AN ABSTRACT OF THE THESIS OF

ALAN GARD HEATH (Name) for the Ph. D. (Degree) in Physiology (Major)

Date thesis is presented August 5, 1963

Title PHYSIOLOGICAL ALTERATIONS FOLLOWING SEVERE HYPOXIC STRESS IN TWO SPECIES OF FRESH-WATER FISH.

Abstract approved (Major Professor)

Bluegill sunfish, Lepomia macrochirus, and cutthroat trout, Salmo clarki, were exposed to a rapidly declining oxygen tension for a period of time sufficient to just cause loss of equilibrium in the fish. During recovery from the stress, measurements of oxygen uptake, blood lactic acid, blood glucose, liver glycogen, muscle glycogen, and hemoglobin or red blood cell counts were made at fixed intervals of time.

Hypoxic stress caused an immediate rise in blood lactate and oxygen uptake in both species with the trout showing the greatest increases. This suggests a greater degree of oxygen debt in the latter, even though the stress routine used on this species was not as prolonged. A delayed peak in the blood lactate observed in the trout but not in the bluegill indicated a possible impairment of circulatory function in the former. The blood lactate returned to

normal after two and five hours in the bluegill and cutthroat respectively. The return of the oxygen uptake to control levels required eight and ten hours respectively.

An immediate mobilization of the carbohydrate stores occurred during the stress in both the bluegill and trout. The blood glucose concentration rose greatly in the bluegill but not in the trout. Mobilization of liver glycogen in the trout without a concomitant rise in glucose suggested utilization of the latter as rapidly as it was formed. During recovery from the hypoxic stress, resynthesis of glycogen stores occurred much more quickly in the bluegills than trout.

Significant changes in hemoglobin or red blood cell numbers did not occur in either species indicating no change in blood water content, or effect on hemopoetic tissues resulting from the stress.

It was concluded that the differences observed between these two species point to possible mechanisms in the bluegill which help it to tolerate acute hypoxic conditions better than the trout.

PHYSIOLOGICAL ALTERATIONS FOLLOWING SEVERE HYPOXIC STRESS IN TWO SPECIES OF FRESH-WATER FISH

by

ALAN GARD HEATH

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

August 1963

APPROVED:

Redacted for Privacy

Associate Professor of Zoology

In Charge of Major

Redacted for Privacy

Chairman of Department of Zoology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 5, 1963

Typed by Nancy Kerley

ACKNOWLEDGMENT

I wish to thank Dr. Austin Pritchard for his very willing advice and constructive criticisms given during the execution of this investigation and preparation of the thesis. In addition, the training and inspiration given me by the faculty of the Department of Zoology during my sojourn here is gratefully acknowledged.

Appreciation is particularly due my wife, Gloria Heath, who assisted during most of the experiments, and existed uncomplainingly at the usual standard of living for graduate students.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PHYSIOLOGICAL ALTERATIONS FOLLOWING SEVERE HYPOXIC STRESS IN TWO SPECIES OF FRESH-WATER FISH

INTRODUCTION

The aquatic habitat often imposes, on the organisms therein, a temporary situation of decreased oxygen concentration. Lakes, for example, will sometimes exhibit a striking diurnal change in oxygen concentration (70; 17, p. 414). During the day the algae and other aquatic plants produce oxygen and consume the carbon dioxide given off by the other organisms, but at night the reverse takes place. Saturation values of less than 15 percent have been recorded at night (70) which would impose considerable anoxic stress on the fish and other organisms in such a pond, particularly at summer temperatures. Other situations in lakes and rivers producing both short and long term diminished oxygen content have been discussed by Ruttner (60, p. 67-74, 197). These include decomposition of organic matter and utilization of oxygen by fish and zooplankton. Thus, many factors can produce conditions of low oxygen content and fresh-water fish are frequently exposed to these situations.

To what extent alterations in oxygen content impose a stress on the animal and what physiological changes occur as a result of this stress are questions that naturally follow. Studies of the effects of prolonged exposure to mild hypoxia yield information on the adaptive abilities and mechanisms of fish, whereas investigations involving

brief but severe hypoxic stress may help to clarify the general mechanisms of stress reactions in fish. The latter approach is of primary interest here.

Many workers have been interested in the minimum level of oxygen that fish can tolerate. Probably the easiest method to use in determining this concentration is to seal the fish in a container and allow it to remove oxygen from the water until it dies, at which time a sample of the water is taken for oxygen analysis. The concentration of oxygen at death is referred to as the residual level and is often considered to be the lethal concentration. This method has been used with minor modifications by many workers (55; 13; 22; 12, p. 84-97). Other investigators (e. g. 24; 63; 18) have kept water flowing through the containers holding the fish and altered the oxygen content by equilibration with nitrogen. Field observations by Jahoda (42), Pearse and Actenberg (56), Moore (53), and Odum and Caldwell (54) have given further information on oxygen concentrations sustained by various species.

Correlations between habitat and minimum oxygen requirements have been frequently observed. Forms from slow moving warm water, such as Perca flavesces, carp, catfish, and smallmouth bass show noticably less sensitivity (i. e. lower residual oxygen tension) than do members of the salmonid family and other groups that inhabit fast-moving cold water. (See 12, p. 84-97 for comparison

of small-mouth bass with trout.) Marine species have been investigated by Hall (32) who found a good correlation between residual oxygen tensions and the activity of the fish. Sluggish fish (e. g. puffer) had decidedly lower asphyxial oxygen tensions than active pelagic forms such as the mackerel.

Another approach to the problem of evaluating the effect of lowered oxygen concentrations has been to determine the metabolic rate at various oxygen tensions. The phenomenon of "respiratory dependence," as pointed out by Fry (23, p. 37), is a situation where the oxygen uptake of the fish decreases with decreasing oxygen concentration. Complete respiratory dependence of the standard metabolic rate over the entire oxygen saturation range is rare. A more common picture is one in which metabolic rate is fairly stable over a certain range of oxygen tensions down to a point (referred to usually as the "critical oxygen tension") below which the metabolic rate becomes dependent on oxygen concentration. This relationship has been observed in both marine and fresh-water fish (31; 11; 58). Occasionally an increase in the metabolic rate has been observed at the critical tension due possibly to augmented muscular movements of the respiratory apparatus producing a demand for more oxygen (e. g. 11).

Respiratory dependence implies that at concentrations of oxygen below the critical oxygen tension, the fish must not be

obtaining sufficient oxygen to meet its minimum metabolic demands. This is, of course, assuming that the standard metabolic rate approaches the true basal rate, an assumption that is probably not justified (23, p. 30-31). In this regard, Fry (21, p. 43) has discussed the fact that respiratory dependence occurs over a much wider range of oxygen pressures if the active metabolic rate is used rather than the standard rate. Consequently, if the standard rate were higher than the basal rate it would tend to make the critical oxygen tension higher. This is probably the case in some early studies where the investigators measured the metabolic rate of their fish in the first few hours after putting them in the respiratory chambers. Fry (23, p. 43) has pointed out that handling can induce near maximum oxygen uptake which might not return to normal for several hours.

Fry and his students (27; 63; 26) have concentrated on measuring the active metabolic rate of various species of fish at different oxygen concentrations. With this type of data, concentrations of oxygen that cause some limitation of the activity of the fish can be established. The concentration of oxygen that brings about a reduction of the active metabolic rate to that of the standard rate Fry has called the "level of no excess activity" (21, p. 45). According to Fry (23, p. 42) the level of no excess activity can be taken as a conservative estimate of the asphyxial level. Theoretically, at this

concentration of oxygen, the fish could exist but could not sustain any activity without some degree of anaerobiosis.

Fry (23, p. 36) states that "there is no evidence for extensive anaerobiosis in fish." While this may be true for most fish, Blazka (11) has recently demonstrated a very dramatic ability of at least one species, crucian carp, to withstand complete anoxia for as long as five and one-half months. Moreover, in the same work it was observed that trout could undergo a short period of hypoxia and then repay a supposed "oxygen debt" incurred during the stress by respiring at a substantially higher than normal rate immediately following.

When animal tissues are subjected to reduced oxygen tensions, they do not necessarily reduce their energy needs. In order to meet these needs, the tissue will metabolize anaerobically causing an accumulation of intermediates, the most important ones being lactic and pyruvic acid (69, p. 396-400).

The fact that lactate appears as a result of muscular exercise has been known since the turn of the century (9, p. 100). In 1920 Meyerhof (as cited by Black, loc. cit.) showed that the source of lactic acid was muscle glycogen. Margaria, et al. (47, p. 689-745) investigated the "oxygen debt" mechanisms in mammals pointing out that the prolonged high respiratory rate following a period of exercise is for the purpose of oxidizing a portion of the accumulated

lactate and converting another part of it to liver glycogen. Thus lactate has come to be thought of as the "security for the oxygen debt." Further work on the physiology of exercise has yielded many papers in the last 20 or so years. These have been summarized by Robinson (59, p. 494-525).

Recently there have been attempts to reexamine the whole question of an oxygen debt mechanism. Alpert and Root (1, p. 461) found no consistent relationship between the amount of lactate utilized and the excess oxygen consumption, which casts doubt on the idea of lactate serving as the "security" for the oxygen debt. Drury and Wick (19) have provided evidence of a much greater oxidation of lactic acid in extra-hepatic tissues (rather than conversion to glycogen) than was previously believed to occur. The excellent work of Alpert et al. (2) has further demonstrated that lactate is not, as such, the reason for the increased oxygen consumption following exercise. Their studies were concerned with the effects of short term hypoxia on the time course of lactate and oxygen uptake changes in dogs. In these experiments, hypoxia produced increased lactate levels but decreased oxygen uptake during the recovery period.

The alterations in energy stores of fish, as a result of hypoxia or exercise stress, has received the attention of many workers. As more precise and convenient techniques for the estimation of glycogen and blood glucose have been developed, these have

been applied to the whole problem of metabolism in fish. Black (9, p. 89-124) has reviewed much of this work with emphasis on the studies involving exercise.

Leivestad et al. (46), Scholander (62), and Barrett and Conner (4) determined the changes in lactic acid of fish that had been exposed to air. As would be expected, air exposure caused increases in muscle lactic acid, but the blood lactate did not rise until the fish were placed back in the water. This was apparently due to circulatory system responses similar to those in diving mammals, a point that will be discussed further in a later section.

Blazka (11) placed crucian carp and trout in deoxygenated tap water for a period of time and then sampled them for muscle lactate and fatty acids. He found no change in muscle lactate as a result of exposure to anoxic water conditions but did observe increases in short chain fatty acids.

Several early investigators (48, p. 6-7; 64; 67; 44, p. 460-491) attempted to evaluate the various factors affecting the blood sugar level of fish. This work has been reviewed by Kiermier (44, p. 460-491), who points out that temperature, nutrition, narcosis, drugs, and muscular exercise can all cause significant alterations in fish blood sugar concentrations. Asphyxia, produced by exposing the fish to air for one to two minutes, usually caused a large increase in blood sugar within 30 minutes from the beginning of the stress

and this hyperglycemia persisted for one to several days in the fish returned to well aerated water. Their data are very variable and the uniformity of the degree of stress is poor. It is not known how much of the effect is caused by asphyxiation and how much by the muscular exertions of the fish as it flops on the floor.

Middlesworth, Kline, and Britton (50) investigated the regulation of blood sugar concentration in rats exposed to anoxic conditions. Generally, anoxia produces hyperglycemia in rats but many factors affect the degree of this response, the most important of which is the absorptive condition of the animal. Starvation for 12 hours followed by anoxic stress produces a hypoglycemic condition, even though the glycogen stores are adequate. These workers presented evidence that the hyperglycemic reaction to anoxia is a result of excitation of the symphathico-adrenal mechanism.

Claude Bernard (1876, as cited by Black, 9) was the first to show the relationship of liver glycogen to blood sugar. In spite of this knowledge, it is noteworthy that with the exception of McCormick and Macleod (48, p. 1-29) early workers on fish biochemistry seem to have payed little attention to it. McCormick and Macleod, using sculpins, found a direct correlation of the increase in glucose, as a result of asphyxia, to the liver glycogen level (p. 6-7). Moreover, fish that showed no hyperglycemia also had very little or no liver glycogen.

Kilborn and Macleod (45), Dill (16), and Greene (28) can be cited as examples of the earlier efforts to establish normal glycogen concentrations in fish and to observe changes in the glycogen content during situations such as the spawning migration of salmon. More recently Fontaine and Hatey (20) have continued these studies and Cordier (14) has specifically investigated the effects of asphyxia on the phosphorylase enzymes of fish muscle. Systematic investigations of the effects of anoxic water conditions on fish glycogen levels appear to be lacking.

STATEMENT OF PROBLEM

The presence of interspecific variability of fish in their ability to withstand conditions of diminished oxygen supply is well known. This should not be surprising considering the wide variety of aquatic habitats available. The cold, fast-running streams rarely, if ever, impose a problem of inadequate oxygen on the fish living in them. Fish native to this type of habitat (e. g. trout) would not be expected to be adapted to conditions of low oxygen. On the other hand, fish from warm-water ponds, often choked with algae, are frequently subjected to hypoxic conditions and would be expected to show greater resistance to this sort of stress.

The purpose of this study was to investigate the energy stores and metabolism of two species of fish in relation to short term but severe hypoxic stress. The two species chosen were the bluegill sunfish, Lepomis macrochirus Raf., which is native to warm-water rivers and ponds, and cutthroat trout, Salmo clarki, which are found in cold fast-flowing streams and rivers.

The fish have been subjected to a pre-determined period of severe hypoxic stress and then allowed to recover in well aerated water. During the recovery period, metabolic rate, blood lactate, blood glucose, liver and muscle glycogen, and hemoglobin and/or red blood cell numbers have been measured at intervals of time in

order to observe the changes caused by this stress, and associated with recovery from it.

METHODS AND MATERIALS

Bluegill sunfish, L. macrochirus, were obtained by hook and line from Triangle Lake in western Oregon. They were acclimated to laboratory conditions for a period of at least one and one half weeks before being used in any experiments. During this time, the fish were kept in 35 gallon tanks provided with a continuous flow of aerated, dechlorinated city water. They were fed liver every other day. The experiments on the bluegills were performed during the summers of 1961 and 1962. All runs were made at $20 \pm 1^{\circ}$ C which approximates the summer temperatures of the epilimnion zone of Triangle Lake.

Yearling cutthroat trout, Salmo clarki, were obtained from the Oregon State Game Commission hatchery at Alsea, Oregon. They were fed daily on Clark's pellets. The experiments on trout were performed at the hatchery in the fall and early winter of 1962. During this time the temperature of the hatchery water varied between 8 and 12 degrees centigrade and consequently the experimental fish were not all acclimated to exactly the same temperature. The biochemical runs were all made at temperatures between 11 and 12 degrees and the metabolic rate runs at 9-11.5 degrees.

Description of Apparatus

To stress the fish to low oxygen, a system was devised to deliver water of a constant temperature, oxygen content and flow rate to stoppered flasks containing individual fish. In all but one series of experiments, 2.5 liter wide-mouth erlenmeyer flasks (Fernback flasks) were used. (In the determination of "critical oxygen concentration" in bluegills, respirometers made from one liter plastic bottles were employed.) From four to six containers were run at one time.

The oxygen content of the water in the system was reduced by equilibration with nitrogen in 4.5 foot "Plexiglas" columns filled with "rashig rings. Water was delivered to the top of the columns from a reservoir where the temperature was adjusted to a constant value. The water for the flasks was drawn from the bottom of the columns and the oxygen content was adjusted by varying the amount of nitrogen bubbling through the columns.

The outflow of water from the experimental containers was regulated by stopcocks and then delivered to 300 ml water sampling (BOD) bottles. From here the water passed through flowmeters (Manostat Corporation, New York) which continuously monitored the flow-rate in the system. Water samples were taken by shutting off the flow, quickly removing a BOD bottle and replacing it with a full

bottle. The stopcocks were then opened and the flow adjusted to the proper level. The entire sampling procedure took only a few seconds. Oxygen content of the water samples was determined by the unmodified Winkler method, titrations being performed on 50 ml aliquots using a 10 ml semi-micro buret.

In the experiments in which oxygen uptake was determined, a blank flask (not containing a fish) was included in the system. Oxygen uptake rate was computed from the difference in oxygen content between the blank flask and experimental flasks, the flowrate and the weight of the fish.

The experiments on metabolic rate of bluegills following hypoxic stress were performed before flowmeters were available and, consequently, a different method of measuring oxygen uptake was employed. In this method, water was allowed to flow through the flasks at a fairly rapid rate (approximately 8 liters per hour). When a measurement of oxygen uptake was desired, the outflow of the flask was sampled and the flow was then stopped. After a 30 minute interval, a water sample was removed. The water flow was then resumed until another measurement was desired, at which time the procedure was repeated. The difference in oxygen concentration between the initial and final sample, the volume of the flask, and the weight of the fish were used to calculate the metabolic rate. Subsequent tests showed that metabolic rates of bluegill obtained by

this procedure were similar to those obtained by the continuous-flow method.

Blood and Tissue Sampling Procedure

Blood was drawn from the caudal artery into a heparin-rinsed syringe, after severing the caudal peduncle with a sharp knife. One tenth ml aliquots were taken for blood lactate and blood sugar analyses. Smaller aliquots were then taken for hemoglobin or RBC number determinations. The Barker-Summerson (3) method was used for estimating blood lactate, the glucose oxidase ("Glucostat") method for blood glucose, and the acid hematin method for hemoglobin. Protein free filtrates were made quickly for the lactate and glucose determinations because of the well-known glycolytic activity of red blood cells. All blood analyses were completed on the day of the experiments.

After the blood had been drawn, the fish was stunned by a blow on the head. Tissues for glycogen analysis were then removed, weighed wet on a Sauter torsion balance, and transferred to 5 ml of boiling 60 percent KOH. These were boiled for two to four hours, cooled, stoppered, and stored in the refrigerator for analysis at a later date. In most cases the entire liver was used for liver glycogen determination. For the muscle glycogen measurements a strip of muscle (approximately 800 mg) was taken from just below the

dorsal fin. Glycogen was determined by the Montgomery (52) method after precipitation with hot ethanol.

The time taken to capture the fish, draw the blood, and prepare the tissue samples took from three to five minutes. Black et al. (8, p. 489) has found that there is no significant relationship between the sampling time and glycogen levels in rainbow trout up to at least five and one half minutes, indicating that changes during this time due to struggling or autolysis were slight.

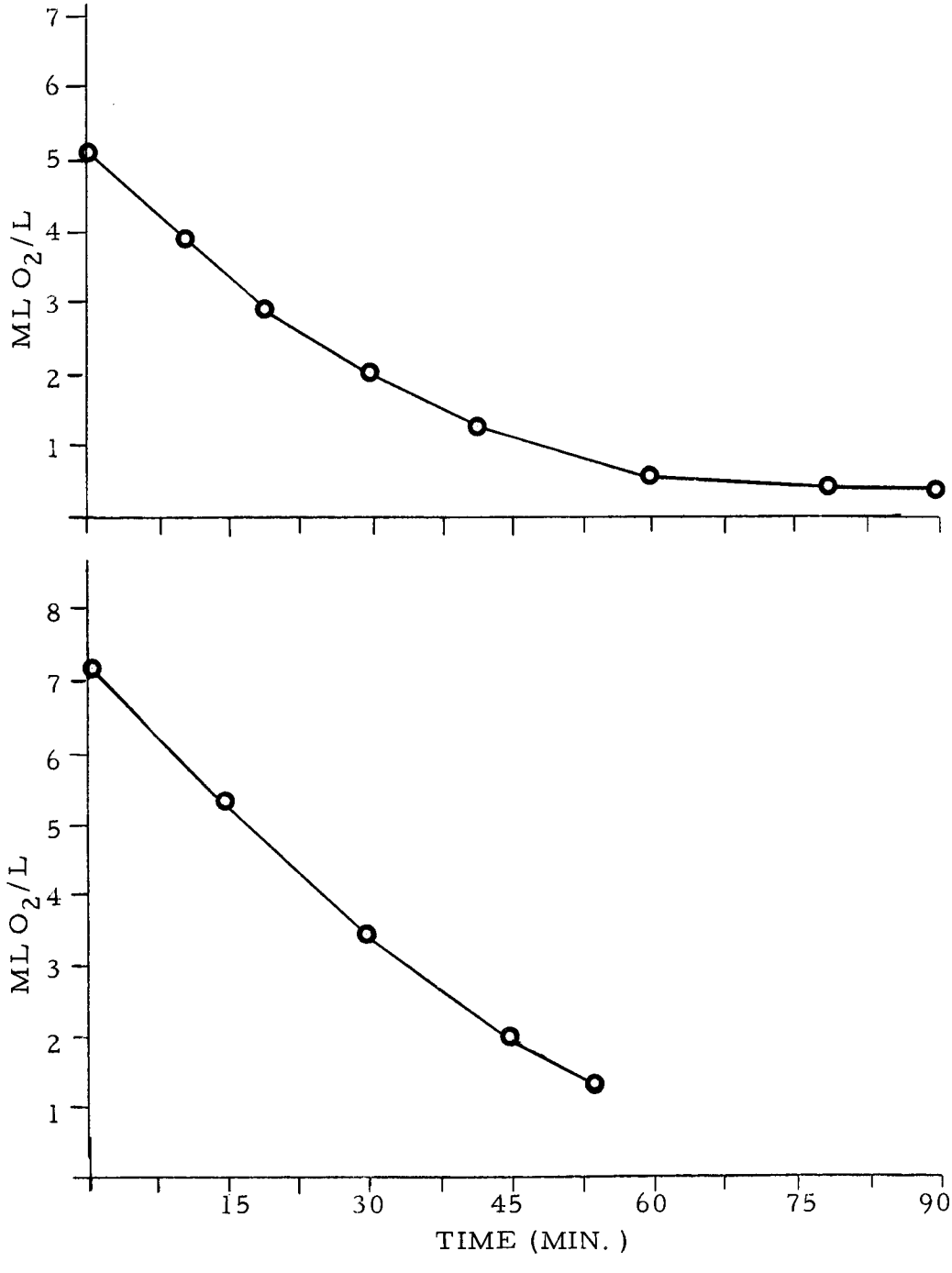
Experimental Protocol

The protocol for an experiment in which biochemical measurements were made is described below:

1. The fish were netted from the holding tanks and placed in the flasks the evening before a run was to begin. The flasks were wrapped with a dark cloth to prevent visual disturbance of the fish.
2. Water was allowed to flow through the flasks overnight.
3. In the morning, one or two control fish were removed and sampled for blood and tissue analysis.
4. The flow rates through the flasks were adjusted to about five liters per hour and the nitrogen was turned on "full," providing the flasks with near oxygen-free water. Figures 1 and 2 show the change in oxygen content of the

Figure 1. Decline of oxygen concentration during stress routine - bluegill experiments.

Figure 2. Decline of oxygen concentration during stress routine - cutthroat trout experiments.


7
6
5
4
3
2
1
ML O_2/L
8
7
6
5
4
3
2
1
ML O_2/L
15
30
45
60
75
90
TIME (MIN.)

outflowing water from the flasks during the "stress" period of the bluegills and trout respectively. This gives an approximate value for the oxygen level of the water within the flasks which is probably slightly lower than the values given in the graphs because of incomplete turnover.

5. After 90 minutes in the case of the bluegills and 55 minutes in the case of the trout, the nitrogen was shut off and the flasks flushed rapidly for 15 minutes with oxygen-saturated water.
6. At fixed intervals of time during the recovery period a single fish was removed from its container and samples of blood and tissue quickly taken.

The protocol in which oxygen uptake was measured before and after the stress was identical to the above, except that serial determinations of metabolic rate were made on each fish throughout the entire recovery period.

RESULTS

Behavioral Responses to Hypoxic Stress

Observations were made of fish as they were subjected to a steadily declining oxygen tension in order to determine the maximum degree of hypoxia they could withstand. From this information, the stress procedures outlined earlier and illustrated in Figures 1 and 2 were formulated.

Bluegills. A mirror was arranged so the fish could be observed without disturbing them. Adjustments of water flow rates, nitrogen flow, etc. were made as outlined in the methods section and then the fish were observed as the oxygen in the flasks declined in concentration. At first, while the water was near saturation, the fish remained quite calm. After 45 minutes, they began to show restlessness and deeper respiratory movements. At this time the oxygen concentration was about one ml per liter. After one hour, at which time the oxygen concentration had declined to about 0.4 ml per liter, they were definitely showing signs of stress but still exhibited no loss of equilibrium. After 90 minutes, the fish were beginning to show convulsive jerks and loss of equilibrium. On a few occasions, a fish exhibited respiratory collapse and died, but this was not common unless the time interval exceeded 90 minutes.

Trout. The alterations in behavior were similar except that the fish lost equilibrium very quickly after the concentration of oxygen fell below about 1.25 ml per liter, thus demonstrating in a qualitative fashion a difference between the bluegills and cutthroat trout in their tolerance to hypoxic conditions.

Several authors have observed loss of equilibrium in fish exposed to lethal concentrations of oxygen (e.g. 63, p. 403). This is almost invariably followed by a few violent movements ("leaps") and then respiratory collapse. Since similar behavior patterns were observed in both bluegills and trout, it would seem that an exposure to hypoxic conditions for a period of time sufficient to just induce loss of equilibrium could be considered as severe hypoxic stress and this criterion has been followed in this investigation.

Oxygen Uptake Studies

Preliminary experiments were performed to determine the presence or absence of endogenous metabolic rate cycles. Since stress experiments were planned for the hours 9:00 A.M. to 10:00 P.M., serial determinations of "standard oxygen uptake," under constant subdued light, were made during this time interval. No significant changes of metabolic rate occurred in the bluegill between those hours (Table 1 in Appendix). The trout, on the other hand, under the same conditions seemed to show a higher rate of oxygen

uptake between 6:00 and 10:00 P. M. (Table 2 in Appendix). The difference is small, however, and would not appreciably affect the trend of the recovery oxygen uptake curve (Figure 6).

Spencer (65, p. 119-132) was unable to find any consistant rhythm of activity in the bluegill. Graham (27, p. 274) cites unpublished data by A. H. Lawrie showing a clear cut cycle in speckled trout reaching its peak at noon and dropping to a minimum at midnight. Had the cutthroat trout in our study been exposed to normal daily photoperiod, they might have shown a similar phenomenon, as young trout and salmon "are habituated to sleep during the hours of darkness when food is less likely to be available" (35, p. 101).

An attempt was made to determine critical oxygen concentrations for both species of fish by measuring their oxygen uptake at several different oxygen tensions. One and one half hours was allowed between each change in oxygen concentration and two determinations per fish were made at each level. These data are presented in Figures 3 to 5 where individual runs are shown.

At oxygen concentrations below saturation, the bluegills maintained a fairly constant metabolic rate (respiratory independence) down to an oxygen level of 1. 5 ml per liter (Figure 3). Below this critical concentration, metabolic rate declined rapidly (respiratory dependence). The data for the cutthroat trout are quite variable and

Figure 3. Effect of oxygen concentration on rate of oxygen uptake in bluegill sunfish. Circles are means of two determinations.

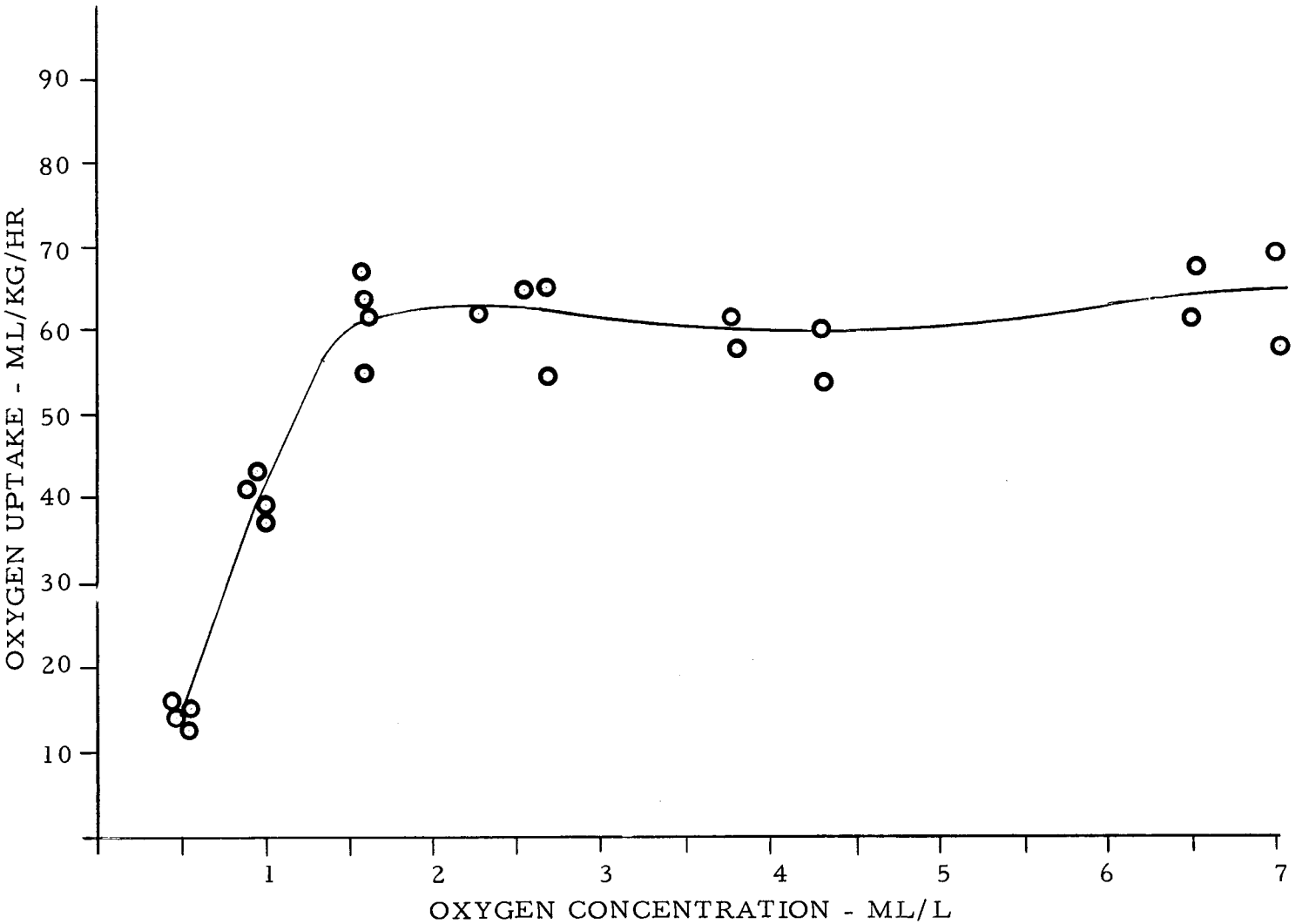

OXYGEN UPTAKE - ML/KG/HR
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
OXYGEN CONCENTRATION - ML/L

Figure 4. Effect of oxygen concentration on rate of oxygen uptake in cutthroat trout. Each symbol represents a different fish and is the mean of two determinations.

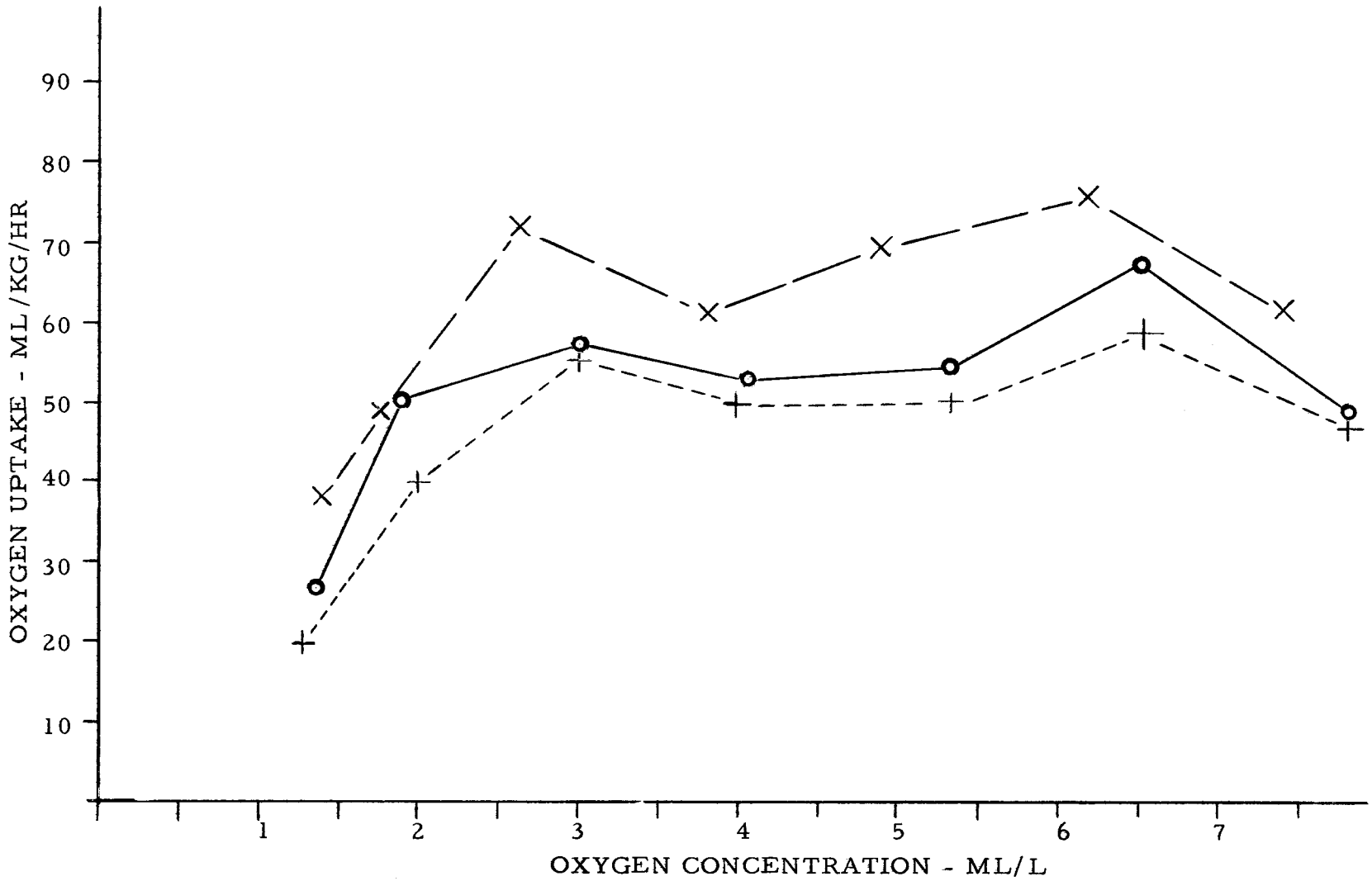
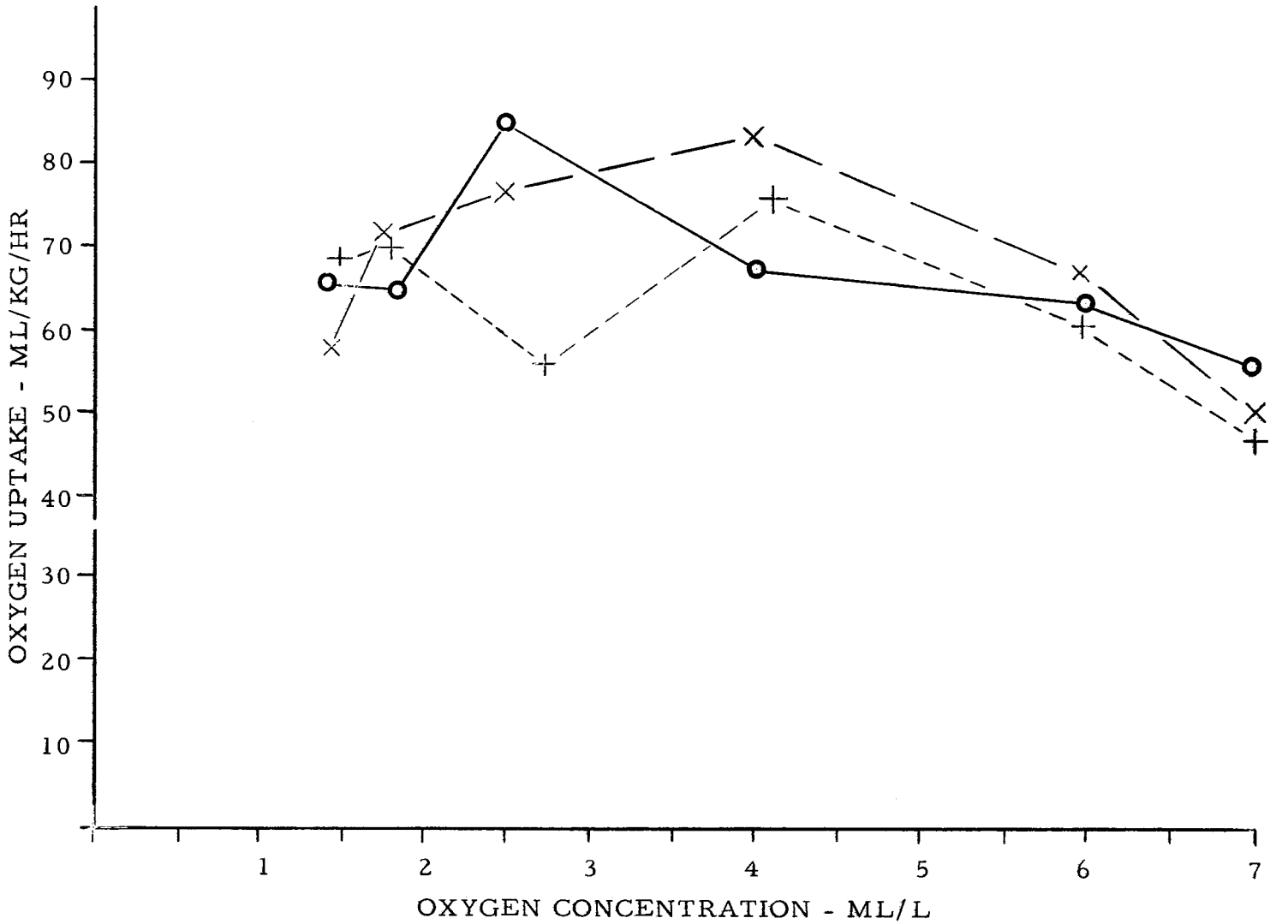

OXYGEN UPTAKE - ML/KG/HR
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
OXYGEN CONCENTRATION - ML/L

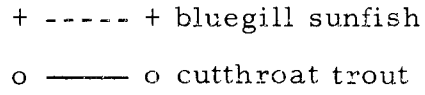
Figure 5. Effect of oxygen concentration on rate of oxygen uptake in cutthroat trout. Each symbol represents a different fish and is the mean of two determinations.


OXYGEN UPTAKE - ML/KG/HR
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
OXYGEN CONCENTRATION - ML/L

it is difficult to see a clear relationship between rate of oxygen consumption and oxygen concentration. In one of the two runs shown (Figure 4), all three fish showed a steadily declining oxygen uptake rate below an oxygen concentration of 2.5 to 3.0 ml per liter. In this range, however, the metabolic rate of each fish is higher than the rate in air-saturated water, indicating perhaps the metabolic cost of increased ventilation at this lowered oxygen concentration. In the other run shown (Figure 5) there is no indication of a critical oxygen concentration and, in fact, at the lowest oxygen concentration tested, the metabolic rate of the fish were higher than the rate in air-saturated water. It is believed that part of the variability in this series of experiments may be due to incomplete exchange of water in the large experimental flasks, when the oxygen content of the incoming water was varied.

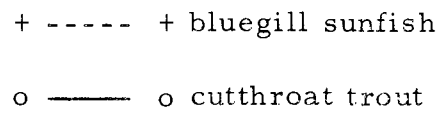
The data on the recovery oxygen uptake of bluegills and cutthroats, following hypoxic stress, is illustrated in Figure 6. In bluegills a peak metabolic rate (50 percent above pre-stress levels) is reached immediately after the stress, and is followed by a slow decline to the control level in approximately eight hours. The maximum recovery uptake in cutthroat is not, in contrast to the situation in bluegills, reached until five hours after the exposure to hypoxia. Furthermore, the peak uptake is considerably more elevated (100 percent) above the pre-stress level than was the case of the bluegill.

Figure 6. Changes in the rate of oxygen uptake following hypoxic stress.

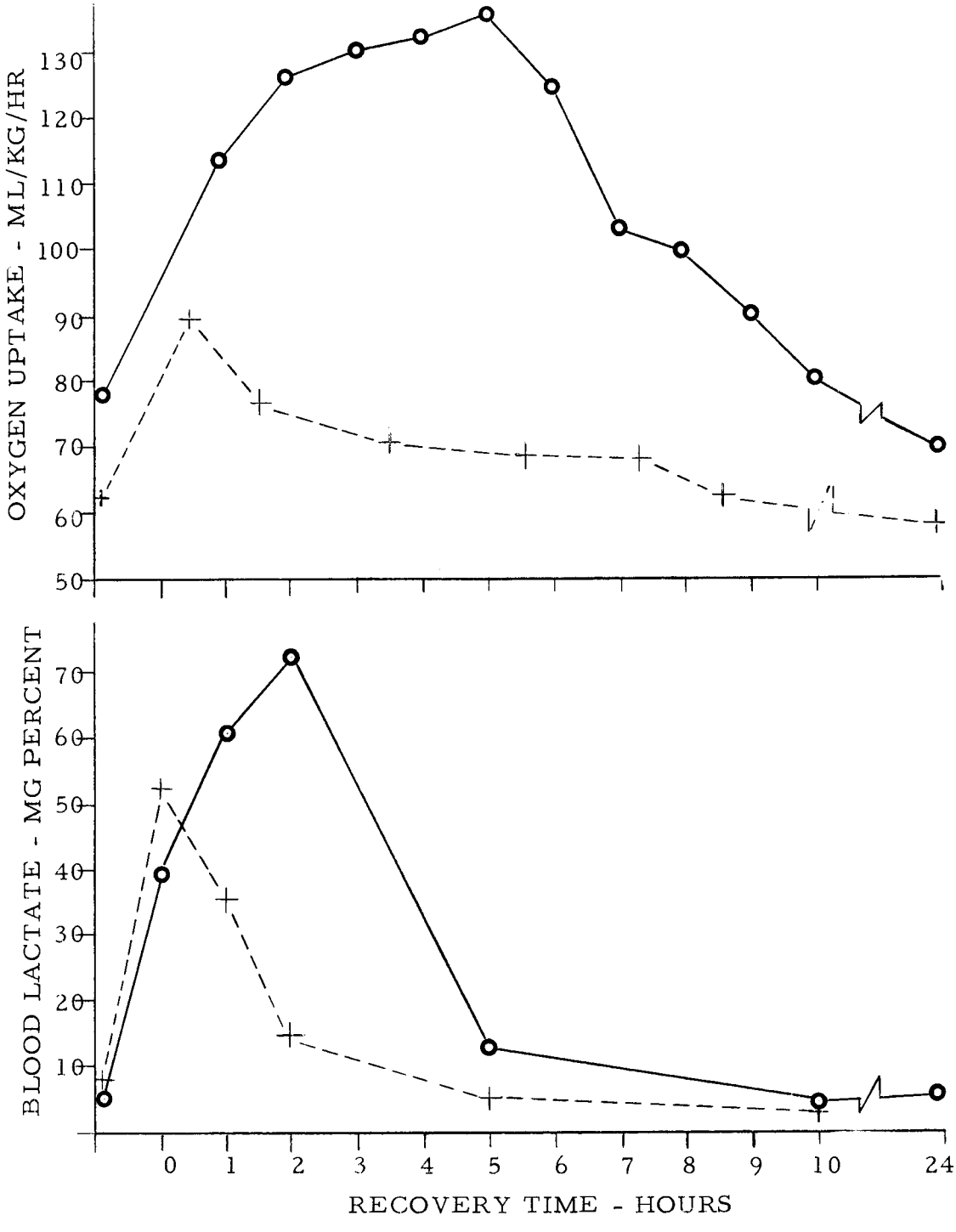


The symbols represent the mean values; the first points on the graph are the pre-stress rates.

Figure 7. Changes in blood lactate concentration following hypoxic stress.



The first points on the graph are the pre-stress concentrations of lactate. All are the means of several determinations.


OXYGEN UPTAKE - ML/KG/HR
130
120
110
100
90
80
70
60
50
BLOOD LACTATE - MG PERCENT
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
24
RECOVERY TIME - HOURS

After ten hours recovery, the rate has fallen to near the control level.

Blood Lactic Acid

As seen in Table 1 and Figure 7, the lactic acid in bluegills increases during the hypoxic stress period from a control level of 8.4 mg percent to a maximum of 52.7 mg percent found immediately after the stress. It declines during the recovery period to near the control concentration in three hours and thereafter remains constant.

The blood lactic acid recovery data for cutthroat are recorded in Table 2 and also illustrated in Figure 7. The concentration increases from a control level of 6 mg percent to a concentration of 40 mg percent immediately following the stress, and then continues to rise during the recovery period until a maximum of 72 mg percent is reached two hours after the stress. It returns to slightly above the control level in five hours. The concentration at 10 and 24 hours recovery remains near the control level.

Blood Glucose

The blood glucose of the normal bluegill sunfish averaged 43 mg percent. Hypoxia caused a rise in the concentration to a level of 135 mg percent, an increase to over three times the control value. Following the stress, blood glucose slowly declined, still

Table 1. Body weights and levels of lactic acid, glucose, hemoglobin, muscle glycogen and liver glycogen in bluegill sunfish before, and during recovery from severe hypoxia stress. All values are the mean ± standard error. The number of samples is in parenthesis.

	Control	Immediately Following	1 Hour	2 Hours	5 Hours	10 Hours
Body Weight (Grams)	29.1± 4.4 (5)	27.1± 1.8 (6)	25.9± 5.2 (6)	31.2± 3.7 (6)	34.7± 6.8 (6)	24.4± 2.8 (6)
Blood Lactic Acid (Mg percent)	8.4± 1.6 (5)	52.7± 5.2 (8)	35.9± 6.8 (6)	13.5± 2.4 (6)	7.3± 0.9 (7)	7.2± 0.9 (6)
Blood Glucose (Mg percent)	43.2± 3.2 (8)	134.9±14.0 (9)	134.7±17.5 (5)	110.3±14.3 (7)	89.1±13.5 (7)	69.1± 7.2 (9)
Hemoglobin (Gram percent)	8.79±1.15 (7)	9.53±0.75 (7)	10.50±1.20 (7)	10.39±1.52 (5)	8.85±1.56 (7)	9.62±1.51 (6)
Muscle Glycogen (Mg/100 g)	46.4±15.7 (7)	17.9± 5.1 (7)	21.7± 6.6 (7)	26.2± 6.3 (7)	56.5±18.7 (6)	39.8± 3.6 (5)
Liver Glycogen (Mg/100 g)	217.1±36.1 (7)	66.0±19.0 (6)	42.2±16.0 (7)	139.0±40.9 (7)	202.8±102.9 (5)	168.8±16.2 (6)

Table 2. Body weights and levels of lactic acid, glucose, muscle glycogen, liver glycogen, and red blood cell count in cutthroat trout before, and during recovery from, severe hypoxic stress. All values are the mean ± standard error. The number of samples is in parenthesis.

	Control	Immediately Following	1 Hour	2 Hours	5 Hours	10 Hours	24 Hours
Body Weight (Grams)	18.9± 2.4 (7)	20.3± 1.9 (5)	21.9± 1.9 (5)	24.1 (2)	24.3± 3.1 (6)	21.3± 1.7 (6)	
Blood Lactic Acid (Mg percent)	5.6± 1.3 (8)	39.9± 6.7 (8)	60.6±13.0 (8)	72.3±11.2 (5)	13.1± 1.3 (8)	5.5± 0.7 (7)	6.0± 2.1 (6)
Blood Glucose (Mg percent)	63.1± 3.1 (9)	48.0± 5.7 (8)	66.7±10.8 (7)	67.6±10.3 (5)	67.8±10.0 (7)	72.5± 4.3 (7)	56.2±10.9 (7)
Muscle Glycogen (Mg/100 g)	65.1±15.7 (10)	14.6± 1.4 (7)	40.9± 6.2 (7)	33.9±19.3 (5)	25.3± 3.1 (8)	50.2±17.0 (8)	28.3± 5.4 (7)
Liver Glycogen (Mg/100 g)	127.0±22.0 (9)	5.5±11.5 (7)	24.5± 8.4 (8)	6.6± 1.4 (4)	53.6±26.6 (7)	42.0±24.4 (6)	51.8±22.3 (6)
RBC Count (Millions/mm^3)	1.28±0.08 (6)	1.40±0.2 (4)	1.29 (4)	1.30 (3)	1.33 (6)	1.22 (3)	1.24 (2)

not having reached the control level after ten hours recovery (Figure 8). A markedly different pattern was seen in the cutthroat trout (Figure 8). Hypoxic stress produced a decrease in blood glucose, from a control value of 63 mg percent to a level of 48 mg percent immediately following the stress. There was a return to the control concentration in one hour, after which there appeared to be very little change in blood glucose until a slight, but probably insignificant, decrease occurred after 24 hours recovery.

Muscle Glycogen

As seen in Figure 9 and Tables 1 and 2, the control concentrations of muscle glycogen averaged 46 mg per 100 grams of tissue in the bluegills and 65 mg per 100 grams of tissue in the cutthroat trout. These values are somewhat lower than those given by Black et al. (9) for one and one-half year old rainbow trout and considerably lower than the values given by Miller et al. (51), and by Hochachka and Sinclair (37), also for one and one-half year old rainbows. They are higher than those observed by Heifetz (34) who found concentrations of 1.8 to 8.6 mg per 100 grams in largemouth bass and crappie.

Hypoxic stress caused an immediate and severe reduction in muscle glycogen concentration in both bluegills and trout. The utilization of glycogen was so great in some specimens that detection

Figure 8. Changes in blood glucose concentration following hypoxic stress.

+ ----- + bluegill sunfish

o ——— o cutthroat trout

The first points on the graph are the pre-stress concentrations of glucose. All are the means of several determinations.

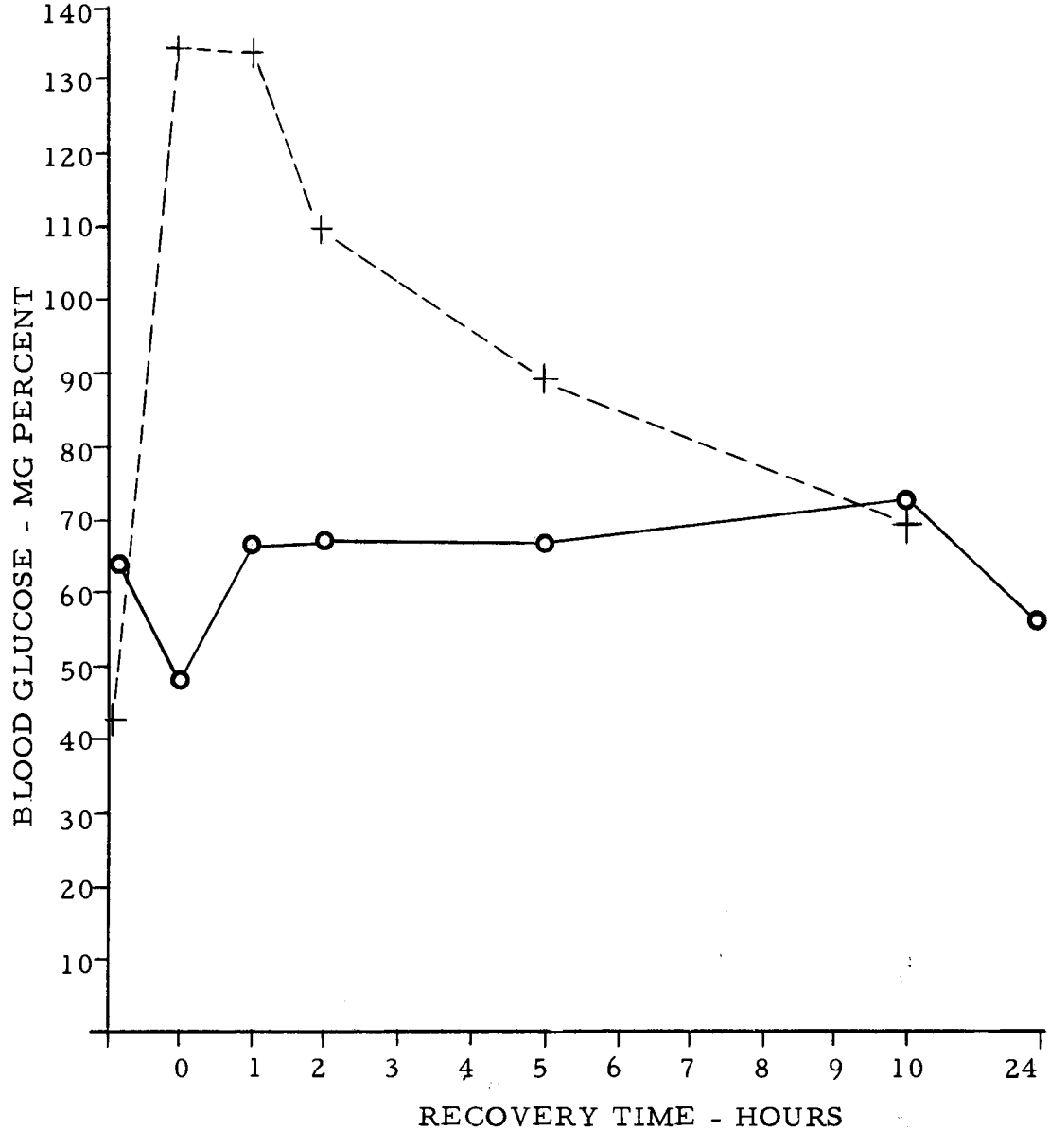

140
130
120
110
100
90
80
70
60
50
40
30
20
10
BLOOD GLUCOSE - MG PERCENT
0
1
2
3
4
5
6
7
8
9
10
24
RECOVERY TIME - HOURS

Figure 9. Changes in muscle glycogen concentration following hypoxic stress.

+ ----- + bluegill sunfish

o ——— o cutthroat trout

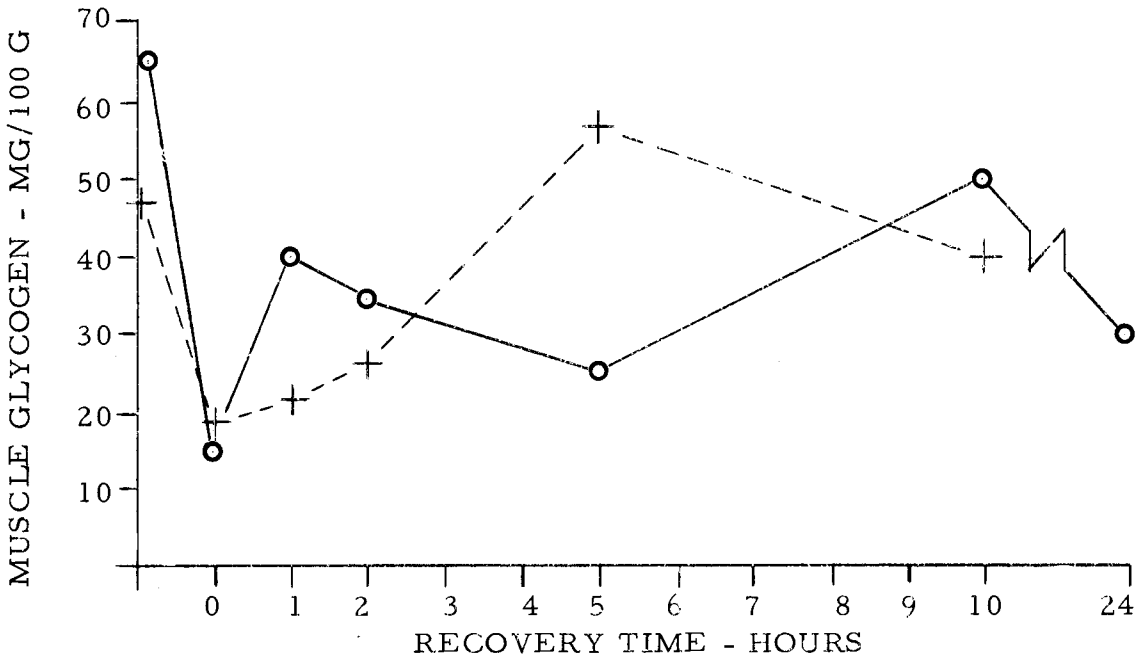
The first points on the graph are the pre-stress concentrations of glycogen. All are the means of several determinations.

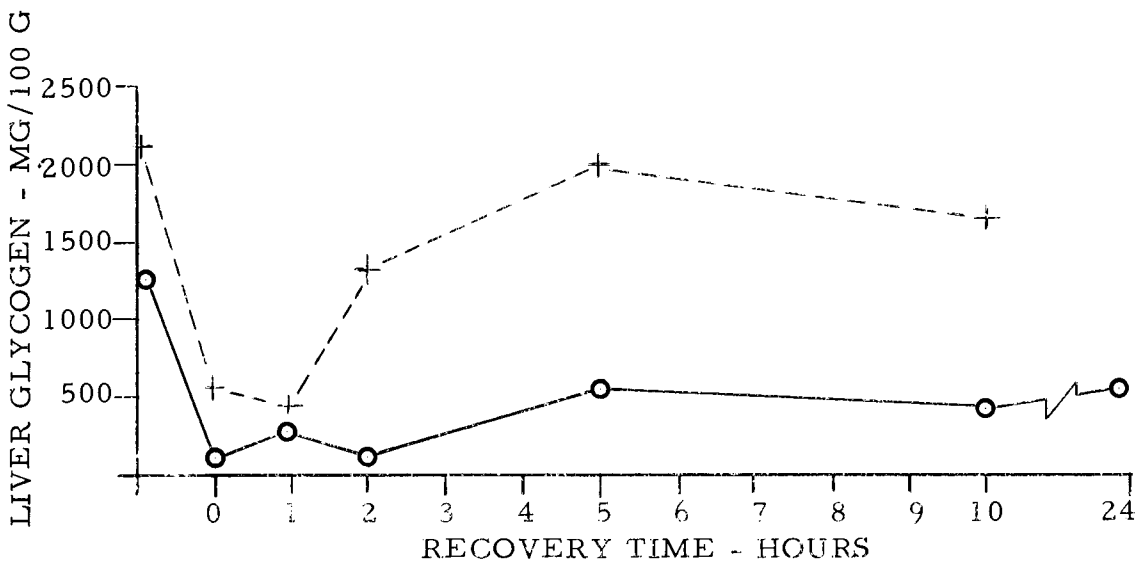
Figure 10. Changes in liver glycogen concentration following hypoxic stress.

+ ----- + bluegill sunfish

o ——— o cutthroat trout

The first points on the graph are the pre-stress concentrations of glycogen. All are the means of several determinations.


MUSCLE GLYCOGEN - MG/100 G
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
24
RECOVERY TIME - HOURS


LIVER GLYCOGEN - MG/100 G
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
10
24
RECOVERY TIME - HOURS

of glycogen in muscles was difficult. This agrees with the observations by Black et al. (9, p. 93) on the effects of 15 minutes of strenuous exercise in Kamloops trout, in which a reduction of muscle glycogen to a level about 1/5 that of the control was observed.

During recovery, the muscle glycogen of the bluegill increased steadily in concentration, reaching a level slightly higher than the controls in five hours. This was followed by a slight, probably insignificant, decline in glycogen concentration in fish sampled at ten hours.

The recovery of muscle glycogen in cutthroat trout following hypoxia was more variable. There was a marked rise in glycogen during the first hour of recovery and then a leveling off at a concentration somewhat below the control level. Fish sampled after 24 hours of recovery still had less than half the control concentration of muscle glycogen.

Liver Glycogen

The control concentrations of liver glycogen averaged about 2100 mg per 100 grams and 1275 mg per 100 grams in the bluegills and cutthroat trout respectively. These are somewhat low (particularly for the trout) compared with values published by other workers. The low levels in both muscle and liver were first observed when the glycogen samples for the trout were analyzed, about three months

after the experimental runs had been completed and it was felt that perhaps these did not represent normal values; therefore, ten additional fish were sampled directly out of the holding tanks. These data are presented in Table 3 of the Appendix and show that the concentrations of glycogen in these fish were much higher than in the fish that had been put in the flasks for 12 hours previous to an experimental run. Handling of the animals was the probable reason for this reduction of glycogen in the latter.

Hypoxia resulted in a marked depletion of liver glycogen in both species (Figure 10). During the recovery period, there was a resynthesis of glycogen in the bluegill to approximately the control level in five hours. The cutthroats showed a gradual increase in liver glycogen but this did not approach the control value even 24 hours after the stress.

The great variability of the liver glycogen levels for both species is evident from the relatively large standard errors (Tables 1 and 2). This seems to be quite characteristic of fish liver glycogen (e.g. 8, p. 487-500; 10, p. 409-436; 37, p. 127-136). Average control values reported by these workers vary from around 820 mg per 100 grams in one and one-half year old Kamloops trout (9, p. 94) to 10,033 mg per 100 grams in rainbow trout (37, p. 127-136). These investigators also found very large individual variation.

Hemoglobin and Red Blood Cells

Tables 1 and 2 summarize the hematological data for both species and show that no significant change in either hemoglobin or RBC number occurred as a result of hypoxic stress in these forms. There is a slight rise in RBC's seen in the cutthroats immediately after the stress. This elevated level is not, however, maintained during the recovery period.

Some investigators have observed increases in hemoglobin and/or red blood cells (e. g. 29; 30) while others have found no change or a decrease (e. g. 68; 57) following asphyxiation of fish. Where increases have been obtained, they have generally been attributed to release of blood cells from hemopoetic tissue or contraction of the spleen. Severe exercise can produce slight increases in hemoglobin, which has been attributed to a loss of blood water (10, p. 412-413). The decrease in red blood cell count observed by Westfall (68) following asphyxia was shown to be due to stagnation of blood in the gills.

DISCUSSION

The principle aim of this study has been to characterize the physiological responses of two species of fish, from different ecological situations, to a brief but severe hypoxic stress. The stress employed was an arbitrary one, limited by the qualitative criterion of loss of equilibrium. The fish were not subjected to complete anoxia, but rather to a rapidly declining oxygen concentration. The preliminary tests in which oxygen uptake rates were determined at different oxygen concentrations (Figures 3-5) indicate that for at least part of the oxygen stress regime, the fish were subjected to oxygen concentrations less than that necessary to maintain a normal standard metabolic rate. In the case of the bluegills, the fish were exposed to less than 1.5 ml per liter oxygen concentration (below which oxygen consumption is dependent on oxygen concentration) for about 50 minutes. Because of their greater sensitivity to low oxygen, the trout could not be subjected to less than 1.25 ml per liter oxygen concentration without losing equilibrium, and thus the oxygen stress regime was shorter (55 minutes) for the trout than for the bluegill (90 minutes). It is of some interest that in the afore-mentioned metabolic rate experiments, a clear-cut "critical" oxygen concentration was not obtained for cutthroat trout. In fact, in one experiment (Figure 4) the metabolic rate appeared to

be independent of the oxygen concentration of the medium down to about 1. 5 ml per liter, whereas in a second experiment (Figure 5) "metabolic dependence" was shown below 2. 5 ml per liter oxygen concentration. Below 1. 5 ml per liter concentration it was not possible to determine oxygen consumption accurately, as the trout showed obvious signs of distress and many died shortly thereafter. These experiments and observations, though not extensive, do seem to point to marked differences in the efficiency of the respiratory apparatus in the two species.

The efficiency of the respiratory apparatus of fish is difficult to assess, although recently there has been some extensive work in this area (61, p. 817-862; 41, p. 275-361). The data of Saunders' indicate that fish from warm sluggish waters show a higher percentage utilization of respired water at high respiratory volumes (amount of water passing over the gills per unit time) than do forms from fast-flowing cold streams (loc. cit. p. 829). High respiratory volumes are found when fish are exposed to water low in oxygen. However, carp and bullheads showed less of an increase in respiratory volume at low oxygen levels than did the suckers from fast-flowing streams. Since increases in respiratory volume presumably require energy, an ability to remove oxygen with a minimum of effort would be of benefit to the fish under conditions of reduced oxygen content. These observations suggest that

perhaps the bluegill, being a warm-water fish, might be able to remove oxygen from water of low oxygen concentrations with less expenditure of energy than does the cutthroat trout. This might endow the former with a greater resistance to hypoxia conditions.

Interrelations of Blood Lactate and Muscle Glycogen

During the period of hypoxic stress, the bluegills accumulated lactic acid in their tissues, which subsequently diffused rapidly into the blood stream. This is evidenced by the relatively high level of lactate found in the blood at the termination of the stress. The concomitant drop in muscle glycogen (Figure 9) indicates that the source of the lactate was primarily glycogen, as would be expected from the early work of Meyerhof (1920) cited by Alpert et al. (2, p. 585). This relation between lactate and muscle glycogen was also demonstrated by Black et al. (9, p. 93-96) for various species of salmonids that had been subjected to strenuous exercise.

The lactate recovery pattern in cutthroats (Figure 7) differed from that seen in the bluegills in that there was a delayed rise of the blood lactate during the recovery period. Whereas the bluegill showed a return of the lactic acid level to near that of the control in two hours of recovery, the cutthroat trout exhibited its highest level of lactate at this time. The fact that the muscle glycogen in the cutthroat trout does not decline further during the recovery period

suggests that its conversion into lactic acid has been slowed, or even halted completely, following the stress. Therefore, the rising level of blood lactate after the stress may be attributed to a relatively slow movement of lactic acid from the tissues into the blood stream. Black (8, p. 128-129) has suggested that cold temperatures would reduce the diffusion rate of lactic acid from the tissues into the blood stream in fishes. However, more recent work has shown this may not be as important as originally believed (10, p. 429-436). Of some significance may be the findings by several workers (46; 62; 25) that asphyxiation of fishes by exposure to air causes a drop in heart rate and a general reduction in circulatory efficiency. Moreover, the aforementioned workers show that during exposure to air muscle lactic acid increases rapidly, but the blood lactate does not increase significantly until the fish are returned to water. At this time the heart rate again returns to normal, and the tissues are flushed with blood. In the light of these findings, it is possible that hypoxia induces a greater reduction in circulatory efficiency in the trout than in the bluegill.

It is interesting to note that another type of stress in bluegill, namely severe muscular exercise, is followed by a delayed rise in blood lactate (33). Thus it is tempting to suggest that, with the bluegill at any rate, exercise stress causes a greater reduction in circulatory function than does hypoxic stress. Hayashi (as cited by

Black, 10, p. 429) has studied the effects of fatigue on circulation in fish. His data show a considerable impairment of circulation during, and for two hours following exercise. The studies by the authors mentioned earlier on the effects of asphyxiation on circulation showed an apparent resumption of normal circulation immediately following air exposure. Whether more severe asphyxiation, or more to the point, acute hypoxic stress, would cause a prolonged circulatory impairment is still an open question.

The bluegill and cutthroat differ in the maximum level of lactic acid that is accumulated in the blood as a result of hypoxic stress. Although the duration of hypoxia for the trout was considerably less than that for the bluegills, the maximum lactic acid levels during the recovery period were actually somewhat higher in the trout, suggesting a greater amount of tissue anaerobiosis. It should be noted, however, that the presence of lactic acid is not necessarily an indication of tissue hypoxia. Huckabee (38) has demonstrated that lactate concentration in mammalian tissue is governed by two factors; pyruvate concentration and the state of oxidation of diphosphopyridine nucleotide (DPN). They are related to one another by the following equation:

$$(\text{Lactate}) = (\text{Pyruvate}) \times K \frac{\text{DPNH}}{\text{DPN}}$$

Only the DPNH/DPN ratio varies with the oxygen concentration, so measurements of either it or the levels of pyruvate are necessary to

evaluate how much of the lactate formed is a result of oxygen deficiency (called "excess lactate"). In human subjects, breathing gas mixtures of 13 percent oxygen can produce a decrease in blood oxygen tension without any tissue hypoxia being evident, even though slight increases in lactate were found. When the oxygen in the respired air is reduced to ten percent or lower, however, tissue hypoxia is indicated by the accumulation of "excess lactate" (40). Thus it cannot be stated unequivocally that, under the hypoxic stresses used in this study, the trout showed more tissue anaerobiosis than the bluegill. The fact remains, of course, that the trout accumulates more post-stress lactate than the bluegill, be it "excess lactate" or not, even though not subjected to as severe a stress. From a practical standpoint, this may be important since it has been suggested by Black that high levels of lactate may be a cause of fish mortality due to a disturbance of the acid-base balance, alterations in shape and volume of red blood cells, and a reduction of oxygen and carbon dioxide combining capacity of the blood (7).

It could well be that the levels of lactate formed are limited by the amount of fuel available. In both species, the muscle glycogen was severely depleted as a result of the stress. Black and his associates have found levels of blood lactic acid in excess of 160 mg percent in lake and Kamloops trout that had been severely exercised for 15 minutes (9, p. 105). As mentioned earlier, their fish had

higher initial levels of muscle glycogen than did those in the present study. Furthermore, their experiments (9, p. 94) showed an almost complete utilization of this glycogen during the exercise. Since handling of our fish before the hypoxia experiments would have reduced their initial levels of glycogen, exposure of these two species to hypoxic stress without previous handling might prove interesting.

The importance of carbohydrates as a source of energy for sudden spurts of swimming has been emphasized by Black et al. (10, p. 427). The rapid decline in muscle glycogen during severe muscular activity, and the well known slowness of protein and lipid catabolic reactions support this conclusion. The initial level of muscle glycogen is of considerable importance then, in that it probably sets a limit on the extent of stress that a fish can undergo. Furthermore, the concentrations seen in fish tissues are low in comparison with corresponding mammalian tissues, excepting the relatively high concentrations of cardiac glycogen found in fish (15). The virtual depletion of the muscle glycogen stores by exposure to hypoxic conditions (Figure 9) would indicate that for a period of time following such a stress, the fish would be unable to engage in any strenuous activity such as capturing food or evading predators.

In both species, blood lactate returns quickly to the normal level after the peak values are achieved. This is in contrast to the situation in exercised fish (9, p. 103-110), and may reflect

differences in circulatory responses to the two types of treatment, as mentioned earlier. If normal circulation is restored sooner following a short period of hypoxia than occurs following muscle fatigue, the removal of lactate by extrahepatic and hepatic tissues would be hastened.

Relationship of Blood Lactate to Oxygen Consumption

Figures 6 and 7 show a decided rise in oxygen consumption in parallel with the increased amounts of blood lactate in both species. During recovery, however, the lactate declines relatively quickly to the resting level while the metabolic rate is still slightly elevated above the pre-stress level in the bluegills and maximally elevated in the trout. The concept of an oxygen debt, originally formulated by Hill and his associates (2), gave lactic acid the role of security for the debt in that it supposedly forced its repayment. The excess oxygen consumed following exercise was thought to be used for the oxidation and resynthesis to glycogen of the accumulated lactate. Thus, it was assumed that the oxygen consumption would parallel the lactate changes during recovery from exercise. The lack of correlation of these two paramenters during recovery, however, has been well established in mammals and in fish (47, p. 689-715; 39; 36; 33).

The shape of the recovery oxygen consumption curve has

been considered a measure of the oxygen debt by most workers in this field. Huckabee (40) showed that this may indeed be justified if "excess lactate" is considered. This parameter and recovery oxygen consumption values were closely related in his investigations of hypoxic stress in men and dogs. Alpert et al. (2), however, report no increased oxygen consumption following hypoxia in dogs and hypothesize the presence of a "metabolic governor." These findings are similar to those of Blazka (11) on crucian carp, except that he found no change in lactate while Alpert et al. observed the usual increase. On the other hand, Blazka's data on trout show compensatory oxygen consumption following the hypoxic stress, again however, with no lactate changes.

In the present study, an increased oxygen uptake in both species occurred following the stress (Figure 6). The trout, however, exhibited a noticeably greater increase than did the bluegill and this corresponds with the higher levels of blood lactate seen in the former following the stress (Figure 7). The data on oxygen uptake agree qualitatively with those of Blazka (11) in that the species adapted to low oxygen conditions (bluegill) apparently developed less of an oxygen debt than did the species not adapted to these conditions (trout).

During recovery, the oxygen uptake in both forms declined to the pre-stress level several hours after the blood lactate had

returned to normal (Figures 6 and 7). According to Huckabee (40), however, oxygen consumption should decline to the control level slightly before the total lactate reaches normal. In this regard, it is conceivable that the prolonged high level of oxygen uptake is due not only to the oxygen debt incurred, but also to the excitement of the stress. Many workers on fish respiration have mentioned the elevated oxygen uptake that occurs when a fish is disturbed in any way (23, p. 30-35) and, in addition, this higher level may persist for several hours even though the fish is apparently quiescent. This would be a difficult thing to evaluate because we do not know at present what causes the increased rate. There may be alterations in muscle tonus associated with excitement, and these would not be detected by observations or measurements of activity.

Blood Glucose and Muscle and Liver Glycogen

During recovery from hypoxia both the bluegills and cutthroat trout showed a gradual restoration of muscle glycogen. Although the data are variable, the bluegills seemed to accomplish this considerably faster than did the trout. The source of muscle glycogen is considered to be blood glucose (9, p. 90) and, it should be noted that the bluegills had extremely high concentrations of glucose in their blood immediately following the stress while the trout showed relatively normal glucose levels during this time. Moreover, the

decline of glucose in the bluegills during recovery is paralleled by a rise in muscle glycogen.

The lack of a rise in blood sugar in the cutthroat trout as a result of hypoxia is difficult to explain, and is contrary to the response observed by other workers on fish and mammals (40, p. 461-491; 50). However, Middlesworth (50) found a hypo-glycemic response to anoxia in rats fasted for 12 hours, even though their carbohydrate stores seemed adequate. McCormick and Macleod (48) have suggested that the extent of hyper-glycemia in asphyxiated fish is proportional to the initial levels of liver glycogen.

Figure 10 shows that most of the liver glycogen was mobilized by the end of the hypoxic stress in both species. The high level of blood glucose in the bluegills immediately after the stress is readily explained by this mobilization. Presumably, the liver glycogen in the cutthroat must have also been converted to glucose, but the lack of a rise in blood glucose suggests that it was utilized in extra-hepatic tissue as rapidly as it was produced. In addition, the relatively low initial glycogen levels probably limited the amount of hexose produced.

Synthesis of liver glycogen from lactic acid has not yet been established in fish. For that matter, the significance of this reaction sequence in mammals has been recently questioned (19). During recovery from hypoxia, the bluegills, and to a lesser extent

the trout, showed a restoration of liver glycogen. It is impossible to tell, however, whether the glycogen was synthesized from lactic acid or blood glucose or both. Carbohydrate metabolism is, of course, in a dynamic state of flux, and samples obtained of the various stores show only the situation at a particular time and do not give information on rates of conversion of one substance to another.

General Conclusions

The data presented on the effects of hypoxia on the energy stores and metabolism of two species of fish from contrasting types of ecological habitat contributes to the question of how fish from warm-water ponds are able to survive conditions of reduced oxygen better than forms native to fast flowing cold-waters. It is hypothesized that the bluegill, a warm-water fish, makes more efficient use of its fuel reserves under the hypoxic conditions used in this study than does the trout. This is evident from the more rapid recovery of glycogen reserves in the bluegill as contrasted to the trout. Furthermore, the trout accumulated a greater quantity of lactic acid during hypoxic stress than does the bluegill. This could reflect a greater energy demand at low oxygen concentrations on the part of the trout, which it is unable to meet effectively by oxidation of pyruvate. As a consequence, it rapidly converts its glycogen stores

to lactic acid in an attempt to obtain energy anaerobically.

Although no direct information was obtained on the functioning of the circulatory system in these forms, indirect evidence suggests a more severe impairment of its efficiency in the cutthroat trout than occurred in the bluegill as a result of hypoxic stress, and points to a possible direction for fruitful research in the future.

SUMMARY

1. Bluegill sunfish and cutthroat trout were compared in their physiological responses to severe hypoxia stress. Measurements of metabolic rate, blood lactic acid, blood glucose, liver glycogen, muscle glycogen, and hemoglobin or red blood cell counts were made on fish that had been exposed for a short time to a very low level of oxygen. Changes in these parameters were followed during the recovery period.

2. The hypoxic stress caused an immediate mobilization of the carbohydrate stores in both species of fish and a concomitant accumulation of blood lactic acid. Blood glucose increased greatly in the bluegills but not in the cutthroat trout.

3. The delayed rise in blood lactic acid in the trout, but not in the bluegill, following the stress suggests differences in the circulatory responses to hypoxia in these two species.

4. In both species, oxygen uptake changes during the first portion of recovery from hypoxia paralleled the lactate changes, however, during the later stages of recovery the blood lactate returned to normal sooner than did the oxygen uptake.

5. Resynthesis of glycogen stores occurred much more quickly in the bluegills than trout following hypoxic stress.

6. Significant changes in hemoglobin or red blood cell numbers were not apparent in either species indicating no change in blood water content, or effect on hemopoetic tissues resulting from the hypoxia.

7. It is suggested that the bluegill makes more efficient use of its fuel reserves under conditions of acute hypoxia and that this may enable it to survive conditions of low oxygen better than does the trout.

BIBLIOGRAPHY

1. Alpert, Norman R. and Walter S. Root. Relationship between excess respiratory metabolism and utilization of intravenously infused sodium racemic lactate and sodium L (-) lactate. American Journal of Physiology 177:455-462. 1954.

2. Alpert, Norman R., Herbert Kayne and Winona Haslett. Relationship among recovery oxygen, oxygen missed, lactate production and lactate removal during and following severe hypoxia in the unanesthetized dog. American Journal of Physiology 192:585-591. 1958.

3. Barker, S. B. and William H. Summerson. The colorimetric determination of lactic acid in biological material. Journal of Biological Chemistry 138:535-554. 1941.

4. Barrett, Izadore and Anne Robertson Connor. Blood lactate in yellowfin tuna, Neothunnus macropterus, and skipjack, Katsuwonus pelamis, following capture and tagging. Bulletin of the Inter-American Tropical Tuna Commission 6 (6):233-280. 1962.

5. Black, Edgar C. Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity I. Kamloops trout, Salmo gairdneri. Journal of the Fishery Research Board of Canada 14:117-134, 1957.

6. _____. Energy stores and metabolism in relation to muscular activity in fishes. In: The Investigation of Fish-Power Problems, H. R. MacMillan Lectures in Fisheries, ed. by P. A. Larkin. Vancouver, University of British Columbia, 1958. p. 51-67.

7. _____. Hyperactivity as a lethal factor in fish. Journal of the Fishery Research Board of Canada 15:573-586. 1958.

8. Black, Edgar C. et al. Alterations in glycogen, glucose, and lactate in rainbow and Kamloops trout, Salmo gairdneri, following muscular activity. Journal of the Fishery Research Board of Canada 17:487-500. 1960.

9. Black, Edgar C., Anne C. Robertson and Robert R. Parker. Some aspects of carbohydrate metabolism in fish. In: Comparative Physiology of Carbohydrate Metabolism in Heterothermic Animals, ed. by Arthur W. Martin. Seattle, University of Washington Press, 1961. p. 89-124.

10. Black, Edgar C. et al. Changes in glycogen, pyruvate and lactate in rainbow trout (Salmo gairdneri) during and following muscular activity. Journal of the Fishery Research Board of Canada 19: 409-436. 1962.

11. Blazka, P. The anaerobic metabolism of fish. Physiological Zoology 31:117-128. 1958.

12. Burdick, G. E. et al. Lethal oxygen concentrations for trout and small-mouth bass. New York Fish and Game Journal 1:84-97. 1954.

13. Chapman, W. N. Effects of a decreased oxygen supply on sockeye and chinook salmon. Transactions of the American Fishery Society 69:197-204. 1939.

14. Cordier, D. and A. Duveau. Action de l'anoxie et de l' asphyxie sur la phosphorolyse du glycogène rousculaire chez la grenoiulle (Rana esculenta). Comptes Rendus De Société Biologique (Paris) 152:84-87. 1958.

15. Cordier, G. Recherches sur le taux du glycogène cardiaque chez le poissons d'eau douce. Comparison avec les poissons marins. Comptes Rendus De Société Biologique (Paris) 153: 435-437. 1959.

16. Dill, D. B. A chemical study of the California sardine Sardinia caerulea. Journal of Biological Chemistry 48:93-103. 1921.

17. Doudoroff, Peter. Water quality requirements of fishes and effects of toxic substances. In: The Physiology of Fishes, vol. 1. Margaret E. Brown (ed) New York, Academic Press, 1957. p. 403-430.

18. Downing, Kathleen M. Influence of dissolved oxygen concentration on the toxicity of KCN to rainbow trout. Journal of Experimental Biology 31:161-164. 1954.

19. Drury, Douglas R. and Arne N. Wick. Metabolism of lactic acid in the intact rabbit. American Journal of Physiology 184: 304-308. 1956.

20. Fontaine, M. and J. Hatey. Contribution à l'etude du métabolisme glucidique du saumon Salmo salar L. à diverses étapes de son développement et de ses migrations. Physiologia Comparata et Oecologia 3:37-52. 1953.

21. Fry, F. E. J. Effects of the environment on animal activity. Toronto, 1947. 62 p. (Publication of the Ontario Fisheries Research Laboratory No. 68)

22. Fry, F. E. J., V. S. Black and E. C. Black. Influence of temperature on the asphyxiation of young goldfish (Carassius auratus L.) under various tensions of oxygen and carbon dioxide. Biological Bulletin 92:217-224. 1947.

23. Fry, F. E. J. The aquatic respiration of fish. In: The physiology of fishes, vol. 1. Margaret E. Brown (ed.) New York, Academic Press, 1957. p. 1-69.

24. Gardner, J. A. and C. Leetham. On the respiratory exchange in fresh-water fish. II. On brown trout. Biochemical Journal 8:591-597. 1914.

25. Garey, W. F. Cardiac responses of fishes in asphyxial environments. Biological Bulletin 122:362-368. 1962.

26. Gibson, E. S. and F. E. J. Fry. The performance of the lake trout, Salvelinus namaycush, at various levels of temperature and oxygen pressure. Canadian Journal of Zoology 32:252-260. 1954.

27. Graham, J. M. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, Salvelinus fontinalis. Canadian Journal of Research D27: 270-288. 1949.

28. Greene, Charles W. Carbohydrate content of king salmon tissues during the spawning migration. Journal of Biological Chemistry 48:429-436. 1921.

29. Hall, F. G., I. E. Gray and S. Lepkovsky. The influence of asphyxiation on the blood constituents of marine fishes. Journal of Biological Chemistry 67:549-554. 1926.

30. Hall, F. G. and I. E. Gray. The hemoglobin concentration of the blood of marine fishes. Journal of Biological Chemistry 81:589-594. 1929.

31. Hall, F. G. The influence of varying oxygen tension upon the rate of oxygen consumption in marine fishes. American Journal of Physiology 88:212-218. 1929.

32. _____. The ability of the common mackerel and certain other marine fishes to remove dissolved oxygen from sea water. American Journal of Physiology 93:417-421. 1930.

33. Heath, Alan G. and Austin W. Pritchard. Changes in the metabolic rate and blood lactic acid of bluegill sunfish, Lepomis macrochirus, Raf. following severe muscular activity. Physiological Zoology 35:323-329. 1962.

34. Heifetz, Harold. Storage of glycogen in fish muscle. Progressive Fish Culturist 55:33-34. 1941.

35. Hoar, W. S. Diurnal variation in feeding activity of young salmon and trout. Journal of the Fishery Research Board of Canada 6:90-101. 1942.

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

37. Hochachka, P. W. and A. C. Sinclair. Glycogen stores in trout tissues before and after stream planting. Journal of the Fishery Research Board of Canada 19:127-136. 1962.

38. Huckabee, W. E. Relationships of pyruvate and lactate during anaerobic metabolism. I. Effects of infusion of pyruvate or glucose and of hyperventilation. Journal of Clinical Investigation 37:244-254. 1958.

39. _____. Relationships of pyruvates and lactates during anaerobic metabolism. II. Exercise and formation of oxygen debt. Journal of Clinical Investigation 37:255-263. 1958.

40. _____. Relationships of pyruvate and lactate during anaerobic metabolism. III. Effect of breathing low-oxygen gases. Journal of Clinical Investigation 37:244-254. 1958.

41. Hughes, G. M. and G. Shelton. Respiratory mechanisms and their nervous control in fish. Advances in Comparative Physiology and Biochemistry 1:275-364. 1962.

42. Jahoda, W. J. Survival of brook trout in water of low oxygen content. Journal of Wildlife Management 11:96-97. 1947.

43. Johlin, J. M. and F. B. Morland. Studies on the blood picture of the turtle after complete anoxia. Journal of Biological Chemistry 103:107-114. 1933.

44. Kiermeir, Anna. Uber den Blutzucker der Süsswasserfische. Zeitschrift für Vergleichende Physiologie 27:460-491. 1939.

45. Kilborn, L. G. and J. J. R. Macleod. Observations on the glycogen content of certain invertebrates and fishes. Quarterly Journal of Experimental Physiology 12:317-330. 1919.

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

47. Margaria, R., H. T. Edwards and D. B. Dill. The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. American Journal of Physiology 106:689-715. 1933.

48. McCormick, N. A. and J. J. R. Macleod. The effect on the blood sugar of fish of various conditions including the removal of the principal islets. Proceedings of the Royal Society of London, Series B 98:1-29. 1925.

49. Meyer, Helga. Die Atmung von Uranoscopus scaber in ihrer Abhändigkeit von Sauerstoffdruck, von pH and von der Temperatur in Aussenmedium. Zeitschrift für Vergleichende Physiologie 22:435-449. 1935.

50. Middlesworth, L. Van, R. F. Kline and S. W. Britton. Carbohydrate regulation under severe anoxic conditions. American Journal of Physiology 140:474-482. 1943.

51. Miller, R. B., A. C. Sinclair and P. W. Hochachka. Diet, glycogen reserves and resistance to fatigue in hatchery rainbow trout. Journal of the Fishery Research Board of Canada 16:321-328. 1959.

52. Montgomery, Rex. The determination of glycogen. Archives of Biochemistry and Biophysics 67:378-386. 1957.

53. Moore, W. G. Field studies on the oxygen requirements of certain freshwater fishes. Ecology 23:319-329. 1942.

54. Odum, Howard T. and David K. Caldwell. Fish respiration in the natural gradient of an anaerobic spring in Florida. Copeia, 1955, p. 104-106.

55. Paton, D. N. Observation on the amount of dissolved oxygen in water required by young Salmonidae. Proceedings of the Royal Society of Edinburgh 24:145-150. 1903.

56. Pearse, A. S. and H. Actenberg. Habits of yellow perch in Wisconsin lakes. Bulletin of the U. S. Bureau of Fisheries 36:295-366. 1920.

57. Philips, M. M. The effects of asphyxia upon the red cell content of trout blood. Copeia, 1947, p. 183-186.

58. Prosser, C. Ladd et al. Acclimation of goldfish to low concentrations of oxygen. Physiological Zoology 30:137-141. 1957.

59. Robinson, Sid. Physiology of muscular exercise. In: Philip Bard's Medical Physiology, 11th ed. St. Louis, Mosby, 1961. p. 494-525.

60. Ruttner, Franz. Fundamentals of limnology. Toronto, University of Toronto Press, 1953. 242 p.

61. Saunders, Richard L. The irrigation of the gills in fishes. II. Efficiency of oxygen uptake in relation to respiratory flow, activity and concentrations of oxygen and carbon dioxide. Canadian Journal of Zoology 40:817-862. 1962.

62. Scholander, P. F., Edda Bradstreet and W. F. Garey. Lactic acid response in the grunion. Comparative Biochemistry and Physiology 6:201-203. 1962.

63. Shepard, M. P. Resistance and tolerance of young speckled trout (Salvelinus fontinalis) to oxygen lack, with special reference to low oxygen acclimation. Journal of the Fishery Research Board of Canada 12:387-446. 1955.

64. Simpson, W. W. The effects of asphyxia and isletectomy on the blood sugar of Myxocephalus and Ameiurus. American Journal of Physiology 77:409-418. 1926.

65. Spencer, W. P. Diurnal activity rhythms in freshwater fishes. Ohio Journal of Science 39:119-132. 1939.

66. Van Liere, E. Anoxia, its effect on the body. Chicago, University of Chicago Press, 1942. 269 p.

67. Vorhauer, Hans. Untersuchungen über den Blutzucker bie Karpfen (Cyprinidae). Biochemische Zeitschrift 296:90-98.

68. Westfall, B. A. Specific gravity of fish blood during rapidly developed anoxia. Journal of Cellular and Comparative Physiology 22:177-186. 1943.

69. White, Abraham et al. Principles of biochemistry. New York, McGraw-Hill, 1959. 1149 p.

70. Wiebe, A. A. Diurnal variations in the amount of dissolved oxygen, alkalinity and free NH_3 in certain fish ponds at Fairport, (Iowa). The Ohio Journal of Science 31:120-126. 1931.

APPENDIX

Table 1. Average rates of oxygen consumption at different times of day in bluegill sunfish (N = 4).

Time	O_2 Uptake (Ml/Kg/Hr)
10:15	39.8
11:45	40.6
1:45	35.1
3:15	38.3
4:45	46.6
6:15	41.4
8:45	45.3

Table 2. Average rates of oxygen consumption at different times of day in cutthroat trout (N = 5).

Time	O_2 Uptake Ml/Kg/Hr	Time	O_2 Uptake Ml/Kg/Hr
9:45	58.0	4:45	72.3
10:45	64.0	5:45	69.8
11:45	61.6	6:45	73.3
12:45	61.8	7:45	66.3
1:45	62.1	8:45	75.0
2:45	67.5	9:45	67.0
3:45	64.7	next morning	63.3

Table 3. Concentrations of muscle and liver glycogen in cutthroat trout taken directly from hatchery trough.

Muscle Glycogen (Mg/100 g)	Liver Glycogen (Mg/100 g)	Body Weight (Grams)
238	7600	61
348	9134	93
226	12298	63
406	7207	38
588	13129	56
649	4725	90
234	9595	84
445	11563	45
358	3595	56
154	5290	62
Average:		
365	8414	64.8