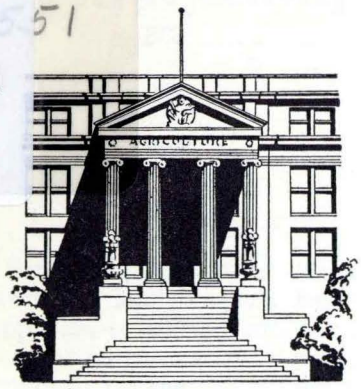

Hyperactivity, Blood Lactic Acid and Mortality in Channel Catfish

by Charles W. Caillouet, Jr.

Department of Zoology and Entomology

AGRICULTURE AND HOME ECONOMICS EXPERIMENT STATION
IOWA STATE UNIVERSITY of Science and Technology

RESEARCH BULLETIN 551 APRIL 1967 AMES, IOWA

CONTENTS

SUMMARY

Mortality in submature channel catfish, *Ictalurus punctatus* (Rafinesque), caught in the Mississippi River and stocked in Iowa streams and ponds, led to this investigation in 1961-1963. The major findings can be summarized as follows:

The mean concentration of lactic acid in the blood of unexercised channel catfish varied from 2.3 to 7.6 mg/100 ml of whole blood. That of fish subjected individually to forced exercise varied from 21.1 to 44.1 mg/100 ml after 5 minutes of exercise and from 59.1 to 67.6 mg/100 ml after 15 minutes of exercise. A lower concentration of lactic acid, 28.2 mg/100 ml, for fish exercised 15 minutes in a group indicates that fish exercised in a group were not subjected to as strenuous muscular activity as were those exercised individually. Mean lactic acid values were usually greater at higher temperatures, both for unexercised and exercised fish.

The degree of muscular activity manifested by individual fish during forced exercise decreased during exercise. The fish were judged exhausted in an average of less than 5 minutes. The average time required for exhaustion was less at higher temperatures.

Capturing catfish with electric shocker or hoop nets and subsequent handling of the fish elevated the lactic acid concentration of the blood to a greater extent than did forced exercise for 15 minutes. Fatigue, loss of equilibrium and mortality were associated with high mean values of lactic acid in fish collected with hoop nets.

Variation in lactic acid concentration among individual fish increased during forced muscular activity. It was hypothesized that such variation might result from differences among individuals in muscle glycogen concentration before activity or differences in fitness among individuals or both.

Hyperactivity, Blood Lactic Acid and Mortality in Channel Catfish[1]

by Charles W. Caillouet, Jr.[2]

For years submature channel catfish, *Ictalurus punctatus* (Rafinesque), have been collected with hoop nets, primarily from the Mississippi River, and transported to numerous stocking sites in Iowa. Attempts to restock the Middle Raccoon River in central Iowa with these fish, after the resident fish population was eradicated with rotenone, were largely unsuccessful (Harrison, 1960) and indicated that this practice needed to be examined. It was hypothesized that strenuous muscular activity in captured, handled and transported fish might cause fatigue, accumulation of lactic acid in the blood and consequent mortality.

Mortality associated with hyperactivity in fishes and its relation to elevated blood lactic acid concentration have been discussed extensively by Black (1967c; 1958a,b), Black et al. (1959) Black, Robertson and Parker (1961), and Black, Manning and Hayasha (1966). Muscular contraction *in vivo* under anaerobic conditions depends ultimately upon glycolysis, as a result of which lactic acid is formed from muscle glycogen (Needham, 1960). The lactic acid readily diffuses into the extracellular fluids, including the blood, and under aerobic conditions, is oxidized to carbon dioxide and water via the tricarboxylic acid cycle or is resynthesized to glucose or glycogen (Guyton, 1961; Prosser and Brown, 1961). Most of the lactic acid is oxidized by extrahepatic tissues (Drury and Wick, 1956), particularly the heart (Guyton, 1961).

The purposes of this investigation were to (1) observe changes in lactic acid concentration in the blood of submature channel catfish subjected to forced muscular activity under laboratory conditions and under conditions of capture and handling in the field and (2) determine if increased concentration of lactic acid in the blood is associated with mortality in these fish.

[1]Project 1373, Iowa Agriculture and Home Economics Experiment Station. Iowa Cooperative Fishery Unit, sponsored by the Iowa State Conservation Commission and Iowa State University of Science and Technology, with the cooperation of the Bureau of Sport Fisheries and Wildlife, U. S. Department of Interior. This project was supported in part by Predoctoral Fellowship GPM-18,515 from the Division of General Medical Sciences, National Institutes of Health, U. S. Public Health Service. This reports a portion of the author's Ph. D. dissertation (Caillouet, 1964b).

[2]Formerly graduate assistant and N.I.H. predoctoral fellow, Department of Zoology and Entomology, Iowa State University of Science and Technology. Now Assistant Professor of Zoology, University of Southwestern Louisiana, Lafayette.

Materials and Methods

Materials and methods are reported in detail because blood lactic acid values may be influenced by handling and condition of the fish, method of blood sampling and procedure of chemical analysis of the blood. Some of these effects have been measured in this investigation.

Data on size, collection and blood sampling of fish are summarized in table 1. Experiments can be considered in three categories: (1) experiments to test blood sampling and lactic acid analysis procedures (experiment 1 and other tests), (2) laboratory experiments to determine the effects of forced muscular activity on blood lactic acid (experiments 2-6) and (3) observations on the effects of capture and handling of fish in the field on blood lactic acid (experiments 7-10). Blood sampling in all experiments was conducted during daylight hours.

Fish Used in Experiments

Fish for the laboratory experiments were collected with hoop nets baited with spoiled processed cheese. On the day of collection they were transported from various locations in the Des Moines River (table 1) in two aerated, 150-gallon, trailer tanks to the laboratory, Cooperative Fisheries Research Unit, Iowa State University, Ames.

After transportation, the fish were transferred to a 200-gallon holding tank and were allowed to rest. Tap water entered an adjacent 60-gallon tank (used for exercising fish in some experiments) and was continuously siphoned into the holding tank. Overflow tubes removed excess water from both tanks. When dechlorination was necessary, water was passed through a vertical column containing glass beads, while air was bubbled up through the beads. The water warmed slightly while flowing through the two tanks exposed to room temperature. Water in the holding tank was less than 2 C warmer than that in the adjacent tank. Water in the tanks was aerated.

The fish were allowed to rest in the holding tank for at least 2 days before being used. They were usually used within 1 week of capture, though one group (experiment 3) was held for 12 days. Fish that died or showed injury were removed. The holding tank was covered to limit disturbance of the fish.

The fish were not fed, but cheese remained in the gastrointestinal tract of many fish for several days

Table 1. Size, collection and blood sampling of submature channel catfish and preparation and storage of protein-free blood filtrates.

Experiment number	Channel catfish[a] Number of fish	Total length mm	Weight, grams	Collection[b] Date	Location	Blood sampling[c] time in minutes Date	Lactic acid[d]	Total[e]	Preparation of protein-free filtrates 10% trichloroacetic acid[f]	Hours lapsed before filtration	Storage time days[g]
1	12	274± 8.9 (239-333)	139±15.0 (82-257)	7/25/63	Des Moines River, Polk County	7/27/63	—	1.4±0.18 (0.6-2.4)	9 ml, C	0.25	10
2	25	314± 3.4 (284-345)	245± 9.0 (167-379)	6/4/62	Des Moines River, Humboldt County	6/6/62	1.0±0.11 (0.3-2.5)	2.3±0.12 (1.5-3.8)	4.5 ml, C	2.0	7-12
3	19	251± 4.2 (224-307)	116± 7.6 (83-231)	6/4/62	Des Moines River, Humboldt County	6/16/62	0.6±0.07 (0.2-1.5)	1.0±0.07 (0.7-2.1)	4.5 ml, U	0.25	4-7
4	26	274± 5.3 (236-340)	158± 9.0 (108-283)	6/20/62	Des Moines River, Humboldt County	6/22/62	1.4±0.23 (0.3-4.3)	1.9±0.26 (0.6-4.9)	4.5 ml, C	0.25	4-6
5	20	256± 2.0 (239-279)	120± 3.1 (95-149)	6/20/62	Des Moines River, Humboldt County	6/25/62	0.9±0.13 (0.2-2.4)	1.8±0.15 (0.9-3.6)	4.5 ml, C	0.25	10-11
6	12	223± 8.8 (193-284)	80±10.0 (52-157)	7/30/63	Des Moines River, Polk County	8/2/63	0.9±0.14 (0.3-2.1)	1.7±0.15 (1.1-2.9)	9 ml, C	0.25	5-6
7	26	230± 5.0 (175-274)	88± 6.4 (32-146)	5/29/61	Middle Raccoon River, Dallas County	5/29/61	0.5±0.07 (0.1-1.6)	—	9 ml, U	4.25	1
8	16	257±16.3 (178-386)	154±30.8 (46-458)	6/14-15/61	Des Moines River, Boone County	6/17/61	0.4±0.08 (0.1-0.9)	—	9 ml, U	2.75	5
9	62	240± 2.9 (145-310)	111± 4.3 (23-253)	7/15,17-18/61	Mississippi River, Allamakee County	7/17-18/61	—	1.0±0.06 (0.2-2.5)	9 ml, U	2.75	2
10	14	237± 4.5 (213-282)	108± 5.5 (72-163)	7/24/61	Lake View Ponds, Sac County	7/24/61	0.5±0.03 (0.3-0.7)	—	9 ml, U	0.5	2

[a]Values represent the mean, followed by standard error, with range in parentheses.
[b]Fish were collected with hoop nets for all experiments except 7 and 10 in which electric shocking was employed.
[c]Blood sampling was done at Iowa State University except that sampling was done at or near the collection site for experiments 7, 9 and 10.
[d]Time lapse from insertion of the hypodermic needle to ejection of blood into trichloroacetic acid.
[e]Time lapse from removal of fish from tank to completion of sampling.
[f]U=uncooled; C=cooled (near 6 C).
[g]Filtrates for experiments 1-6 were frozen immediately and stored at -25 C. Filtrates in experiment 7 were cooled 4.5 hours, frozen and stored at -14 C. Filtrates for experiment 9 were kept at 20 C for 0-11.5 hours, cooled for 5-6.5 hours and frozen at -14 C. Filtrates for experiments 8 and 10 were frozen immediately and stored at -14 C.

after capture. Black et al. (1961) indicated that both muscle and liver glycogen are restored more rapidly when fish are fed during recovery from muscular activity. Other details of laboratory experiments are reported later.

Blood Lactic Acid

Each fish was removed from the water and was stunned by a blow on the head. While the fish was held with a moist cloth, the operculum was lifted (see Perkins, 1957), and blood was taken by cardiac puncture. The volume of blood taken was 1 ml or less.

Blood was extracted with 2-ml Luer syringes, into which aqueous ammonium heparinate had been drawn and from which the excess anticoagulant had been thoroughly ejected. The heparinate preparation (produced by Clay-Adams, Inc., New York, N. Y.) contained 1,000 USP heparin units/ml.

After blood extraction, the syringe was inverted, and the plunger was carefully pushed upward to the nearest 0.1-ml graduation to expel air bubbles and small excesses of blood. The blood sample was immediately ejected into a polyethylene vial containing 10% (weight/volume) trichloroacetic acid solution. Blood was deproteinized by treatment with trichloroacetic acid and filtration. Filtrates were analyzed for lactic acid by the colorimetric method of Barker and Summerson as outlined in Hawk, Oser and Summerson (1954). Lactic acid values were expressed in mg/100 ml of whole blood to the nearest 0.1 mg/100 ml.

To determine the effect of time that fish were out of water on concentration of lactic acid in their blood, unexercised fish were removed from water (18 C) and were stunned by a blow on the head. Blood samples were taken from individual fish left out of water (room temperature, 27 C) for 0, 2 or 4 minutes (experiment 1). The three treatments were randomly assigned to 12 fish, 4 fish per treatment. Each fish was treated individually. Because time was required for sampling, the actual time that a fish was out of water was slightly greater than the designated time, but the three treatments were distinctly different.

A significant[3] linear relationship was found between blood lactic acid and time (table 2). The approximate average rate of increase in blood lactic acid was 1 mg/100 ml/minute the fish was left out of water. In other experiments, sampling was completed in less than 6 minutes, and the average was less than 3 minutes.

In the 1961 experiments (table 1), the trichloroacetic acid solution was not cooled before receiving channel catfish blood to be deproteinized. In 1962 and 1963, cold (near 6 C) trichloroacetic acid was used to reduce possible change in blood lactic acid concentration (as suggested by Friedemann and Haugen, 1942, 1943). Blood-trichloroacetic acid mixtures were often filtered within 15 minutes after blood extraction, but in some cases, several hours intervened. Protein-free filtrates, collected in clean polyethylene vials, were either frozen immediately or first cooled (iced or refrigerated) and then frozen, except in experiment 9 in which, because of a malfunction in refrigeration, 11.5 hours elapsed before filtrates were cooled. Filtrates were stored at -14 or -25 C until analyzed for lactic acid.

To evaluate the effect of the time between sampling of blood and filtration of the blood-trichloroacetic acid (uncooled) mixture and the effect of storage of protein-free filtrates in the frozen condition, the following experiment was conducted. A 2-ml blood sample was mixed with 18 ml of 10% trichloroacetic acid at 23 C. The mixture was divided into two parts; one was filtered immediately, and the other was filtered after 2 hours at 23 C. The two filtrates were each subdivided into two portions; blood lactic acid analysis was begun immediately on one, and the other was analyzed after storing 35 days at -14 C. Lactic acid determinations were made on three aliquots from each portion of protein-free filtrate.

Three orthogonal comparisons (Snedecor, 1956) were made among the four treatments (table 3). The comparisons were: immediate filtration compared with filtration after 2 hours at 23 C, immediate analysis compared with analysis after 35 days at -14 C and the interaction. Only the comparison between immediate and later filtration was significant. Blood-trichloroacetic acid mixtures left standing 2

Table 2 . Blood lactic acid (mg/100 ml) in submature channel catfish left out of water (experiment 1).

Time out of water, minutes	Mean	SE[a]	Range	Number of fish	CV[b]
0	3.3	1.22	1.4-6.8	4	74
2	3.5	0.43	2.8-4.7	4	24
4	7.2	1.16	4.0-9.4	4	32

[a]Standard error = standard deviation/number$^{\frac{1}{2}}$
[b]Coefficient of variation = standard deviation (100)/mean.

	Analysis of variance			
Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Periods	2	38.86	19.43	4.81*
Linear	(1)	(30.81)	30.81	7.63*
Quadratic	(1)	(8.05)	8.05	1.99
Individual fish	9	36.39	4.04	
Total	11	75.25		

*Indicates significance at the 0.95 confidence level.

[3]Refers throughout this paper to the 0.95 confidence level.

Table 3. Concentration of lactic acid (mg/100 ml) after preparation and storage of protein-free filtrates of blood-trichloroacetic acid mixtures.

Treatment	Mean	SE	Range	Number of fish	CV
Filtered immediately					
Analysis begun immediately	9.3	0.46	8.4-9.9	3	8
Analyzed after storage for 35 days at -14 C	10.6	0.74	9.7-12.1	3	12
Filtered after 2 hours at 23 C					
Analysis begun immediately	8.1	0.35	7.6-8.8	3	8
Analyzed after storage for 35 days at -14 C	9.2	0.58	8.1-10.1	3	11

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatments	3	9.47	3.16	3.43
Filtration	(1)	(5.20)	5.20	5.65*
Storage	(1)	(4.20)	4.20	4.56
Interaction	(1)	(0.07)	0.07	0.08
Determinations	8	7.34	0.92	
Total	11	16.81		

*Indicates significance at the 0.95 confidence level.

hours at 23 C (uncooled) before filtration averaged 1.3 mg/100 ml less lactic acid than did those filtered immediately. Storage of the protein-free filtrates at -14 C for 35 days, more than twice as long as usually employed, caused no demonstrable change in blood lactic acid concentration.

The dilution of blood with the protein-precipitating agent should be kept constant (Hawk et al., 1954). This was not always possible in studies of blood lactic acid in fishes (see Parker et al., 1959; Caillouet, 1964a). With constant trichloroacetic acid volume, calculated blood lactic acid values presumably are subject to increasing variability with decrease in blood volume used. Preparation of sufficient numbers of vials, each containing an equal volume of trichloroacetic acid solution, facilitated subsequent blood sampling. Since trichloroacetic acid volume was constant in a given experiment (viz., 9 ml when 1 ml or less of blood was extracted and 4.5 ml when 0.5 ml or less was extracted), differences in volume of blood extracted resulted in variation in dilution. It was difficult to secure the same volume of blood from each submature channel catfish, probably because of relatively low blood volumes and impaired circulation (see Leivestad et al., 1957; Scholander et al., 1962). The effect of variation in dilution, though not evaluated, may also have involved variation in efficiency of deproteinization. Dilution was 1:10 or greater.

For determination of blood lactic acid by the Barker-Summerson method, a "Spectronic 20" spectrophotometer (Bausch and Lomb Optical Co., Rochester, N.Y.) was used with 3/4-inch test tubes (in 1961) or 1/2-inch test tubes (in 1962 and 1963). Lactic acid standards were prepared from lithium lactate produced by Hartman-Leddon Co., Philadelphia, Pa. Throughout the study, lactic acid determinations were made on duplicate aliquots from many individual protein-free filtrates. These duplicate determinations were grouped into two categories: those made with 3/4-inch test tubes and those made with 1/2-inch test tubes. Within each category, duplicates were grouped according to their means into several classes (tables 4 and 5). The variance and standard deviation of duplicates provided measures of precision of the Barker-Summerson method. Bartlett's test (Snedecor, 1956) failed to detect significant heterogeneity among the variances within different classes of duplicate determinations made with 3/4-inch test tubes. An F-test (Snedecor, 1956) indicated a significant difference between the variances of duplicates within the two classes of duplicate determinations made with 1/2-inch test tubes. Relative precision of the method increased with increases in the measured quantity of lactic acid, since the standard deviation remained relatively constant as the mean increased (tables 4 and 5).

Variances for duplicate determinations made with 3/4-inch and 1/2-inch test tubes, classes combined (tables 4 and 5), were significantly different as

Table 4. Partitioning of variance of duplicate Barker-Summerson blood lactic acid (mg/100 ml) determinations made with 3/4-inch Bausch and Lomb test tubes.

Class mg/100 ml	Source of variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation
0-39	Samples	7	1,651.38	235.91	
	Duplicate determinations	8	121.29	15.16	3.89
40-79	Samples	5	816.61	163.32	
	Duplicate determinations	6	94.89	15.82	3.98
80-119	Samples	2	661.98	330.99	
	Duplicate determinations	3	161.99	54.00	7.35
160-199	Samples	0	—	—	
	Duplicate determinations	1	24.50	24.50	4.95
Combined	Samples	17	47,250.90	2,779.46	
	Duplicate determinations	18	402.67	22.37	4.73

determined by an F-test. This test was approximate because of the difference between variances for duplicates within the two classes of duplicate determinations made with 1/2-inch test tubes; however, the calculated F-value for the test greatly exceeded the tabular F-value. The greater precision obtained with 1/2-inch test tubes may have been due in part to experience and improvement in technique.

A direct test was made between 3/4-inch and 1/2-inch test tubes. Duplicate aliquots from each of five solutions of lactic acid (representing a range in lactic acid concentration from 12.5 to 62.5 mg/100 ml) were analyzed by the Barker-Summerson method, and absorbancy of the final colored solutions was measured both in 3/4-inch and 1/2-inch test tubes. An F-test indicated that the variance for duplicate determinations made with 3/4-inch test tubes was significantly greater than that for duplicate determinations made with 1/2-inch test tubes (table 6). There was no detectable difference between the two types of tubes with regard to the mean lactic acid concentration.

Protein-free filtrates of channel catfish blood were typically colorless, but green filtrates were observed in a few cases. Upon adding concentrated sulfuric acid to the copper-lime supernatants produced from such filtrates in the Barker-Summerson method, a yellow rather than colorless solution resulted, and the final color was orange-green rather than violet. It was suspected that during blood sampling the hypodermic needle was accidentally inserted through the heart into the liver or gall bladder and that the blood sample thereby became contaminated with bile. Graphed absorption spectra of colored solutions produced from a green filtrate and from a filtrate from blood into which bile was intentionally introduced were similar. Both absorption spectra differed from that determined for the standard solution; i. e., their maximum absorbancy was near 390 mμ rather than 570 mμ. This suggested that bile had produced the green in some filtrates. Interference with the Barker-Summerson method was evident. Green filtrates and any copper-lime supernatants that turned yellow on addition of sulfuric acid were discarded. Barrett and Connor (1962) noted that the protein-free filtrates from the skipjack tuna, *Euthynnus pelamis,* were an unusual pale green. They concluded that the color caused no interference with the Barker-Summerson procedure.

Unexercised Condition and Forced Exercise

Caillouet (1964b, table 1) reviewed studies that showed that lactic acid accumulates in the blood of fish during and after muscular activity. Earlier findings were supported by recent studies (Black,

Table 5. Partitioning of variance of duplicate Barker-Summerson blood lactic acid (mg/100 ml) determinations made with 1/2-inch Bausch and Lomb test tubes.

Class mg/100 ml	Source of variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation
0-39	Samples	97	32,406.52	334.09	
	Duplicate determinations	98	145.19	1.48	1.22
40-79[a]	Samples	44	11,057.54	251.31	
	Duplicate determinations	45	105.39	2.34	1.53
Combined	Samples	142	135,563.44	954.67	
	Duplicate determinations	143	250.58	1.75	1.32

[a]Includes a single duplicate with mean 80 mg/100 ml.

Table 6. Analysis of variance of duplicate Barker-Summerson lactic acid determinations made with 3/4-inch and 1/2-inch Bausch and Lomb test tubes.[a]

Test tubes	Source of variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	F
3/4-inch	Solutions	4	2,885.44	721.36		
	Duplicate determinations	5	13.35	2.67	1.64	
1/2-inch	Solutions	4	3,353.94	838.48		
	Duplicate determinations	5	1.34	0.27	0.52	9.89
Combined	Test tubes	1	1.30	1.30		0.22
	Solutions	4	6,215.95	1,553.99		
	Error	4	23.43	5.86		
	Duplicate determinations	10	14.69	1.47	1.21	

[a]Lactic acid concentration of the solutions was expressed in mg/100 ml of whole blood for comparison with tables 4 and 5; a 1:10 dilution was assumed.

Table 7. Blood lactic acid concentration (mg/100 ml) in submature channel catfish unexercised (15 C) and after forced exercise (14 C) (experiment 2).

Treatment	Mean	SE	Range	Number of fish	CV
Unexercised	2.8	1.65	0.4-12.7	7	156
Exercised 5 minutes	27.9	2.94	17.9-36.8	6	26
Exercised 10 minutes	52.7	2.61	45.7-63.4	7	13
Exercised 15 minutes	59.1	4.29	50.3-72.7	5	16

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatments	3	12,622.65	4,207.55	86.01*
Individuals	21	1,027.40	48.92	
Total	24	13,650.05		

*Indicates significance at the 0.95 confidence level.

Table 8. Blood lactic acid concentration (mg/100 ml) in submature channel catfish unexercised (19 C) and after forced exercise (18 C) (experiment 4).

Treatment[a]	Mean	SE	Range	Number of fish	CV
Exercised 5 minutes	44.1	4.45	26.6-60.9	7	27
Exercised 10 minutes	56.5	4.54	40.2-76.4	7	21
Exercised 15 minutes	67.6	3.56	58.2-79.6	6	13

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatments	2	1,795.12	897.56	7.34*
Individuals	17	2,078.95	122.29	
Total	19	3,874.07		

[a]Data for unexercised fish were discarded due to an error made in blood lactic acid analyses.
*Indicates significance at the 0.95 confidence level.

Table 9. Blood lactic acid concentration (mg/100 ml) in submature channel catfish unexercised (19 C) and after forced exercise (17 C) (experiment 5).

Treatment	Mean	SE	Range	Number of fish	CV
Unexercised	3.9	0.63	0.5-6.7	10	51
Exercised 5 minutes	37.8	1.94	24.8-46.1	10	16

Manning and Hayashi, 1966; Hayashi, Green and Black, 1964; Stevens and Black, 1966; Wendt, 1964, 1965). However, the family Ictaluridae has received only limited attention (Black, 1955; Dean and Goodnight, 1964).

Secondat and Diaz (1942) were the first to determine blood lactic acid levels of "resting" fish. They also observed the changes in blood lactic acid concentration in tench, *Tinca tinca*, subjected to 15 minutes of forced exercise. Black (1955) proposed that the term "unexercised" be used rather than "resting," since the resting condition was difficult to assess. He used the unexercised condition merely as a control to be compared with the exercised condition. The 15-minute period of exercise used by Secondat and Diaz (1942) and by Black (1955) has been useful for comparing changes in blood lactic acid accompanying muscular activity in many fish species.

Laboratory experiments were conducted to determine blood lactic acid concentrations of submature channel catfish in the unexercised condition and during and after 15 minutes of forced exercise. These experiments provided a basis for evaluating studies on the effects of capture and handling of channel catfish.

Fish Treated Individually

In experiments in which fish were treated individually, a fish was netted from the holding tank and was either blood sampled immediately (unexercised) or was transferred to the exercise tank (20x40 inches) containing approximately 6 inches of water. The fish was chased (exercised) for the designated period, and a blood sample was taken. Timing of the exercise began when the fish was removed from the holding tank. Fish exercised individually seemed to exert maximal muscular activity since full attention was devoted to chasing each fish.

Treatments were assigned to individual fish at random, and the person treating the fish was not informed of the particular treatment until the instant the fish was removed from the holding tank. Thus, possible increased activity from disturbance of fish remaining in the holding tank was not confounded with treatment effects. Because sex of the fish was determined after blood was drawn, the effect of sex was not confounded with treatment effects.

Because water temperature in the holding tank was slightly warmer than that in the exercise tank, both temperatures are given. The maximum period of exercise, 15 minutes, may not have been sufficient time for reduction in body temperature, and increased metabolism during exercise may have counteracted such reduction.

Four experiments were conducted. Experiments 2 and 4 consisted of four treatments: unexercised and 5, 10 and 15 minutes of forced exercise. Experiment 5 consisted of two treatments: unexercised and 5

minutes of forced exercise. Experiment 6 consisted of six treatments: unexercised and 1, 2, 3, 4 and 5 minutes of forced exercise.

Differences in mean blood lactic acid concentrations of male and female fish, tested separately within each treatment (in experiments 2, 4 and 5) were inconsistent as well as nonsignificant, and therefore, sex of the fish was disregarded. Variability in blood lactic acid among fish appeared to increase after forced exercise. Bartlett's test (Snedecor, 1956) failed to show significant heterogeneity among variances of the treatments in experiments 2 and 4. It did, however, reveal significant heterogeneity in experiment 6. Significant heterogeneity was detected with an F-test in experiment 5. Variation among fish in relation to the mean (i. e., the coefficient of variation) decreased with increase in mean blood lactic acid level (tables 7, 8 and 9) but increased in experiment 6 (table 10).

Analyses of variance of the data from experiments 2 and 4 (tables 7 and 8) indicated a significant increase in mean blood lactic acid with increased duration of forced exercise. A t-test, with adjustments for heterogeneous variance (Snedecor, 1956), indicated a significant increase in the mean blood lactic acid concentration of exercised fish in experiment 5 (table 9). Blood lactic acid increased significantly with increased exercise in experiment 6 (table 10). The mean increase in blood lactic acid during 5 minutes of forced exercise was approximately tenfold in experiments 2 and 5 (tables 7 and 9) and sixfold in experiment 6 (table 10). The mean blood lactic acid concentration after 5 minutes of forced exercise (tables 7, 8 and 9) increased with temperature; however, the mean value, 21.1 mg/100 ml, from experiment 6 (table 10) did not follow the trend.

In experiments 2, 4 and 5, measurements were made of fish behavior. The period of vigorous activity was measured as the time from the beginning of exercise to the first sign of fatigue. During the exercise period, attempts were made to grasp the dorsal spine between thumb and forefinger and in this way retain the fish. When exercising vigorously, the fish could not be retained. Indeed, the spine could inflict a painful cut or puncture wound if not readily released. First sign of fatigue was considered that condition in which the fish could first be retained. After the first sign of fatigue, the fish continued to swim, though only moderately, until exhausted; i. e., until the fish would not readily right itself when turned dorsal side down in the tank.

There was some indication that vigorous muscular activity was maintained longer at lower water temperatures (table 11). Exhaustion occurred within an average of less than 5 minutes in all three experiments. Muscular activity decreased during exercise.

Fish Treated in a Group

Experiment 3 consisted of two treatments: unexercised and 15 minutes of forced exercise. Blood sam-

Table 10. Blood lactic acid concentration (mg/100 ml) in submature channel catfish unexercised (20 C) and after forced exercise (18 C) (experiment 6).

Treatment	Mean	SE	Range	Number of fish	CV
Unexercised	3.7	0.50	3.2-4.2	2	19
Exercised 1 minute	5.4	0.45	5.0-5.9	2	12
Exercised 2 minutes	17.8	1.05	16.7-18.8	2	8
Exercised 3 minutes	20.4	3.75	16.7-24.2	2	26
Exercised 4 minutes	28.1	12.50	15.6-40.6	2	63
Exercised 5 minutes	21.1	9.40	11.7-30.5	2	63

Analysis of variance[a]

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatments	5	1.2088	0.2418	7.39*
Individuals	6	0.1964	0.0327	
Total	11	1.4052		

[a]Since the standard deviations and means of treatments were approximately proportional, the data were transformed to logarithms (Snedecor, 1956) before analysis of variance.
*Indicates significance at the 0.95 confidence level.

Table 11. Time lapse (minutes) from the beginning of forced exercise to the first sign of fatigue and to exhaustion in submature channel catfish.

Experiment number	Water temperature, C		Mean	SE	Range	Number of fish
2	14	1st sign fatigue	2.4	0.20	1.0-5.0	18
		exhaustion	4.4	0.38	2.0-10.0	18
5	17	1st sign fatigue	1.8	0.11	1.2-2.3	10
		exhaustion	3.6	0.16	2.8-4.4	10
4	18	1st sign fatigue	2.2	0.13	1.4-3.3	20
		exhaustion	3.7	0.16	2.6-5.0	20

Table 12. Blood lactic acid concentration (mg/100 ml) in submature channel catfish unexercised (18 C) and after forced exercise in a group (18 C) (experiment 3) and after 5 minutes of forced exercise in a group (19 C) and 10 minutes rest (part of experiment 4).[a]

Treatment	Mean	SE	Range	Number of fish	CV
Unexercised	2.3	0.32	1.2-4.1	9	42
Exercised 15 minutes	28.2	3.58	12.7-40.0	10	40
Exercised 5 minutes, rested 10 minutes	52.8	6.04	27.5-72.0	6	28

[a]No significant differences were detected between the mean blood lactic acid levels of male and female fish; therefore, data were combined.

ples were taken first from the group of unexercised fish, netted one after the other from the holding tank. The remaining fish were then forced to swim simultaneously in the holding tank in which the water level was lowered to approximately 6 inches. Blood samples were obtained from these fish, netted one after the other, within 30 minutes after exercise.

The mean blood lactic acid concentration of fish exercised simultaneously for 15 minutes (table 12) was approximately half as great as that for fish exercised individually for 15 minutes (tables 7 and 8), but was significantly greater than for the unexercised fish. Although the fish were chased to induce exercise, it was not possible to cause maximal muscular activity in all individuals because full attention could not be devoted to all fish simultaneously. Therefore, the degree of muscular activity in fish exercised in a group was not believed as strenuous as that in fish exercised individually. An F-test indicated significant difference between treatment variances, but the increased variation among individual fish after forced exercise may have been partly the result of further changes during the period in which blood samples were being taken (table 12). Coefficients of variation were similar for unexercised and exercised fish (table 12).

In experiment 4, after individual treatment of certain fish (table 8), a remaining group was exercised for 5 minutes in the holding tank (19 C) and was allowed to rest for at least 10 minutes before blood was taken. The mean blood lactic acid value (table 12) did not differ significantly from that of fish exercised individually for 5 minutes (table 8), but variation among the rested fish was greater. This suggested that further changes in blood lactic acid took place during the periods of rest and blood sampling.

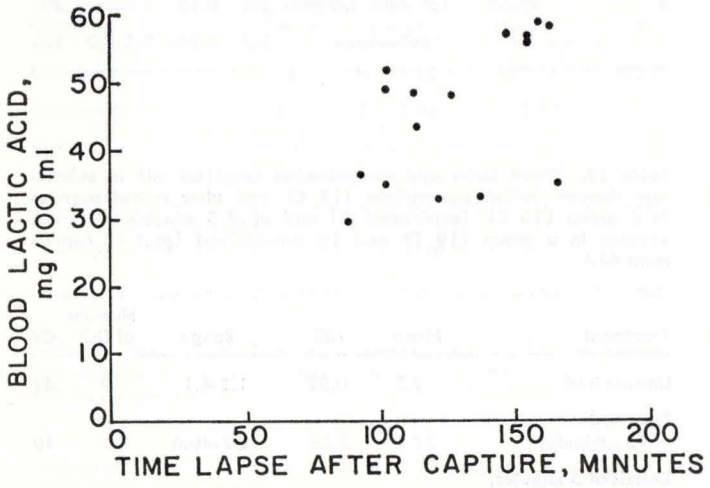

Fig. 1. Blood lactic acid concentration in submature channel catfish after capture with electric shocker, finclipping and handling. Each point represents a different fish. Fish were held in a tub containing unaerated river water, 19 C.

Capture and Handling

Many studies (Caillouet, 1946b, table 1) have demonstrated that blood lactic acid in fishes can be elevated by several procedures of capture and handling. However, there is only limited information on the effects of capture by such commonly used methods as electric shocking and netting with hoop nets.

Electric Shocking and Handling

Nakatani (1957), Heath and Pritchard (1962) and Wendt (1965) used intermittent electric shock to induce vigorous activity in fish for studies of blood lactic acid. Blancheteau et al. (1961), in a review of neurophysiological effects of electricity on fish, stated that motor facilitation and tetanic contraction of the muscles were caused by electric shock. Such forced muscular contraction undoubtedly leads to increased blood lactic acid. Johnson, Nakatani and Felton (1956), in a study of coho salmon, *Oncorhynchus kisutch,* observed an increase in muscle lactic acid in fish subjected to electric shock, but they were unable to distinguish between the direct effect of shocking and the indirect effect of activity induced by the electric current and other stimuli.

A 230-volt, alternating current, Homelite generator equipped with immersible electrodes was used to capture catfish from ponds at Lake View, Iowa (experiment 10), 3 days after the fish had been transported to the ponds by truck from the Iowa State Conservation Commission Fish Hatchery at Lansing, Iowa, a distance of more than 200 miles. Shocked fish were transferred to a 20-gallon tub containing fresh pond water (25-27 C) and were sampled within 30 minutes after capture. Since the correlation coefficient for the relationship between blood lactic acid concentration and time lapse after capture was not significantly different from 0, the mean blood lactic acid value of 79.5 mg/100 ml (SE = 6.09 mg/100 ml; range = 44.7-125.5 mg/100 ml) was calculated for the 14 fish. This value was higher than the means for fish exercised 15 minutes under laboratory conditions (tables 7 and 8).

Catfish were collected for 2 hours from the Middle Raccoon River (experiment 7) below the dam at Redfield, Iowa, with the same electric shocker. Some fish were finclipped and placed in a 20-gallon tub containing fresh river water (19 C). Blood was taken from these fish from 1.5 to 2.8 hours after capture (fig. 1). The lactic acid concentration of the blood increased with the time the fish were retained in the tub after capture and finclipping (the correlation coefficient, $r = 0.52$, is significant at the 95 percent level of confidence).

Other catfish were placed in tubs and transferred to two 160-gallon live-tanks containing aerated river water (19 C). After 3 hours, these tanks and fish were transported 15 minutes by truck, and most of

the catfish were released above the dam. Blood samples were taken from 10 catfish within an hour after transportation. Their average blood lactic acid concentration was 18.4 mg/100 ml (SE = 3.84 mg/100 ml; range=5.2-41.3 mg/100 ml), which was significantly lower than that of the 16 catfish finclipped and held in the tub, 45.6 mg/100 ml (SE= 2.62 mg/100 ml; range = 29.2-58.4 mg/100 ml).

The effect of electric shocking alone on the blood lactic acid in catfish cannot be determined from these experiments. Several factors may have contributed to the high lactic acid values. Fish dipnetted from the water after they were stunned were briefly out of the water. The catfish were somewhat crowded in the tub, and dissolved oxygen concentration was lowered, although fresh water was introduced at intervals. No mortality was observed in these experiments. However, in fish-shocking operations on the Des Moines River, Boone County, in June 1961 individual fish (several species, including channel catfish) with high blood lactic acid concentrations died after capture.

Capture With Hoop Nets and Handling

Capture of fish with hoop nets may be expected to cause an increase in blood lactic acid, particularly when the fish are exposed to anoxia while being removed from the nets. A brief period of struggling in air caused production of lactic acid in the muscle of cod, *Gadus callarius,* and of California grunion, *Leuresthes tenuis,* and the lactic acid diffused rapidly into the blood when the fish were returned to water (Leivestad et al., 1957; Scholander et al., 1962).

Throughout this study, mortality occurred in channel catfish transported to the laboratory from the Des Moines River. On one occasion (experiment 8), blood samples were taken during the time when considerable mortality was occurring. The fish had been captured with hoop nets baited with cheese and were transported 25 miles in two 150-gallon, aerated live-tanks and a 20-gallon nonaerated tub. After transportation, the catfish were subjected to additional handling when they were weighed and measured for another research project. The fish were then transferred to the holding tank in the laboratory. Because regurgitated cheese fouled the water, the fish were transferred several times to fresh water. The tanks were well aerated. Water temperature was 16 C at the time of sampling, 2-3 days after capture and transportation. Blood was obtained from fish that exhibited fatigue, from some that had lost equilibrium and from others that died (sampled before rigor mortis). Cessation of opercular movement was assumed to indicate death.

Since the number of fish was small and there seemed little difference in blood lactic acid concentration between males and females, data for the sexes were combined (table 13). Bartlett's test indicated significant heterogeneity of variance among the three groups of fish. Since the coefficient of variation was relatively constant, a logarithmic transformation (Snedecor, 1956) was used in an analysis of variance that detected significant differences among groups. A multiple-range test (Kramer, 1956) showed no significant difference between mean blood lactic acid concentrations of fish exhibiting fatigue and those that lost equilibrium, but the means of these two groups were significantly lower than those of dead fish. The means of all three groups were higher than those of fish subjected to 15 minutes forced exercise in the laboratory (tables 7 and 8).

Table 13. Blood lactic acid concentration (mg/100 ml) in submature channel catfish after capture with hoop nets, transportation and handling at 16 C (experiment 8).

Group	Mean	SE	Range	Number of fish	CV
Exhibited fatigue	75.9	9.50	39.1-112.3	8	35
Lost equilibrium	104.1	14.62	66.4-138.9	5	31
Dead	200.9	42.34	157.2-285.6	3	36

Analysis of variance of logarithmically transformed data

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Groups	2	0.4085	0.2042	8.23*
Individuals	13	0.3223	0.0248	
Total	15	0.7308		

*Indicates significance at the 0.95 confidence level.

Experiment 9 involved channel catfish caught in hoop nets in the Mississippi River near Lansing, Iowa. The nets were set for 2 days, and thus, the catfish may have entered the nets anytime during the period. Blood samples for one group were taken within 1 hour after the fish were removed from the nets. These fish were first placed in a live-well with river water (25 C) flowing through it, and then they were transferred to a 20-gallon tub of fresh river water just before sampling.

Blood was taken from a second group after the fish had been in the live-well (25 C) about 4 hours.

A third group, collected when river water temperature was 24 C, was transferred within 6 hours from the live-well to 150-gallon live-tanks containing water continually pumped from the river and then to 300-gallon hatchery tanks containing river water that was replaced within 3 hours by colder (16 C) artesian water. Blood samples were taken 6 to 14 hours after the fish were removed from the nets.

A fourth group, collected when river water temperature was 26 C, was handled as was the third group, but blood was not sampled until 60-65 hours after the fish were removed from the nets. At this time, samples were taken from dead fish (sampled

before or during rigor mortis), from fish that had lost equilibrium and from fish that showed signs of fatigue.

Individual t-tests were made on differences between mean blood lactic acid concentrations of male and female fish in each group. Significant differences were detected between sexes only in fish that exhibited fatigue and that lost equilibrium at 60-65 hours. Females had the higher mean concentration in the former group, and males had the higher mean in the latter group. Because of these inconsistencies, data from the sexes were combined (table 14). Bartlett's test detected significant heterogeneity of variance among the groups. Because the coefficient of variation was relatively constant, a logarithmic transformation was used for the analysis of variance that indicated significant differences among groups. A multiple-range test (Kramer, 1956) indicated which of these differences were significant (table 15).

Table 14. Blood lactic acid concentration (mg/100 ml) in submature channel catfish after removal from hoop nets and after various types of treatment (experiment 9).

Group[a]	Mean	SE	Range	Number of fish	CV
Appeared normal (25 C), first hour	47.4	3.85	29.8- 71.4	10	26
Appeared normal (25 C), 4 hours	65.4	5.30	41.1- 89.7	10	26
Appeared normal (16 C), 6-14 hours	19.4	2.86	9.0- 36.8	10	47
Exhibited fatigue (16 C), 60-65 hours	17.5	1.96	4.8- 31.5	12	39
Lost equilibrium (16 C), 60-65 hours	35.5	3.96	17.0- 55.9	11	37
Dead (16 C), 60-65 hours	78.3	11.85	28.2-132.6	9	45

Analysis of variance of logarithmically transformed data

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Groups	5	3.9286	0.7857	24.71*
Individuals	56	1.7790	0.0318	
Total	61	5.7076		

[a]Condition of fish, water temperature, and time after removal of fish from hoop nets.

*Indicates significance at the 0.95 confidence level.

The mean blood lactic acid concentrations 1 to 4 hours after removal of fish from the hoop nets (table 14) was near that of catfish exercised 10 to 15 minutes in the laboratory (tables 7 and 8). The mean concentrations were significantly lower after the fish had been in the hatchery tanks for some hours, but were higher than those of unexercised fish in the laboratory. No difference in blood lactic acid was noted between the catfish that appeared normal at 6-14 hours and those that showed fatigue at 60-65 hours. At 60-65 hours, however, many of the fish had lost equilibrium or died. The group that lost equilibrium had a mean blood lactic acid concentration of 35.5 mg/100 ml about twice that of the group that showed fatigue, but not significantly different from the group sampled within an hour after removal from the hoop nets. The dead catfish had the highest mean blood lactic acid concentration, 78.3 mg/100 ml, but this was not significantly different from the 65.4 mg/100 ml reported for the group sampled 4 hours after removal from the hoop nets. Catfish that died after transportation from the Des Moines River (table 13) had mean blood lactic acid concentrations over twice as great as those that died in the Mississippi River studies (table 14).

In the Mississippi River study, stiffening developed in the propulsive lateral muscles of some fish that lost equilibrium as well as in some that died. No significant difference was detected between mean blood lactic acid levels of live or dead fish that exhibited stiffening and those that did not (table 16).

All hoopnetted fish had abraded and inflamed maxillaries and mandibles, the mucous coat of the body was partially disrupted, and fins were frayed, supposedly from contact with the nets in attempts to escape and from repeated handling. The blood of fish sampled 60-65 hours after netting seemed darker

Table 15. Results of multiple-range test among mean blood lactic acid concentrations (mg/100 ml) of groups of submature channel catfish captured with hoop nets and handled (from table 14).

	Group[a]					
	Exhibited fatigue (16 C), 60-65 hours	Appeared normal (16 C), 6-14 hours	Lost equilibrium (16 C), 60-65 hours	Appeared normal (25 C), first hour	Appeared normal (25 C), first 4 hours	Dead (16 C), 60-65 hours
Mean[b]	17.5	19.4	35.5	47.4	65.4	78.3

[a]Condition of fish, water temperature and time after removal of fish from hoop nets.

[b]Any two means not underscored by the same line are significantly different, and any two means underscored by the same line are not significantly different.

and more viscous than normal. These fish were not readily responsive to vibrations (caused by striking the metal tank) and had lost the tendency to school. About 170 of the approximately 400 fish died in 2 days. Fish sampled 6-14 hours after removal from nets were readily responsive to vibrations and were actively schooling. Only 20 of the approximately 400 were dead after 1 day in the tank. At time of mortality about 7,500 largemouth bass fingerlings, *Micropterus salmoides,* were maintained in an adjacent tank provided with the same artesian water, and only 25 died.

The accumulation of blood lactic acid in these catfish probably occurred, for the most part, during handling, transportation and crowding after removal from the hoop nets. However, the abraded condition of the fish removed from nets indicated that the fish were active while in the nets.

Blood Lactic Acid Concentrations After Comparable Activity

In several studies, blood lactic acid concentration has been measured on fish after 15 minutes of forced exercise. Forced exercise for 15 minutes was sufficient to cause exhaustion in channel catfish. This was observed in other species (see Caillouet, 1964b, table 1). Exercising individual fish seems to cause a greater degree of muscular activity than does exercising a group of fish. Although exhaustion, as defined in this report, is reached in less than 15 minutes, the blood lactic acid concentrations were not as high in catfish exercised for 15 minutes in the laboratory as in the catfish captured, transported and handled in the field. Comparisons with data published on other species are most convenient at the unexercised or 15-minute exercise levels, however.

Blood lactic acid data for black bullheads were adapted from the studies of Black (1955), Dean and Goodnight (1964) and Caillouet (unpublished data) for comparison with those of the present investigation (table 17). The mean blood lactic acid concentration was lower, in most cases, in unexercised channel catfish than in unexercised black bullheads. The mean for fish exercised 15 minutes was higher in channel catfish than in the bullhead, except for bullheads exercised at 5 C. The mean blood-lactic acid values of salmonids, unexercised and after 15 minutes of forced exercise (Black, 1955, 1957a,b,c; Black et al., 1959, 1962, 1966), were usually greater than corresponding values obtained for channel catfish in the present investigation, but this was not without exception (Miller et al., 1959; Black et al., 1960). In a study by Black (1955), blood lactic acid was lower in the black bullhead than in rainbow trout, both unexercised and after 15 minutes of forced exercise.

Black (1955) related the lactic acid response in several fishes, including the black bullhead, to the magnitude of the Bohr effect, the decrease in oxygen-combining capacity of the blood with increase in carbon dioxide tension. He concluded that the black bullhead is well adapted to withstand appreciable in-

Table 16. Blood lactic acid concentration (mg/100 ml) and stiffening of propulsive lateral muscle in submature channel catfish that lost equilibrium or died 60-65 hours after removal from hoop nets and handling (16 C) (experiment 9).

Group	Mean	SE	Range	Number of fish	CV
Lost equilibrium					
No muscle stiffening	30.9	5.22	17.0-55.9	7	45
Muscle stiffening	43.5	3.83	33.7-49.6	4	18
Dead					
No muscle stiffening	74.3	13.24	28.2-132.6	7	47
Muscle stiffening	92.3	33.50	58.8-125.8	2	51

Table 17. Blood lactic acid concentration (mg/100 ml) in catfishes unexercised and subjected to 15 minutes of forced exercise.

Genus and species	Water temperature, C	Unexercised			Exercised			Author and date
		Mean	SE	Number of fish	Mean	SE	Number of fish	
Ictalurus melas	11.5	9.0	2.27	10	33.1	3.68	11	Black, 1955
	11.5	6.4	—	2	37.4	11.70	8	
	5	45.9	0.60	20	100.6	0.97	20	Dean and Goodnight, 1964
	20	10.1	0.37	15	48.0	0.97	15	
	21	5.5	1.10	6	42.3	2.69	6	Caillouet,[a] to be published
Ictalurus punctatus	14-15	2.8	1.65	7	59.1	4.29	5	This study[b]

[a]Fish were exercised 17 minutes.

[b]Data from experiment 2, table 7; this was the only experiment including both unexercised fish and those subjected to 15 minutes of forced exercise.

creases in blood acid. Haws and Goodnight (1962) found the Bohr effect greater in channel catfish than in brown bullhead, *Ictalurus nebulosus.*

Temperature and Blood Lactic Acid Concentration

Mean blood lactic acid concentration of submature channel catfish in the unexercised condition increased with acclimation temperature (table 18). In all cases, the mean blood lactic acid concentration of unexercised catfish was less than 10 mg/100 ml. In exercised fish, there also was evidence of greater blood lactic acid concentrations at higher temperatures (tables 7, 8 and 9).

Black (1957a) made the first direct test of the effect of temperature on blood lactic acid in fishes. He compared the mean blood lactic acid levels of two groups of rainbow trout exercised for 15 minutes at different acclimation temperatures, 11.5 C and 20.0 C. No significant difference was detected. Denyes and Joseph (1956) determined blood lactic acid concentrations of largemouth bass acclimated to several temperatures between 5 C and 35 C. The highest individual lactic acid values and greatest variability in lactic acid among fish were associated with lower temperatures. Caillouet (1964a) noted higher individual lactic acid values and greater variation in lactic acid among individual carp at 24 C than at 1 C.

The study by Dean and Goodnight (1964) of four species produced the following results: In the black bullhead and white crappie, *Pomoxis annularis,* the mean blood lactic acid level was higher at 5 C than at 20 C, both in unexercised and exercised fish. In the bluegill, *Lepomis macrochirus,* mean blood lactic acid concentration was greater at 5 C than at 20 C in unexercised fish and was less at 5 C than at 20 C in exercised fish. In largemouth bass, the mean blood lactic acid level was lower at 5 C than at 20 C in unexercised fish and was higher at 5 C than at 20 C in exercised fish. Wendt (1964) reported higher mean blood lactic acid values in immature brook trout, *Salvelinus fontinalis,* exercised 15 minutes at 15 C than in mature brook trout exercised 15 minutes at 5 C. Mean blood lactic acid concentration of Atlantic salmon, *Salmo salar,* exercised to exhaustion was higher at 15 C than at 0.2 C (Wendt, 1965).

In the present study, mean blood lactic acid concentration was usually greater at higher temperatures, both in unexercised and exercised channel catfish. Exhaustion occurred more rapidly at higher temperatures.

A major difficulty in interpretation of the effect of temperature on blood lactic acid in fishes is that the different temperatures have been investigated simultaneously in only two studies, Black (1957a) and Denyes and Joseph (1956). Activity in fishes (Fry, 1947; Fry and Hart, 1948), rates of enzymic reactions (Black et al., 1961) and rate of lactic acid diffusion (Johnson et al., 1945) may be expected to increase as temperature increases within the nonlethal range. Presumably, greater production of lactic acid would occur at higher temperatures, both in unexercised and exercised fish. However, a shift from one metabolic pathway to another with change in temperature (Hochachka and Hayes, 1962) may also be involved. Black et al. (1961) indicated a need for further study of the effect of temperature on lactic acid production and metabolism in fishes.

Table 18. Blood lactic acid concentration (mg/100 ml) in unexercised submature channel catfish at different temperatures.[a]

Experiment number	Water temperature, C	Mean	SE	Range	Number of fish	CV
2	15	2.8	1.65	0.4-12.7	7	156
3	18	2.3	0.32	1.2- 4.1	9	42
1	18	3.3	1.22	1.4- 6.8	4	74
5	19	3.9	0.63	0.5- 6.7	10	51
6	20	3.7	0.50	3.2- 4.2	2	19
-	20	7.6	0.85	6.8- 8.5	2	16
-	22	6.8	1.34	4.4-10.4	4	40

[a]Data for the last two experiments were from studies that will be reported elsewhere.

Heterogeneity of Variance in Blood Lactic Acid Data from Fishes

Variation in blood lactic acid concentration among individual channel catfish tended to increase in exercised fish. This change in variation was sufficient, in some cases, to cause heterogeneous variance. Two factors, other than characteristic variation among individual fish, may have influenced these results; *viz.,* a slight decrease in precision of the Barker-Summerson method with increase in the measured quantity of lactic acid (tables 4 and 5) and the multiplicative effect of variation in dilution of blood samples. In the former case, while absolute precision decreased slightly, relative precision increased with increase in the measured quantity of lactic acid. In the latter case, relative precision in measurement of smaller quantities of blood was less than that for larger quantities. Smaller quantities of blood were diluted to a greater extent. Thus, high lactic acid concentrations would be distorted to a greater extent than low concentrations by variation in dilution.

Caillouet (1964a) noted that variation in blood lactic acid concentration among individual carp increased during forced exercise. This heterogeneity of variance occurred whether blood volume was constant or not. Greater variation among fish was evident with variation in dilution of blood samples, but this was also associated with higher acclimation temperature for the carp. Since the carp were exer-

cised simultaneously rather than individually, further changes may have occurred during the blood sampling period. Similar changes may have contributed to the heterogeneity of variance observed in channel catfish exercised in a group (table 12). Variation in blood lactic acid concentration among individual fish has increased during exercise of the fish in most studies (see Caillouet, 1964b). It is assumed, therefore, that changing or heterogeneous variation discussed by Caillouet (1964a) and in this report represents, for the most part, characteristic variation among individual fish.

Secondat and Diaz (1942) recognized that variation in blood lactic acid concentration among individual fish in the unexercised condition was slight and that greater variation was exhibited after forced exercise. Since that time, nearly every study of blood lactic acid in fishes has provided data demonstrating that variation among individual fish in the unexercised condition was low, that it increased after hyperactivity and that it decreased during recovery.

Black (1956, p. 22) reported that blood lactic acid "subsided erratically to approach the unexercised level . . . ." Black (1957a, p. 127) observed "considerable variation in the data." Parker and Black (1959, p. 98) noted "large variation . . . ." "An apparent . . . decrease in range of lactate values as fish were held for longer periods" was recognized by Parker et al. (1959, p. 436). Barrett and Connor (1962, p. 239) observed that "the great variation in the blood lactate levels of individual [fish] . . . at any one time was especially noteworthy."

As Nakatani (1957) succinctly stated, the concentration of lactic acid in the blood at any given time is determined principally by (1) the rate at which lactic acid is produced at the site of production, (2) the rate of addition of lactic acid to the blood and (3) the rate of removal of lactic acid from the blood by various tissues.

Black (1957c) suggested that the amount of blood lactic acid produced during exercise was related to the level of muscle glycogen and also implied that variation in blood lactic acid concentration among individual fish was related to variation in muscle glycogen concentration among individual fish. Parker and Black (1959, p. 103) stated that the "large variation in degree of lactic-acid response is thought to reflect individual variation in respect to muscle glycogen level due to either previous environmental experience or genotypic differences, as well as a variable degree of exertion." Miller et al. (1959) suggested that higher blood lactic acid concentrations resulted from higher muscle glycogen reserves.

Black et al. (1960) attributed variation in blood lactic acid concentration among individual fish to failure of certain fish to respond fully to forced exercise. They showed that high blood lactic acid concentrations were associated with low muscle glycogen concentrations and vice versa. A graph of their results (p. 493) showed that the relationship between blood lactic acid and muscle glycogen was curvilinear; i. e., blood lactic acid increased at an increasing rate as muscle glycogen decreased during exercise. A similar relationship between average values of blood lactic acid and of muscle glycogen from rainbow trout in the unexercised condition and during 15 minutes of forced exercise is evident in the data of Miller et al. (1959) and Black et al. (1962). Fish in the unexercised condition exhibited limited variation in blood lactic acid and great variation in muscle glycogen. During forced exercise, variation in blood lactic acid concentration among individual fish increased with a decrease in variation in muscle glycogen. It is suggested that variation in muscle glycogen was converted to variation in blood lactic acid during exercise. This hypothetical interconversion of variation in muscle glycogen to variation in blood lactic acid is shown in fig. 2.

Similar results are evident in the data of Miller et al. (1959, p. 324) and Black et al. (1962, pp. 414 and 423). Thus, high variation in muscle glycogen among individuals may be expected to occur concomitantly with low variation in blood lactic acid when fish are in the unexercised condition. Conversely, when fish are exercised, high variation in blood lactic acid may be expected to occur concomitantly with low variation in muscle glycogen. Stevens and Black (1966) reported a linear relationship between lactic acid concentration and glycogen concentration in the muscle of rainbow trout subjected to intermittent exercise. Thus, curvilinearity in the relationship between blood lactic acid concentration and muscle glycogen concentration probably represents a lag in diffusion of lactic acid from muscle to blood or in circulation of lactic acid to the blood sampling site (usually the heart) or both.

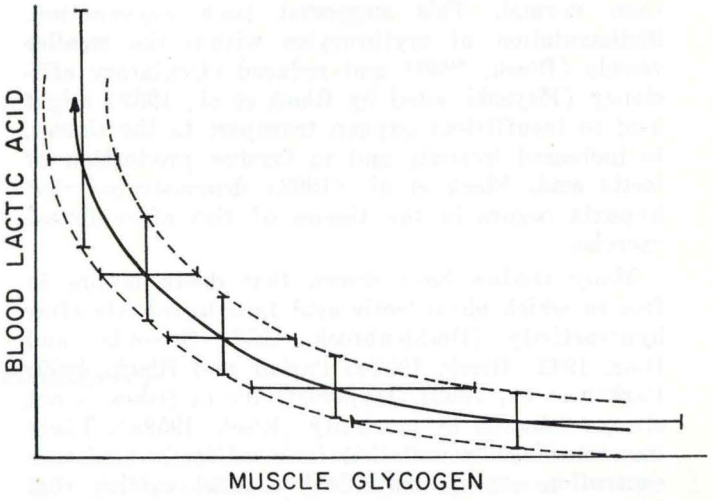

Fig. 2. Hypothetical relationship between blood lactic acid and muscle glycogen during muscular exercise in fish (vertical and horizontal lines represent the range of variation, enclosed by dotted lines, at points representing average values).

This hypothesis requires more direct investigation. The data needed are measurements of muscle glycogen, muscle lactic acid and blood lactic acid in individual fish.

Hochachka (1961) showed that rainbow trout trained by previous exercise were able to acquire an oxygen debt three times as great as that of untrained fish. The trained fish were able to use more muscle glycogen in the production of lactic acid. Thus, variation in fitness among individual fish may contribute to variation in blood lactic acid.

Heterogeneity of variance in blood lactic acid concentration has more than academic importance. Failure to consider such heterogeneity in statistical analyses might lead to erroneous conclusions. Elucidation of this variation may facilitate interpretation of future studies of blood lactic acid in fishes.

Relation of Lactic Acid to Mortality

High blood lactic acid concentration may lead to conditions favorable for further production and continued elevation of lactic acid; i. e., hypoxia in the muscles. Reduction in oxygen capacity of fish blood with increase in lactic acid concentration was demonstrated by Root (1931), Buddenbrock (1938), Black and Irving (1938) and Root and Irving (1943), but Denyes and Joseph (1956) concluded that oxygen capacity improved with increased blood lactic acid concentration. Buddenbrock (1938) suggested that, upon accumulation of lactic acid in the blood and consequent deformation of the erythrocytes and reduction of oxygen capacity, insufficient oxygen would be available to the tissues. He suggested further that this hypoxia might cause additional production of lactic acid that would enhance the already detrimental condition of the blood. It was observed that blood of channel catfish that were fatigued, that lost equilibrium or that died usually was darker than normal. This suggested poor oxygenation. Sedimentation of erythrocytes within the smaller vessels (Black, 1956) and reduced circulatory efficiency (Hayashi, cited by Black et al., 1962) might lead to insufficient oxygen transport to the tissues, to increased hypoxia and to further production of lactic acid. Black et al. (1962) demonstrated that hypoxia occurs in the tissues of fish after forced exercise.

Many studies have shown that death occurs in fish in which blood lactic acid fails to subside after hyperactivity (Buddenbrock, 1938; Secondat and Diaz, 1942; Black, 1957c; Parker and Black, 1959; Parker et al., 1959). Hyperactivity in fishes is not always followed by mortality (Black, 1958a). There was considerable variation in blood lactic acid concentration among individual channel catfish that lost equilibrium or died (tables 13 and 14). The mean blood lactic acid values of catfish that lost equilibrium or died differed greatly in fish collected from the Des Moines River and Mississippi River. In the Mississippi River study, channel catfish that appeared normal a few hours after removal from nets exhibited mean blood lactic acid concentrations similar to those of fish that lost equilibrium or died 60-65 hours after removal from nets (table 15). Thus, loss of equilibrium and death were not associated with a particular level of blood lactic acid. This suggested that there is considerable variation among individual fish in susceptibility to increased blood lactic acid, that prolonged elevation of lactic acid rather than high lactic acid per se is detrimental or that factors other than high blood lactic acid concentraton contributed to the observed loss of equilibrium and death.

No difference in mean blood lactic acid concentration could be demonstrated between dead catfish in a state of rigor mortis and live catfish with stiffening of propulsive lateral muscles (table 16). Physiological contracture, a form of continued muscular contraction that can be brought about by extreme muscular fatigue, seems biochemically similar to rigor mortis; i. e., it occurs as a result of exhaustion of energy compounds necessary for muscle relaxation (Guyton, 1961). Amlacher (1961), in a review of rigor mortis in fishes, emphasized that the cause of rigor is the disappearance of adenosine triphosphate and that rigor mortis begins in less time after death and is of shorter duration when fish undergo strenuous muscular activity before death. The possibility that depletion of adenosine triphosphate during hyperactivity in fishes might lead to physiological contracture seems worthy of further study. Such stiffening may contribute to death (e. g., by occlusion of peripheral circulation). Peripheral circulatory failure can lead to lactic acidosis, which has been shown to cause death in humans (Huckabee, 1961a,b). Indeed, the physiological contracture may become rigor mortis.

Jonas et al. (1962) indicated that death observed in rainbow trout into which either hydrochloric acid or lactic acid had been injected intravenously was caused by the resulting reduction in blood pH. Mortality was similarly related to lowered blood pH in sockeye salmon injected with lactic acid.

In humans, Huckabee (1961a,b) attributed mortality in certain hospital patients to lactic acidosis, which he defined as an accumulation of hydrogen ions and a more acid reaction of the body fluids caused almost entirely by lactic acid. The lactic acid production seemed brought about by widespread tissue hypoxia, though the causes of such hypoxia remained obscure. Only by gradual peripheral circulatory failure in laboratory animals could Huckabee reproduce the chemical syndrome. Danowski (1963) briefly reviewed the lactic acidosis syndromes in humans.

Recent work by Black et al. (1962) and Hayashi (cited by Black et al., 1962) suggested that hypoxia may occur after strenuous exercise in rainbow trout.

This lends support to the hypothesis that death in fishes after hyperactivity may be caused by lactic acidosis. Loss of equilibrium in fish after strenuous activity may represent depression of the central nervous system by accumulated lactic acid. Such depression is the major effect of acidosis that, if severe enough, may lead to disorientation and eventually to coma in humans (Guyton, 1961).

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. Kenneth D. Carlander of the Department of Zoology and Entomology, Iowa State University, who suggested the research problem and provided supervision, assistance and encouragement. Harry M. Harrison, superintendent of biology, Iowa Conservation Commission, and several other Commission personnel gave assistance in collection of specimens and through suggestions for the study. Dr. Edgar C. Black, professor of physiology, University of British Columbia, Vancouver, Canada, provided encouragement and many helpful suggestions through correspondence.

LITERATURE CITED

Amlacher, E. 1961. Rigor mortis in fish. *In*: G. Borgstrom, ed. Fish as food. Vol. 1. pp. 385-409. Academic Press, Inc. N.Y.

Barrett, I., and A. R. Connor. 1962. Blood lactate in yellowfin tuna, *Neothunnus macropterus,* and skipjack, *Katsuwonus pelamis,* following capture and tagging. Inter-Am. Tropical Tuna Comm. Bul. 6:233-280.

Black, E. C. 1955. Blood levels of hemoglobin and lactic acid in some freshwater fishes following exercise. J. Fish. Res. Bd. Canada 12:917-929.

————. 1956. Appearance of lactic acid in the blood of Kamloops and lake trout following live transportation. Canadian Fish Culturist 18:20-27.

————. 1957a. Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. I. Kamloops trout, *Salmo gairdneri.* J. Fish. Res. Bd. Canada 14:117-134.

————. 1957b. Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. II. Lake trout, *Salvelinus namaycush.* J. Fish. Res. Bd. Canada 14:645-649.

————. 1957c. Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. III. Sockeye salmon, *Oncorhynchus nerka.* J. Fish. Res. Bd. Canada 14:807-814.

————. 1958a. Hyperactivity as a lethal factor in fish. J. Fish. Res. Bd. Canada 15:573-586.

————. 1958b. Energy stores and metabolism in relation to muscular activity in fishes. *In*: P. A. Larkin, ed. The investigation of fish-power problems. pp. 51-67. Univ. of British Columbia. Vancouver, B. C.

————, W. G. Chiu, F. D. Forbes and A. Hanslip. 1959. Changes in pH, carbonate and lactate of the blood of yearling Kamloops trout, *Salmo gairdneri,* during and following severe muscular activity. J. Fish. Res. Bd. Canada 16:391-402.

————, A. R. Connor, K. C. Lam and W. G. Chiu. 1962. Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. J. Fish. Res. Bd. Canada 19:409-436.

————, and L. Irving. 1938. The effect of hemolysis upon the affinity of fish blood for oxygen. J. of Cell. Comp. Physiol. 12:255-262.

————, G. T. Manning and K. Hayashi. 1966. Changes in levels of hemoglobin, oxygen, carbon dioxide, pyruvate, and lactate in venous blood of rainbow trout (*Salmo gairdneri*) during and following severe muscular activity. J. Fish. Res. Bd. Canada 23:783-795.

————, A. C. Robertson, A. R. Hanslip and W. G. Chiu. 1960. Alterations in glycogen, glucose and lactate in rainbow and Kamloops trout, *Salmo gairdneri,* following muscular activity. J. Fish. Res. Bd. Canada 17:487-500.

————, A. C. Robertson and R. R. Parker. 1961. Some aspects of carbohydrate metabolism in fish. *In*: A. W. Martin, ed. Comparative physiology of carbohydrate metabolism in heterothermic animals. pp. 89-124. Univ. of Washington Press. Seattle, Wash.

Blancheteau, M., P. Lamarque, G. Mousset and R. Vibert. 1961. étude neurophysiologique de la pêche électrique. Biarritz Centre d'études et de Recherches Scientifiques Bul. 3(3):3-108.

Buddenbrock, W. von. 1938. What physiological problems are of interest to the marine biologist in his studies of the most important species of fish? Part 2. Beobachtungen über das Sterben gefangener Seefische und über den Milchsäuregehalt des Fischblutes. Conseil Permanent Intern. pour L'exploration de la Mer Rapports et Procès-Verbaux des Réunions 101(4):1-7.

Caillouet, C. W., Jr. 1964a. Blood lactic acid concentration of unexercised and exercised mature carp in winter and summer. Iowa State J. Sci. 38:309-322.

————. 1964b. Effect of forced activity on blood lactic acid in channel catfish, *Ictalurus punctatus* (Rafinesque). Ph. D. dissertation. Library, Iowa State Univ., Ames, Iowa. (Microfilm copy No. 64-9257 Univ. Microfilms, Ann Arbor, Mich.).

Danowski, T. S. 1963. The lactic acidosis syndromes. Diabetes 12: 272-279.

Dean, J. M., and C. J. Goodnight. 1964. A comparative study of carbohydrate metabolism in fish as affected by temperature and exercise. Physiol. Zool. 37:280-299.

Denyes, H. A., and J. M. Joseph. 1956. Relationships between temperature and blood oxygen in the largemouth bass. J. Wildl. Mgmt. 20:56-64.

Drury, D. R., and A. N. Wick. 1956. Metabolism of lactic acid in the intact rabbit. Am. J. Physiol. 184:304-308.

Friedemann, T. E., and G. E. Haugen. 1942. Pyruvic acid. I. Collection of blood for the determination of pyruvic and lactic acids. J. Biol. Chem. 144:67-77.

———————— and ————. 1943. Pyruvic acid. II. The determination of keto acids in blood and urine. J. Biol. Chem. 147:415-442.

Fry, F. E. J. 1947. Effects of the environment on animal activity. Ontario Fish Res. Lab. Publ. 68:1-62.

———————— and J. S. Hart. 1948. Cruising speed of goldfish in relation to water temperature. J. Fish. Res. Bd. Canada 7:169-175.

Guyton, A. C. 1961. Textbook of medical physiology. 2nd ed. W. B. Saunders Co., Philadelphia, Pa.

Harrison, H. M. 1960. Progress report-Chemical renovation of the Middle Raccoon River in central Iowa. Iowa State Conserv. Comm. Fish and Game Div. Quart. Biol. Rept. 12(3):47-50.

Hawk, P. B., B. L. Oser and W. H. Summerson. 1954. Practical physiological chemistry. 13th ed. McGraw-Hill Book Co., Inc. N.Y.

Haws, T. G., and C. J. Goodnight. 1962. Some aspects of the hematology of two species of catfish in relation to their habitats. Physiol. Zoöl. 35:8-17.

Hayashi, K., N. W. Green and E. C. Black. 1964. Carbohydrate metabolism during transportation of live rainbow trout, *Salmo gairdneri*. Prefectural Univ. of Mie Faculty of Fish. Rept. 5(1):51-125.

Heath, A. G., and A. W. Pritchard. 1962. Changes in the metabolic rate and blood lactic acid of bluegill sunfish, *Lepomis macrochirus*, Raf. following severe muscular activity. Physiol. Zoöl. 35:323-329.

Hochachka, P. W. 1961. The effect of physical training on oxygen debt and glycogen reserves in trout. Canadian J. Zool. 39:767-776.

————————, and F. R. Hayes. 1962. The effect of temperature acclimation on pathways of glucose metabolism in the trout. Canadian J. Zool. 40:261-270.

Huckabee, W. E. 1961a. Abnormal resting blood lactate. I. The significance of hyperlactatemia in hospitalized patients. Am. J. Med. 30:833-839.

————————. 1961b. Abnormal resting blood lactate. II. Lactic acidosis. Am. J. Med. 30:840-848.

Johnson, R. E., H. T. Edwards, D. B. Dill and J. W. Wilson. 1945. Blood as a physicochemical system. XIII. The distribution of lactate. J. Biol. Chem. 157:461-473.

Johnson, D. E., R. E. Nakatani and S. P. Felton. 1956. The effects of electroshock (a.c.) upon tissue content of inorganic phosphate and lactic acid in yearling silver salmon. Univ. of Wash. School Fish. Tech. Rept. 22 pp. 1-7.

Jonas, R. E. E., H. S. Sehdev and N. Tomlinson. 1962. Blood pH and mortality in rainbow trout (*Salmo gairdnerii*) and sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 19: 619-624.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12:307-310.

Leivestad, H., H. Andersen and P. F. Scholander. 1957. Physiological response to air exposure in codfish. Sci. 126:505.

Miller, R. B., A. C. Sinclair and P. W. Hochachka. 1959. Diet, glycogen reserves and resistance to fatigue in hatchery rainbow trout. J. Fish. Res. Bd. Canada 16:321-328.

Nakatani, R. E. 1957. Changes in the inorganic phosphate and lactate levels in blood plasma and muscle tissue of adult steelhead trout after strenuous swimming. Univ. of Washington School of Fish. Tech. Rept. 30 pp. 1-14.

Needham, D. M. 1960. Biochemistry of muscular action. *In*: G. H. Bourne, ed. The structure and function of muscle. Vol. II. pp. 55-104. Academic Press, Inc. N.Y.

Parker, R. R., and E. C. Black. 1959. Muscular fatigue and mortality in troll-caught chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Bd. Canada 16:95-106.

Parker, R. R., E. C. Black and P. A. Larkin. 1959. Fatigue and mortality in troll-caught Pacific salmon (*Oncorhynchus*). J. Fish. Res. Bd. 16:429-448.

Perkins, F. E. 1957. A field technique for obtaining samples of fish blood serum. Progr. Fish-Culturist 19:144.

Prosser, C. L., and F. A. Brown, Jr. 1961. Comparative animal physiology. 2nd ed. W. B. Saunders Co., Philadelphia, Pa.

Root, R. W. 1931. The respiratory function of the blood of marine fishes. Biol. Bul. 61:427-456.

———————— and L. Irving. 1943. The effect of carbon dioxide and lactic acid on the oxygen-combining power of whole and hemolyzed blood of the marine fish, *Tautoga onitis*. Biol. Bul. 84:207-212.

Scholander, P. F., E. Bradstreet and W. F. Garey. 1962. Lactic acid response in the grunion. Comp. Biochem. and Physiol. 6:201-203.

Secondat, M., and D. Diaz. 1942. Recherches sur la lactacidémie chez le poisson d'eau douce. Académie des Sciences Comptes Rendus Hebdomadaires des Séances 215:71-73.

Snedecor, G. W. 1956. Statistical methods. 5th ed. Iowa State Univ. Press. Ames, Iowa.

Stevens, E. D., and E. C. Black. 1966. The effect of intermittent exercise on carbohydrate metabolism in rainbow trout, *Salmo gairdneri*. J. Fish. Res. Bd. Canada 23(4):471-483.

Wendt, C. 1964. Influence of low temperature on the blood lactate level in *Salvelinus fontinalis* after exercise. Drottningholm Institute of Freshwater Res. Rept. 45:206-210.

————————. 1965. Liver and muscle glycogen and blood lactate in hatchery-reared *Salmo salar* L. following exercise in winter and summer. Drottningholm Institute of Freshwater Res. Rept. 46: 167-184.