74	0.16	0.17
10 August	A	1.20	0.45	0.64	0.11
	B	1.15	0.62	0.38	0.15
21 August	A	1.01	0.48	0.41	0.11
	B	0.91	0.60	0.15	0.16
22 August	A	1.00	0.62	0.26	0.12
	B	0.84	0.35	0.31	0.18
23 August	A	1.01	0.80	0.11	0.10
	B	1.00	0.80	0.10	0.10
24 August	A	0.97	0.66	0.17	0.14
	B	0.82	0.38	0.27	0.17
Mean ($\bar{x}$)	All Tests	1.008	0.611	0.251	0.145
	A	1.052	0.640	0.273	0.135
	B	0.965	0.582	0.228	0.155
S.D. (s_x)	All Tests	0.116	0.166	0.166	0.039
	A	0.089	0.157	0.219	0.048
	B	0.131	0.184	0.108	0.028
S.E. ($s_x/\sqrt{n}$)	All Tests	0.033	0.048	0.048	0.011
	A	0.036	0.064	0.090	0.020
	B	0.054	0.075	0.044	0.012

TABLE A-4. Total length, weight, gill wet weight and gill dry weight for suckers used during the 30-minute exposures to 1 ppm total residual chlorine.

DATE	Fish[1]	Total Length mm	Total Weight g	Wet Gill Weight mg	Dry Gill Weight mg
1971					
2 August	T	129	18.19	138.0	23.5
	T	118	12.18	184.6	24.7
	C	135	17.52	264.3	34.5
	C	115	11.86	133.6	19.0
27 August	T	125	12.77	188.6	32.6
	T	125	14.26	207.5	38.9
	C	123	14.71	219.3	42.2
	C	115	11.01	162.7	32.3
29 August	T	116	11.72	157.4	27.8
	T	110	9.62	156.9	28.5
	C	115	11.61	164.9	31.9
	C	121	13.36	195.4	36.3
30 August	T	80	3.25	60.0	11.0
	T	110	5.70	122.3	22.0
	C	93	6.11	82.0	16.0
	C	94	5.93	78.1	15.0
1 September	T	152	26.20	484.3	78.5
	T	135	20.16	307.6	50.5
	C	130	16.73	261.2	48.1
	C	132	16.83	293.8	54.3
4 September	T	124	13.84	167.1	34.2
	T	111	9.27	147.0	25.8
	C	112	10.43	167.7	30.5
	C	113	9.45	208.0	36.8

TABLE A-4. Continued:

Mean ($\bar{x}$)	All Fish	118.0	12.613	189.68	33.12
	T	119.6	13.097	193.44	33.17
	C	116.5	12.129	185.92	33.08
S.D. (s_x)	All Fish	15.1	5.121	88.79	14.58
	T	17.3	6.257	108.44	17.26
	C	13.2	3.894	68.50	12.10
S.D.($s_x/\sqrt{n}$)	All Fish	3.1	1.045	18.12	2.98
	T	5.0	1.806	31.30	4.98
	C	3.8	1.124	19.77	3.49

[1]T=Test Fish, C=Control Fish.

TABLE A-5. Total length, weight, gill wet weight and gill dry weight for suckers used during the 60-minute exposures to 1 ppm total residual chlorine.

DATE	Fish[1]	Total Length mm	Total Weight g	Wet Gill Weight mg	Dry Gill Weight mg
1971					
26 July	T	132	17.82	177.6	33.4
	T	111	8.54	117.1	20.3
	C	145	20.10	298.8	52.9
	C	110	6.75	102.7	17.6
10 August	T	100	6.34	133.9	23.4
	T	107	8.28	146.3	26.7
	C	108	8.12	163.5	27.6
	C	110	9.30	171.5	28.1
21 August	T	105	9.05	134.8	25.4
	T	112	10.05	167.5	31.4
	C	103	8.06	121.5	21.7
	C	105	8.46	138.5	25.5
22 August	T	121	13.88	209.2	35.6
	T	118	12.53	193.9	35.8
	C	116	10.89	195.5	33.2
	C	110	11.00	161.3	29.4
23 August	T	87	4.68	62.0	11.1
	T	89	4.78	73.8	13.6
	C	90	4.26	85.8	14.6
	C	93	5.00	80.7	14.5
24 August	T[2]	185	52.21	389.4	71.0
				372.6	67.3
	C[2]	172	36.90	234.9	45.6
				247.6	48.3

TABLE A-5. Continued

Mean ($\bar{x}$)	All Fish	115.0	12.591	174.18	31.42
	T	115.2	13.469	181.51	32.92
	C	114.7	11.713	166.86	29.92
S.D. (s_x)	All Fish	24.6	11.334	85.54	15.80
	T	26.7	13.442	102.95	18.70
	C	23.7	9.348	67.70	12.94
S.E. ($s_x/\sqrt{n}$)	All Fish	5.3	2.416	17.46	3.23
	T	8.0	4.053	29.72	5.40
	C	7.2	2.818	19.54	3.74

[1]T = Test Fish, C = Control Fish.
[2]Large fish; gills cut in half (lengthwise) and tested separately.

TABLE B-1. Correction factors (CF), fish weights (g), oxygen uptakes and QO_2 rates[1] of white sucker gill tissue following a 30-minute exposure to 1 ppm total residual chlorine, August 2, 1971.

		TEST				CONTROL			
		18.19 g		12.18 g		17.52 g		11.86 g	
Time (Min)	CF (µl)	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2
30	1.6	22.4	1.906	19.4	1.571	29.6	1.716	19.5	2.053
60	0.3	10.1	0.860	12.5	1.012	20.0	1.159	10.1	1.063
90	0.1	10.5	0.894	12.0	0.972	18.8	1.090	9.0	0.947
120	0.7	3.4	0.289	6.7	0.543	14.8	0.858	5.5	0.579
150	1.6	10.9	0.928	11.6	0.939	14.1	0.817	8.2	0.863
180	0.5	5.4	0.460	7.4	0.599	13.2	0.765	5.9	0.621
210	0.6	6.0	0.511	7.3	0.591	12.8	0.742	6.4	0.674
240	0.9	7.7	0.655	8.7	0.704	11.3	0.655	4.5	0.474
270	0.9	6.1	0.519	6.2	0.502	10.4	0.603	4.6	0.484
300	0.4	5.9	0.502	5.9	0.478	10.3	0.597	5.1	0.537
330	0.3	4.7	0.400	4.2	0.340	7.7	0.446	4.7	0.495
360	0.4	5.9	0.502	5.3	0.429	8.5	0.493	3.8	0.400

[1]Expressed as µl O_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-2. Correction factors (CF), fish weights (g), oxygen uptakes and $Q0_2$ rates[1] of white sucker gill tissue following a 30-minute exposure to 1 ppm total residual chlorine, August 27, 1971.

		TEST				CONTROL			
		12.77 g		14.26 g		14.71 g		11.01 g	
Time (Min)	CF (µl)	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$
30	0.8	35.5	2.178	25.9	1.332	21.3	1.009	27.9	1.728
60	1.4	30.3	1.859	22.4	1.152	14.6	0.692	25.9	1.604
90	1.0	29.2	1.791	23.5	1.208	14.6	0.692	28.2	1.746
120	1.0	28.4	1.742	23.1	1.188	12.4	0.588	26.4	1.635
150	0.4	27.8	1.706	23.2	1.193	14.7	0.697	27.8	1.721
180	1.3	27.7	1.699	22.1	1.136	14.5	0.687	25.4	1.573
210	1.1	26.3	1.613	22.7	1.167	17.5	0.829	27.2	1.684
240	0.5	27.3	1.675	21.6	1.111	19.8	0.886	26.2	1.622
270	1.0	25.7	1.577	20.9	1.075	19.6	0.929	24.7	1.529
300	0.3	25.6	1.571	20.9	1.075	22.1	1.047	26.3	1.628
330	1.2	25.8	1.583	20.7	1.064	20.6	0.976	25.2	1.560
360	0.8	24.4	1.497	20.2	1.039	22.8	1.081	25.0	1.548

[1]Expressed as µl 0_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-3. Correction factors (CF), fish weights (g), oxygen uptakes and QO_2 rates[1] of white sucker gill tissue following a 30-minute exposure to 1 ppm total residual chlorine, August 29, 1971.

		TEST				CONTROL			
		11.72 g		9.62 g		11.61 g		13.36 g	
Time (Min)	CF (µl)	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2
30	1.4	23.3	1.676	32.2	2.260	23.6	1.480	28.2	1.554
60	0.4	19.7	1.417	29.1	2.042	22.6	1.417	25.5	1.405
90	0.4	19.8	1.324	29.3	2.056	21.8	1.367	24.1	1.328
120	0.3	18.0	1.295	28.2	1.979	21.5	1.348	23.9	1.317
150	1.3	19.4	1.396	29.1	2.042	21.0	1.317	22.7	1.251
180	0.5	18.2	1.309	28.8	2.021	19.6	1.229	22.2	1.223
210	0.5	17.7	1.273	27.8	1.951	20.8	1.304	22.1	1.218
240	0.0	18.1	1.302	27.0	1.895	20.1	1.260	21.7	1.196
270	0.4	17.4	1.252	26.1	1.832	18.4	1.154	20.9	1.152
300	0.8	19.0	1.367	27.8	1.951	19.2	1.204	21.0	1.157
330	0.2	18.2	1.309	26.5	1.860	19.0	1.191	21.2	1.168
360	0.7	17.0	1.223	25.4	1.782	18.7	1.172	20.6	1.135

[1]Expressed as µl O_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-4. Correction factors (CF), fish weights (g), oxygen uptakes and $Q0_2$ rates[1] of white sucker gill tissue following a 30-minute exposure to 1 ppm total residual chlorine, August 30, 1971.

		TEST				CONTROL			
		3.25 g		5.70 g		6.11 g		5.93 g	
Time (Min)	CF (µl)	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$	0_2 (µl)	$Q0_2$
30	0.3	26.3	4.782	30.7	2.791	24.6	3.075	25.9	3.453
60	0.6	21.4	3.891	28.1	2.555	19.9	2.487	19.6	2.613
90	1.5	21.3	3.873	26.3	2.391	20.6	2.575	20.1	2.680
120	0.0	18.8	3.418	26.5	2.409	21.0	2.625	19.6	2.613
150	1.0	17.8	3.236	25.4	2.309	18.5	2.313	18.6	2.480
180	1.0	21.8	3.964	26.8	2.436	19.4	2.425	18.9	2.520
210	0.3	20.9	3.800	25.3	2.300	19.2	2.400	18.4	2.453
240	0.2	23.4	4.255	27.4	2.491	18.4	2.300	18.8	2.507
270	0.3	20.8	3.782	25.9	2.355	18.9	2.362	18.6	2.480
300	0.4	21.1	3.836	25.4	2.309	19.0	2.375	18.3	2.440
330	0.6	19.2	3.491	23.0	2.091	18.6	2.325	17.7	2.360
360	0.4	22.2	4.036	26.1	2.373	17.6	2.200	17.9	2.387

[1]Expressed as µl 0_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-5. Correction factors (CF), fish weights (g), oxygen uptakes and QO_2 rates[1] of white sucker gill tissue following a 30-minute exposure to 1 ppm total residual chlorine, September 1, 1971.

		TEST				CONTROL			
		26.20 g		20.16 g		16.73 g		16.83 g	
Time (Min)	CF (µl)	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2
30	0.2	68.2	1.738	38.4	1.521	42.8	1.780	41.6	1.532
60	0.1	57.7	1.470	32.8	1.299	37.8	1.572	37.1	1.366
90	0.5	54.4	1.386	30.9	1.224	36.4	1.514	34.3	1.263
120	0.2	54.1	1.378	32.1	1.271	36.0	1.497	34.0	1.252
150	0.1	53.3	1.358	29.8	1.180	33.5	1.393	33.5	1.234
180	0.4	51.4	1.310	30.5	1.208	35.3	1.468	32.6	1.201
210	0.6	53.5	1.363	30.6	1.212	34.3	1.426	33.2	1.223
240	0.1	48.2	1.228	29.1	1.152	33.2	1.380	31.4	1.157
270	0.3	47.9	1.220	29.2	1.156	31.4	1.306	31.0	1.142
300	0.1	48.3	1.231	29.8	1.180	32.6	1.356	31.6	1.164
330	0.2	48.6	1.238	28.0	1.109	30.9	1.285	30.6	1.127
360	0.1	46.5	1.185	28.4	1.125	29.1	1.210	28.7	1.057

[1]Expressed as µl O_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-6. Correction factors (CF), fish weights (g), oxygen uptakes and QO_2 rates[1] of white sucker gill tissue following a 30-minute exposure to 1 ppm total residual chlorine, September 4, 1971.

		TEST				CONTROL			
		13.84 g		9.27 g		10.43 g		9.45 g	
Time (Min)	CF (µl)	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2
30	0.3	22.5	1.316	24.1	1.868	25.6	1.679	45.5	2.473
60	0.0	23.5	1.374	24.0	1.860	24.9	1.633	40.4	2.196
90	0.2	23.4	1.368	24.0	1.860	24.4	1.600	40.7	2.212
120	0.6	24.9	1.456	24.3	1.884	25.5	1.672	39.7	2.158
150	0.0	23.6	1.380	23.7	1.837	24.8	1.626	39.0	2.120
180	0.7	20.9	1.222	19.7	1.527	21.7	1.423	40.0	2.174
210	0.4	24.0	1.404	25.8	2.000	26.1	1.711	34.8	1.891
240	0.9	22.4	1.310	23.7	1.837	23.3	1.528	38.9	2.114
270	0.3	21.2	1.240	23.7	1.837	23.9	1.567	36.8	2.000
300	0.2	19.6	1.146	20.5	1.589	20.2	1.325	34.6	1.880
330	0.9	25.6	1.497	27.7	2.147	27.3	1.790	40.7	2.212
360	0.2	19.9	1.164	23.7	1.837	22.5	1.475	35.0	1.902

[1]Expressed as µl O_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-7. Correction factors (CF), fish weights (g), oxygen uptakes and $Q0_2$ rates[1] of white sucker gill tissue following a 60-minute exposure to 1 ppm total residual chlorine, July 26, 1971.

		TEST				CONTROL			
		17.82 g		8.54 g		20.10 g		6.75 g	
Time (Min)	CF (µl)	O_2[2] (µl)	$Q0_2$	O_2[2] (µl)	$Q0_2$	O_2[2] (µl)	$Q0_2$	O_2[2] (µl)	$Q0_2$
30	0.3	34.1	2.042	23.1	2.276	36.2	1.369	21.9	2.489
60	0.7	27.7	1.659	19.2	1.892	32.6	1.233	19.5	2.216
90	0.0	35.4	2.120	25.8	2.542	33.8	1.278	22.6	2.568
120	0.0	29.9	1.790	22.8	2.246	32.4	1.225	20.1	2.284
150	0.0	30.4	1.820	22.3	2.197	30.7	1.161	19.7	2.239
180	0.0	32.3	1.934	22.8	2.246	29.9	1.130	19.3	2.193
210	0.0	31.8	1.904	21.7	2.138	27.2	1.028	18.1	2.057
240	0.0	31.8	1.904	21.9	2.158	27.5	1.040	17.2	1.955
270	0.0	32.5	1.946	22.6	2.227	28.7	1.085	20.7	2.352
300	0.0	31.1	1.862	22.0	2.167	25.6	0.968	17.5	1.989
330	0.0	32.3	1.934	20.9	2.059	25.0	0.945	17.9	2.034
360	0.0	29.0	1.737	21.6	2.128	25.6	0.968	17.3	1.966

[1]Expressed as µl 0_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-8. Correction factors (CF), fish weights (g), oxygen uptakes and QO_2 rates[1] of white sucker gill tissue following a 60-minute exposure to 1 ppm total residual chlorine, August 10, 1971.

		TEST				CONTROL			
		6.34 g		8.28 g		8.12 g		9.30 g	
Time (Min)	CF (µl)	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2
30	1.2	26.8	2.291	31.8	2.382	23.6	1.710	28.2	2.007
60	0.4	27.0	2.308	27.0	2.022	24.2	1.754	24.8	1.765
90	0.5	24.9	2.128	27.6	2.067	23.1	1.674	24.9	1.772
120	0.0	24.2	2.068	28.3	2.120	21.9	1.587	24.8	1.765
150	0.4	25.9	2.214	26.5	1.985	22.4	1.623	22.9	1.630
180	0.5	26.8	2.291	28.8	2.157	22.9	1.659	24.7	1.758
210	0.2	22.2	1.897	25.7	1.925	21.1	1.529	23.5	1.673
240	0.0	21.0	1.795	25.9	1.940	21.0	1.522	22.3	1.587
270	0.7	23.3	1.991	26.2	1.963	20.5	1.486	20.6	1.466
300	0.4	23.2	1.983	24.1	1.805	17.3	1.254	18.9	1.345
330	0.0	22.8	1.949	25.6	1.918	21.7	1.572	21.5	1.530
360	0.8	19.4	1.658	25.2	1.888	12.2	0.884	13.5	0.961

[1]Expressed as µl O_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-9. Correction factors (CF), fish weights (g), oxygen uptakes and $Q0_2$ rates[1] of white sucker gill tissue following a 60-minute exposure to 1 ppm total residual chlorine, August 21, 1971.

		TEST				CONTROL			
		9.05 g		10.05 g		8.06 g		8.46 g	
Time (Min)	CF (µl)	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$	0_2[2] (µl)	$Q0_2$
30	0.0	18.2	1.433	24.1	1.535	17.1	1.576	20.2	1.584
60	1.2	14.2	1.118	20.2	1.287	15.9	1.465	18.5	1.451
90	1.5	18.8	1.480	20.9	1.331	16.9	1.558	17.6	1.380
120	0.5	18.4	1.449	22.2	1.414	16.9	1.558	18.0	1.412
150	0.6	18.0	1.417	22.1	1.408	16.9	1.558	17.3	1.357
180	0.2	18.4	1.449	22.5	1.433	17.1	1.576	17.8	1.396
210	0.3	18.9	1.488	20.3	1.293	15.5	1.429	16.7	1.310
240	0.1	19.3	1.520	22.3	1.420	16.7	1.539	17.4	1.365
270	0.2	17.1	1.346	21.3	1.357	16.3	1.502	17.3	1.357
300	0.3	20.0	1.575	21.3	1.357	15.8	1.456	16.8	1.318
330	0.2	19.6	1.543	21.9	1.395	16.2	1.493	17.1	1.341
360	0.2	17.9	1.409	20.5	1.306	15.5	1.429	16.2	1.271

[1]Expressed as µl 0_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-10. Correction factors (CF), fish weights (g), oxygen uptakes and $Q0_2$ rates[1] of white sucker gill tissue following a 60-minute exposure to 1 ppm total residual chlorine, August 22, 1971.

		TEST				CONTROL			
		13.88 g		12.53 g		10.89 g		11.00 g	
Time (Min)	CF (µl)	O_2[2] (µl)	$Q0_2$	O_2[2] (µl)	$Q0_2$	O_2[2] (µl)	$Q0_2$	O_2[2] (µl)	$Q0_2$
30	0.2	36.4	2.045	43.4	2.425	33.2	2.000	38.5	2.619
60	0.2	31.4	1.764	35.2	1.966	22.1	1.331	24.5	1.667
90	1.0	29.4	1.652	34.3	1.916	21.7	1.307	24.3	1.653
120	0.3	29.4	1.652	33.7	1.883	20.6	1.241	22.6	1.537
150	0.1	28.8	1.618	32.7	1.827	21.8	1.313	22.2	1.510
180	0.4	29.7	1.669	32.2	1.799	20.6	1.241	21.6	1.469
210	0.0	28.8	1.618	31.7	1.771	20.1	1.211	20.9	1.422
240	0.2	28.7	1.612	30.1	1.682	19.9	1.199	20.6	1.401
270	0.3	27.1	1.522	31.0	1.732	20.3	1.223	20.3	1.381
300	0.9	27.7	1.556	29.6	1.654	19.2	1.157	20.4	1.388
330	0.6	27.2	1.528	27.4	1.531	19.1	1.151	18.6	1.265
360	0.0	26.8	1.506	28.3	1.581	19.2	1.157	18.1	1.231

[1]Expressed as µl O_2/mg dry gill weight/hr.
[2]Corrected oxygen consumption.

TABLE B-11. Correction factors (CF), fish weights (g), oxygen uptakes and QO_2 rates[1] of white sucker gill tissue following a 60-minute exposure to 1 ppm total residual chlorine, August 23, 1971.

		TEST				CONTROL			
		4.68 g		4.78 g		4.26 g		5.00 g	
Time (Min)	CF (µl)	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2	O_2[2] (µl)	QO_2
30	0.7	7.7	1.387	6.9	1.015	12.5	1.712	15.3	2.110
60	0.2	10.8	1.946	14.4	2.118	15.6	2.137	17.0	2.345
90	0.8	11.6	2.090	1.58	2.324	16.2	2.219	16.7	2.303
120	0.7	13.3	2.396	17.1	2.515	16.3	2.233	16.0	2.207
150	0.4	11.8	2.126	16.2	2.382	18.1	2.479	17.8	2.455
180	1.0	12.5	2.252	16.6	2.441	15.6	2.137	14.7	2.028
210	0.1	13.9	2.505	18.8	2.765	17.5	2.397	16.2	2.234
240	0.7	10.8	1.946	15.9	2.338	16.1	2.205	15.4	2.124
270	0.8	11.3	2.036	16.8	2.471	15.3	2.096	12.8	1.766
300	0.9	13.2	2.378	17.6	2.588	15.3	2.096	14.4	1.986
330	0.7	12.4	2.234	17.0	2.500	16.7	2.288	15.3	2.110
360	0.2	11.5	2.072	16.6	2.441	15.0	2.055	13.1	1.807

[1] Expressed as µl O_2/mg dry gill weight/hr.
[2] Corrected oxygen consumption.

TABLE B-12. Correction factors (CF), fish weights (g), oxygen uptakes and $Q0_2$ rates[1] of white sucker gill tissue following a 60-minute exposure to 1 ppm total residual chlorine, August 24, 1971.

		TEST				CONTROL			
		52.21[2] g				36.90[2] g			
Time (Min)	CF (µl)	0_2[3] (µl)	$Q0_2$	0_2[3] (µl)	$Q0_2$	0_2[3] (µl)	$Q0_2$	0_2[3] (µl)	$Q0_2$
30	0.1	37.5	1.056	25.6	0.761	34.1	1.496	31.1	1.288
60	0.0	35.4	0.997	22.7	0.675	33.1	1.452	26.2	1.085
90	0.0	35.4	0.997	23.8	0.707	32.0	1.404	26.0	1.077
120	0.1	37.5	1.056	25.6	0.761	33.9	1.487	27.6	1.143
150	0.3	30.8	0.868	19.9	0.591	26.7	1.171	21.1	0.874
180	1.0	33.5	0.944	21.7	0.645	29.5	1.294	23.1	0.957
210	0.6	31.0	0.873	20.8	0.618	27.6	1.211	21.6	0.894
240	0.1	31.8	0.896	22.0	0.654	26.9	1.180	23.3	0.965
270	0.2	32.7	0.921	20.9	0.621	29.0	1.272	22.0	0.911
300	0.7	30.4	0.856	21.0	0.624	27.3	1.197	22.0	0.911
330	0.6	31.5	0.887	20.8	0.618	27.6	1.211	21.9	0.907
360	0.1	29.5	0.831	20.4	0.606	26.1	1.145	20.9	0.865

[1] Expressed as µl 0_2/mg dry gill weight/hr.
[2] Fish too large; gills cut in two and 12 readings made on each portion.
[3] Corrected oxygen consumption.