THE INFLUENCE OF CARBON DIOXIDE AND pH ON THE DISSOLVED OXYGEN REQUIREMENTS OF SOME FRESH-WATER FISH

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WILLIAM JOHN McNEIL

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APPROVED:

Redacted for privacy

Head of Department of Fish and Game Management

In Charge of Major

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

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INTRODUCTION

The results of experiments designed to evaluate the influence of free carbon dioxide and of pH or hydrogen ion concentration on the minimum dissolved oxygen requirements of fresh-water fishes are reported in this thesis. In addition, the relative importance of pH and free carbon dioxide as factors influencing the minimum dissolved oxygen requirements of coho salmon and their possible influence on the suitability of natural waters for fish are discussed.

A number of investigators have evaluated the dissolved oxygen requirements of fishes without attempting to establish the possible influence of pH and carbon dioxide variations. Since the pH and carbon dioxide content of fresh waters vary, an appraisal of their effect on the minimum dissolved oxygen requirements of fresh-water fishes is needed for the establishment of a sound, factual basis for pertinent water-quality criteria.

The dissolved oxygen of waters can become depleted as a result of pollution and natural causes. In most instances oxygen is removed from water through oxidative bacterial decomposition of organic matter and by respiring plants and animals. An increase of carbon dioxide and a consequent reduction of the pH value normally accompany this lowering of dissolved oxygen. Furthermore, the pH of natural waters can vary over a wide range due to variations of total alkalinity and acidity.

Results of previous studies with live fish and with fish blood *in vivo* and *in vitro* suggest that pH and carbon dioxide somewhat influence the oxygen requirements of fish. However, the available data on this subject are inconclusive and contradictory, since there is little agreement on the degree of influence or the specific factor responsible. Therefore, further study of the effects of pH variations and carbon dioxide upon the minimum oxygen requirements of fish is needed before reliable water quality criteria can be established for the maintenance of fish life in waters subject to pollution with organic wastes and to other oxygen-depleting influences.

Briefly, the problem was investigated by subjecting live fish to waters whose dissolved oxygen, carbon dioxide, and hydrogen-ion concentrations were artificially varied. By studying the test conditions which produced mortalities, data pertaining to the resistance of fresh-water fish to low dissolved oxygen concentration in the presence of both normal and abnormal pH and free carbon dioxide content were acquired.

A thorough understanding of the role of carbon dioxide and pH in the oxygen requirements of fish has also required a review of the respiratory functions of body fluids, since explanations of the effects of carbon dioxide and hydrogen-ions have often been based upon blood functions. Conversely, studies with live fish have played a part in testing the theories and postulates advanced by those who have worked with the respiratory functions of fish blood.

LITERATURE

Early workers found carbon dioxide to be harmful to fishes. Shelford and Allee (40, pp.262-264) reported that fish avoided carbon dioxide concentrations of 1.5 cc/l (about 3 mg/l) and thought that the free carbon dioxide content of waters was an index of suitability for fish. Wells (44, p.331) demonstrated that large quantities of carbon dioxide (near 200 mg/l) anesthetized active fish very rapidly.

Both Shelford and Wells described the effect of carbon dioxide as a narcotic action. A few years later Krogh and Lietch (24, p.299) subjected fish blood to various carbon dioxide tensions and found that tensions above normal reduced the ability of fish blood to take up oxygen.

More recently workers have undertaken additional studies on the influence of carbon dioxide and pH on live fish and on the respiratory function of fish blood.

Dissolved Oxygen Requirements in Relation to pH and Carbon Dioxide

Before considering the possible influence of free carbon dioxide and pH on the ability of fishes to tolerate low dissolved oxygen, the minimum dissolved oxygen requirements of certain fresh-water fish species are discussed along with some of the factors known to influence tolerance. No attempt is made to present a complete review of the literature on this subject, but several references pertaining mostly to cold-water species are included.

Dissolved Oxygen Requirements

Fresh-water fishes that are typically found in cold water are generally considered to demand higher environmental oxygen concentrations than the warm-water species. Among the investigators who have studied the minimum dissolved oxygen requirements of cold-water fishes, some, including Burdick et al. (7, pp.89-95), sealed fish in jars and measured the concentration of oxygen remaining after the fish had died of anoxia. Others like Gardner and Leetham (15, p.596), Townsend and Earnest (43, pp.348-350), and Davison (9, p.44) used apparatus having continuously flowing water to evaluate the minimum oxygen requirements of fish. Some of the results obtained by these investigators are shown in table I.

According to Shepard (41, pp.425-426) acclimatization of young brook trout to low dissolved oxygen enables them to increase their resistance to further reduction of the dissolved oxygen concentration. He found that their resistance increases slowly, and suggested that "deep-seated" physiological changes are involved. Shepard also showed that the size of brook trout affects the length of time that they can resist dissolved oxygen concentrations which are low enough to cause eventual mortality. Brook trout fry died sooner than fingerlings at lethal oxygen concentrations, but there was apparently no difference between the lowest dissolved oxygen concentrations which could be tolerated indefinitely by these fry and fingerlings.

Davison (9, p.14) tested the influence of temperature on the ability of juvenile salmon to tolerate low oxygen concentrations. He found that the minimum

TABLE I

CRITICAL MINIMUM DISSOLVED OXYGEN CONCENTRATIONS OF SOME FRESH-WATER FISHES

Species	Temperature	Critical Dissolved Oxygen Concentration	Length of Experiments	Investigator
Brook trout	13°C.	1.59 mg/1	2 hrs.	Burdick, _et_ _al_. (1954)
Brook trout	21	2.54	2 hrs.	Burdick, _et_ _al_. (1954)
Brown trout	9	1.42	3 hrs.	Burdick, _et_ _al_. (1954)
Brown trout	21	2.53	3 hrs.	Burdick, _et_ _al_. (1954)
Brown trout	10	0.94		Gardner and Leetham (1914)
Brown trout	25	3.48		Gardner and Leetham (1914)
Rainbow trout	11	1.10	4 hrs.	Burdick, _et_ _al_. (1954)
Rainbow trout	22	1.82	4 hrs.	Burdick, _et_ _al_. (1954)
Coho salmon	11	1.50		Townsend and Earnest (1940)
Coho salmon	18	1.30	5 days	Davison (1954)
Coho salmon	18	2.00	30 days	Davison (1954)
Smallmouth bass	27	1.15	8 hrs.	Burdick, _et_ _al_. (1954)

dissolved oxygen content tolerated by salmon was unaffected by increasing the temperature from 12° C. to 16° C. When the temperature was raised from 18° C. to 22° C., the salmon increased their minimum dissolved oxygen tolerance from about 1.4 mg/l to about 1.7 mg/l.

Tolerance to Low pH

It has been shown that fish have a wide pH tolerance. For instance Creaser (8, pp.246-262) has presented evidence that brook trout are unaffected in their voluntary selection of habitat within a pH range of 4.1 to 9.5. From their extensive review of the literature on the toxicity of industrial wastes, Doudoroff and Katz (10, pp.1451-1452) concluded that pH values between 5.0 and 9.0 are not lethal to most fully developed fresh-water fishes. They also state that resistant species can tolerate much more extreme pH values indefinitely.

Tolerance to High Free Carbon Dioxide

Wells was among the first to conduct studies for the purpose of determining the possible influence of free carbon dioxide on fish. He stated in one of his papers (45, p.557) that carbon dioxide proved quickly fatal to more sensitive fishes at concentrations of 10 cc/l (approximately 20 mg/l). He added that it was doubtful if there were fresh-water fishes that could survive an average yearly carbon dioxide concentration of 6 cc/l (about 12 mg/l). Wells, however, failed to support these assertions with experimental data, and other workers have since contradicted his early statements. Doudoroff and Katz (10, pp.1452-1453) concluded from their review of the literature that carbon dioxide concentrations between 100 and 200 mg/l may be lethal to sensitive species when much dissolved oxygen is present. They also say, "...lower concentrations (100 to 50 p.p.m., or less) may be lethal after prolonged exposure, or may interfere with the utilization of oxygen at low tensions and with normal development."

Several workers have described a narcotic action of free carbon dioxide, e.g. Shelford and Allee (40, pp.262-264) and Wells (44, p.331). Fish (13, p.27) found that 200 mg/l of carbon dioxide anesthetized king salmon and steelhead trout in five minutes.

Influence of pH and Carbon Dioxide on Oxygen Requirements

The effects of pH and free carbon dioxide on the dissolved oxygen requirements of salt water fish have been studied by Hall (18, pp.463-464) and Powers (25, p.315). Hall detected a reduction in the rate of oxygen consumption at lowered pH values, but he also found that the effects from carbon dioxide are more pronounced than those from an inorganic acid at the same pH. Powers added carbon dioxide to the water to test the effect of low pH on herring, mackerel, and other marine species. The oxygen requirements of Power's fish increased noticeably at pH 6.8; however, it was not established whether the alteration of pH or of carbon dioxide tension was responsible for the change.

Several investigators have indicated that pH has some influence on the oxygen requirements of fresh-water fish. For instance, Shelford (39, p.383) showed that reduction of pH from 8.8 to 6.4 shortened survival time of bluegills in water with a low dissolved oxygen content. Wiebe *et al*. (47, pp.442-447) found the optimum pH range for toleration of low dissolved oxygen by bluegills to be from pH 7.0 to pH 8.5; whereas, pH 5.2 to pH 8.5 made little difference in the lethal oxygen concentration for rainbow trout. These authors also claimed that at pH 6.0 7.5 mg/l of dissolved oxygen was the least tolerable concentration for bluegills.

Wiebe reported that the ability of fish to extract oxygen at low pressure can be greatly influenced by pH. However, it appears that neither Wiebe nor Shelford considered the possible influence of carbon dioxide which presumably could have been present in large concentrations under the reported test conditions. It appears that in the experiments reported by Shelford pH was lowered by adding a mineral acid to a rather hard water, a method which would have liberated considerable free carbon dioxide. It is not at all clear in what way Wiebe adjusted the pH of the test medium. In either case it appears that carbon dioxide could have been an important variable which apparently was neglected, and it is likely that increased carbon dioxide concentration, rather than reduced pH, may have been responsible for the observed effects of acidification of the water.

Townsend and Cheyne (42, pp.462-465), who worked with coho salmon, noted that, near the limit of low dissolved oxygen tolerance, increases in the hydrogen-ion concentration produced the same effect as lowering the oxygen. They claimed that alterations in the pH by either mineral acids or carbon dioxide gave rise to the same results for a given pH, and they thought that the effects of pH were not due to changes in the carbon dioxide equilibrium. However, these conclusions were based on few observations and the assumption that the carbon dioxide concentration of the water acidified with mineral acid was very much lower than that of the same water acidified to the same pH with carbon dioxide. Inasmuch as neither the total alkalinity values nor the free carbon dioxide concentrations of the solutions under comparison were reported, the significance of the results of the comparative tests is somewhat questionable. Although the bicarbonate content of the water was said to be small, it appears that the difference in free carbon dioxide concentrations between the waters differently acidified to the same pH may not have been as great as the authors surmised.

Several studies have been undertaken to evaluate the effect of carbon dioxide on the oxygen requirements of fish. Fry and Black (14, p.47) have noted that the ability of fish to utilize dissolved oxygen in the presence of free carbon dioxide varies from species to species. Gutsell (17, p.92) found that carbon dioxide concentrations up to 28 mg/l had no noticeable effect on minimum dissolved oxygen requirements of trout, and his investigations further indicated that waters with concentrations up to 39 mg/l remained suitable for trout provided the dissolved oxygen did not drop below 2 mg/l. Also, Wilding (48, pp.255-262), working with yellow perch and two cyprinids, decided that a carbon dioxide concentration of 13 mg/l had no effect on the asphyxial oxygen concentrations.

Hart (19, p.226) used sealed glass containers to determine the carbon dioxide tension necessary for the asphyxiation of several fresh-water species. He experimentally determined the carbon dioxide tension causing suffocation after a fish had removed one-half of the oxygen from water which was saturated with dissolved oxygen at the beginning of the experiment. Among the fish tested by Hart the gizzard shad was the most sensitive to carbon dioxide, succumbing at a tension of 35 mm. Hg (88 mg/l at the test temperature of 16° C.). Bluegills and largemouth bass reduced the oxygen to one-half of saturation at carbon dioxide tensions of 60 mm. Hg (150 mg/l at the test temperature of 16° C.) and 70 mm. Hg (175 mg/l at the approximate test temperature of 16° C.), respectively. Black, Fry, and Black (4, p.410) also subjected fish in sealed glass containers to known quantities of carbon dioxide. It was found that brook trout, which were among the least resistant species, succumbed at a carbon dioxide tension of 132 mm. Hg (306 mg/l at the approximate test temperature of 18.5° C.) in water saturated with atmospheric oxygen and at about 100 mm. Hg (232 mg/l at the approximate test temperature of 18.5° C.) in water one-half saturated with atmospheric oxygen. These results agree somewhat with the observations of King (23, p.633) who stated that brook, brown, and rainbow trout are not influenced by carbon dioxide concentrations of 200 mg/l. But Doudoroff and

Katz (10, p.1446) have questioned the validity of King's results, noting that serious error may have occurred in the analysis of free carbon dioxide since there was lack of agreement with other available data, particularly data having to do with the resistance to carbon dioxide of rainbow or steelhead trout.

It is apparent from the foregoing summary that the published evidence that minimum dissolved oxygen requirements of fishes can be markedly influenced by pH and/or free carbon dioxide is varied, contradictory, and often inconclusive. Some authors have reported that lowered pH values decrease the tolerance of fresh-water fish to low dissolved oxygen. But there is a possibility that, in interpreting their test results, these authors may have attributed this phenomenon to the wrong factor, i.e. free carbon dioxide may have been chiefly responsible, rather than pH.

There is rather conclusive evidence that free carbon dioxide can have a specific effect (apart from reduced pH) on the minimum dissolved oxygen tolerance of fish. But again it is difficult to establish the levels at which this effect becomes important, and there is insufficient evidence that it is often important practically.

Effects of pH and Carbon Dioxide on Fish Blood *in vivo*

Changes in carbon dioxide and oxygen tensions of the water influence physiological processes according to Powers (27, p.362). Fry and Black (14, p.47) echoed this belief, for they surmised that the ability of fish to utilize oxygen in the presence of carbon dioxide was related to the ability of the blood to hold oxygen in the presence of carbon dioxide.

Powers (26, p.382), Powers and Logan (30, p.344), Powers, Hopkins, and Hickman (29, p.466) and Powers, *et al*. (31, pp.243-244) reported on the ability of fish to adapt to varying environmental carbon dioxide levels through modification of the blood, and several general conclusions resulted. Some of their conclusions were:

1. The blood pH remained fairly constant over a wide range of carbon dioxide concentration.

2. Fish were unable to control the carbon dioxide tension of their blood when subjected to altered environmental carbon dioxide concentration.

3. The alkali reserve of the blood increased to maintain a constant "acidity" (the authors presumably meaning hydrogen-ion concentration) when fish were subjected to abnormally high environmental carbon dioxide concentrations.

There has been, however, disagreement among investigators on the last point. Jobes and Jewell (22, p.182) having statistically analyzed some of Powers' data, found no correlation among the environmental carbon dioxide and pH and the blood alkali reserve.

Another controversial hypothesis advanced by Powers (28, p.280) but contradicted by Baker (3, p.47) is that sudden mortality caused by abnormally high carbon dioxide concentrations can be due to "derangement" rather than to asphyxiation. According to Powers, fish which alternately come to the surface subject themselves to rapid changes in the carbon dioxide concentration, since the surface water contains less of the dissolved gas than the depths. When this occurs, the fish become "deranged" in compensating for rapid changes in carbon dioxide tension by readjustment of the blood alkali reserve.

Results of investigations by Jobes and Jewell (22, p.185) and Powers, Hopkins, and Hickman (29, p.466) disclosed that wide variations in pH of the environment caused little or no change in the blood pH. This was generally true whether the pH was altered by mineral acids or by carbon dioxide.

<u>Effects of pH and Carbon Dioxide on Fish Blood in vitro</u>

Some of the more specific effects of carbon dioxide and pH on fish blood have been studied through experimentation with the blood of several fresh-water and marine fishes *in vitro*. Krogh and Leitch (24, p.299) observed that the blood of several marine and fresh-water species was extremely sensitive to small increases in carbon dioxide. A rapid loss of affinity for oxygen resulted in the presence of carbon dioxide, due to what is known as the Bohr effect. Black and Irving (5, p.356) detected incomplete oxygen saturation of sucker blood at high oxygen pressures when the carbon dioxide tension was as low as 5 mm. Hg. Working with trout, Irving, Black, and Safford (20, p.16) revealed that at 15° C. the blood failed to take up more than one-half of its normal oxygen capacity in the presence of 60 mm. Hg of carbon dioxide. Since the oxygen tension equal to one-half saturation has been considered by physiologists to be the minimum compatible with the exchange of oxygen to the tissues, trout whose blood has been affected to this extent theoretically should die of suffocation.

Root (32, pp.427-456), Root, Black, and Irving (33, p.47 and 37, pp.303-313), and Root and Irving (34, pp.85-96 and 35, pp.307-323) investigated the effect of carbon dioxide on the blood of three marine species (toadfish, sea robin, and tautog). Their findings showed that the higher concentrations of carbon dioxide impaired the oxygen-combining ability of the blood, and they concluded that carbon dioxide had two effects. At low concentrations it exerted an exaggerated Bohr effect on the blood, while at higher concentrations it caused inactivation of some of the blood's oxygen-binding groups.

Other studies with fish blood _in vitro_ have shown that carbon dioxide changes the blood pH. Ferguson and Black (12, p.150) reported some figures on the changes of pH brought about by carbon dioxide in rainbow trout venous blood. At carbon dioxide tensions of 2, 20, and 60 mm. Hg the respective plasma pH values were 7.66, 7.15, and 6.84.

In another paper, Black and Irving (6, p.259) were able to show that lactic acid reduced the oxygen-combining power of sucker blood. Also, in this connection, Root (32, p.441), Green and Root (16, p.386), and Root and Irving (36, pp.209-211) have disclosed that lactic acid affected the oxygen-combining power of the blood of several marine fish much in the same way as did carbon dioxide. They suggested that carbon dioxide and the lactate and bicarbonate ions had no special influence beyond their effect upon the blood's hydrogen-ion concentration. Assuming this to be true, they concluded that the physiological effects of carbon dioxide are due, then, to changes in blood pH.

METHODS

The effects of carbon dioxide and pH on the oxygen requirements of fish were evaluated in both standing and running-water experiments. The two approaches enabled a more thorough investigation into the problem and facilitated the acquisition of corroborative data.

All experimental work was performed at the Oregon State College Fisheries Research Laboratory which is located at Corvallis, Oregon, and is operated by the Department of Fish and Game Management in co-operation with the United States Public Health Service.

Test Animals

Three species of fish were employed. Coho salmon, *Oncorhynchus kisutch* (Walbaum), were used for most of the experimental work, but the steelhead trout, *Salmo gairdneri* Richardson, and the bluegill, *Lepomis macrochirus* (Cuvier and Valenciennes), also were used.

Streams of the Yaquina River drainage supplied all of the juvenile salmon for running-water experiments. It was necessary, however, to obtain salmon from the Oregon State Game Commission's Alsea River hatchery for standing-water experiments. The steelhead trout were furnished by the Marion Forks Hatchery of the Fish Commission of Oregon, and the bluegills were seined from small ponds of the Willamette River flood plain.

Test fish were kept in 250-gallon wooden tanks during their confinement at the laboratory. The tanks were equipped for constant exchange flows of water pumped from Marys River.

The diet of confined fish consisted of a mixture of ground beef liver and marine bottom fish plus dried meal supplements. In summer, when water temperatures were high, the fish were fed daily, whereas in winter the frequency of feeding was reduced to every second or third day.

Running-water Experiments

An apparatus employing a constant exchange flow of water (see figure 1) was used for one series of experiments. The constant water exchange made it possible to subject fish to fairly uniform oxygen and carbon dioxide concentrations and pH for prolonged periods. As a consequence, the effect of carbon dioxide content and pH on the minimum constant oxygen concentrations tolerated by fish for 24 hours could be evaluated after gradual acclimatization of the test fish to the test conditions.

The apparatus used for running-water experiments consisted of several components. There was a water heating jar with a thermostatically controlled immersion heater; there was a chemical pump for introducing bicarbonate solution into the water; there were gas exchange columns which were connected to gas cylinders having two-stage regulators for controlling the amount of nitrogen and carbon dioxide gases introduced into the water; and there were test vessels with attendant water sample bottles.

Four parallel systems, each of which functioned independently of the others, were used. For simplicity only one of the four will be described in detail, since differences among them were slight.

Incoming filtered river water from Marys River first entered a constantly overflowing jar which assured an adequate supply of water for the remainder of the system. The incoming water was vigorously aerated with dispersed compressed air to reduce diurnal fluctuations in its content of dissolved oxygen and carbon dioxide. Water was siphoned from the overflow jar into a heating jar, where a controlled, constant temperature was maintained. After

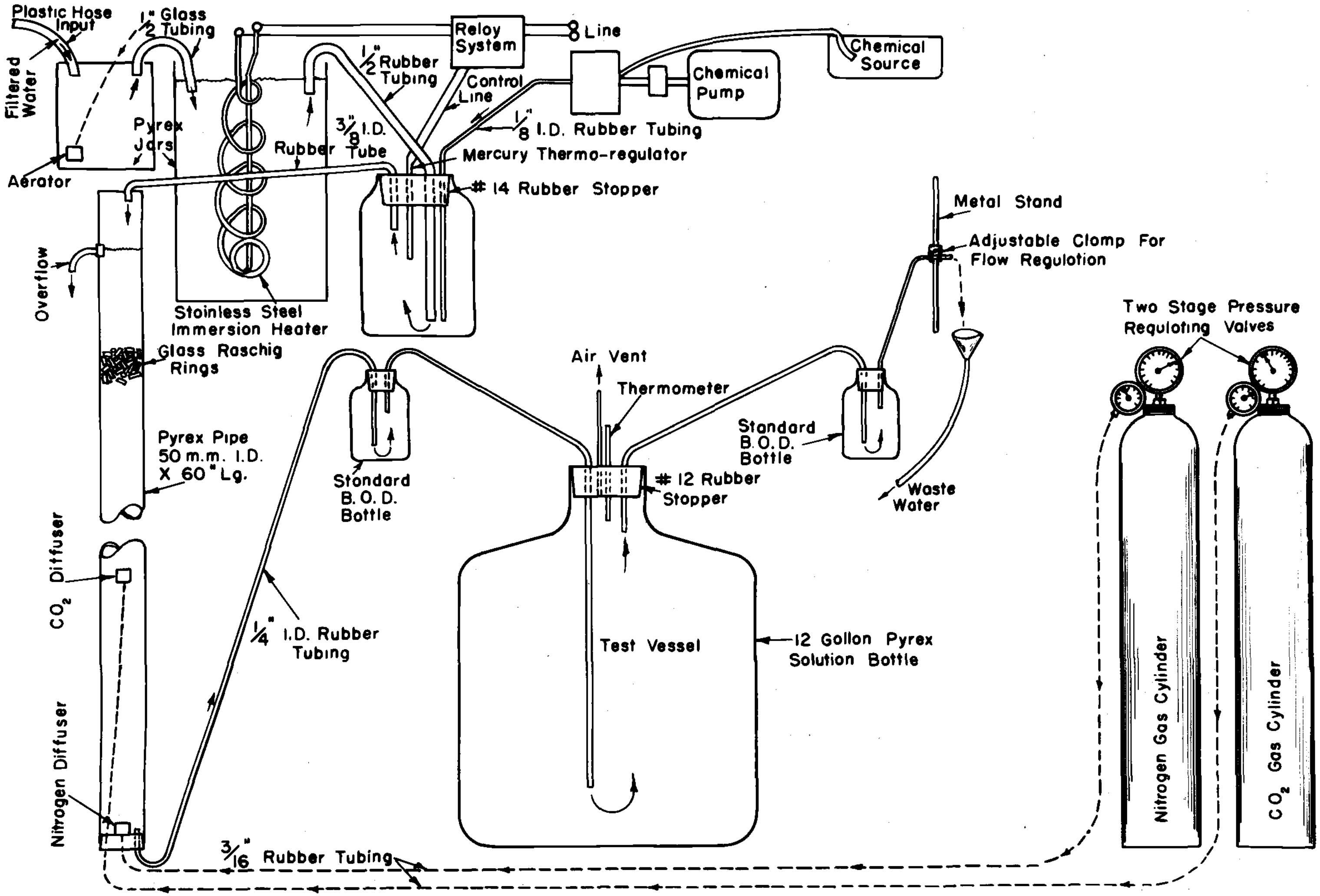

Figure 1. Apparatus for running-water experiments. One of four parallel systems is shown.

leaving the heating jar, water flowed through a small mixing jar while being siphoned into a glass column.

The bicarbonate alkalinity of the river water was increased by pumping a solution of sodium bicarbonate into the mixing jar, and the nitrogen and carbon dioxide gases were dissolved in the water in the glass column. Nitrogen gas was bubbled through the length of the column, and the carbon dioxide gas was bubbled into the water about midway up the column. The dissolved oxygen content of the water was reduced by replacing oxygen with dissolved nitrogen and carbon dioxide. By controlling the amounts of nitrogen and carbon dioxide gases introduced into the column from cylinders by means of two-stage pressure regulators, it was possible to regulate the concentration of dissolved oxygen and carbon dioxide remaining in the water after it had flowed through the column.

The inflow was maintained at a rate slightly in excess of the outflow. The excess water was siphoned away through an overflow siphon located near the top of the column, maintaining a constant head. This made it possible accurately to control the rate of flow through the test vessel.

Upon leaving the column the test water flowed in succession through a sample bottle, a 12-gallon test vessel, a second sample bottle, and an outlet tube. The sample bottles were located to permit the taking of samples for analysis of water entering and leaving the test container. The outlet was arranged to permit the regulation of flow by altering the gravitational head.

Ten fingerling trout or salmon were placed in the test vessel prior to any alteration of water quality. The fish were acclimatized to a test temperature different from their storage temperature for a minimum of three days before being used. Careful attention was given to the selection of healthy, uniform fish for each test.

The oxygen, carbon dioxide, and hydrogen-ion concentrations and the temperature were controlled in each experiment. The oxygen was displaced principally by nitrogen; the carbon dioxide was directly added; and the pH was adjusted by introducing a solution of sodium bicarbonate. Consequently, it was possible to evaluate the independent or joint influence of any of the four variables (dissolved oxygen, carbon dioxide, pH, and temperature) on the survival of fish.

After the selection of test conditions and the start of an experiment, every effort was made to maintain a constancy of the test conditions, irrespective of the mortalities occurring. In other words, no attempt was made to change the dissolved oxygen, carbon dioxide, or hydrogen-ion concentration during the course of a test because the fish were not dying or dying too fast.

In each test the fish had considerable opportunity to acclimatize themselves to changes in water chemistry. The period required to effect a complete change in dissolved oxygen, carbon dioxide, and hydrogen-ion concentration depended upon three factors. These were the size of the test container, the rate of water exchange within the test vessel, and the rate at which the chemicals were added to the test water.

The exchange flow was maintained at 400 ml. per minute in each test container. Thus, after the dissolved oxygen, carbon dioxide, and hydrogen-ion concentration of the inflowing water had been fully adjusted, an additional period of six hours was required to obtain similar conditions within the 12-gallon test vessel. Therefore, the test fish were provided a six-hour period in which they could acclimatize themselves to gradual changes in water chemistry.

The respiring fish caused slight reductions of the dissolved oxygen concentration and the pH and slight increases of the carbon dioxide content of the inflowing test water. All reported concentrations were based on the analysis of samples taken from outflowing water and are believed to be representative of conditions existing within the test container. Frequent adjustments were made in the chemical quality of inflowing test water to compensate for slight decreases in the carbon dioxide content and slight increases in the pH and dissolved oxygen concentration of outflowing water due to the death of fish. It was also necessary to vary the quantity of gases introduced into the test water to compensate for diurnal fluctuations of the quality of river water.

An attempt was made to select dissolved oxygen concentrations which would result in about 50 percent mortality within 24 hours of exposure to the test conditions. In practice, however, mortalities varied from zero to 100 percent, since it was not always possible to predict the conditions that would cause exactly one-half of the fish to die.

At first it was decided that a 48-hour test period should be used for the running-water experiments, but in preliminary tests most if not all of the 48-hour mortality occurred during the initial 24 hours. In addition, the gathering of data became more efficient with the shorter exposure time, so for these reasons a 24-hour test period was selected as the most suitable.

Standing-water Experiments

Standing-water experiments differed from the running-water experiments in two important respects. Firstly, a constant exchange flow was lacking. Secondly, the respiring fish caused progressive reductions in the dissolved oxygen concentration and pH with a corresponding increase in the carbon dioxide content throughout the course of each experiment.

Five-gallon, wide-mouth, Pyrex glass bottles were used as test containers for the standing-water experiments. These were filled with water of the proper temperature which was altered in chemical quality before the introduction of fish. After the fish had been introduced, the bottles were sealed with rubber stoppers.

Water samples were withdrawn by means of a siphon tube inserted through the stopper. The withdrawn water was immediately replaced, and little reaeration occurred from contact of the water surface with the atmosphere. The size of water samples was kept to a minimum to prevent undue alterations in the quality of the test water by the replacement portions.

All standing-water tests were conducted in a constant-temperature room where the ambient temperature was maintained at 20 $\pm$ 1° C. Test fish were acclimatized to the experimental temperature for not less than three days before being used.

The dissolved oxygen was slowly reduced to a critical level by the respiring fish, provided total mortality was not instantaneous. At the beginning of most experiments the dissolved oxygen concentration varied from 6 to 9 mg/l. However, in some cases the initial dissolved oxygen content was reduced to 3 mg/l by bubbling nitrogen gas through the test water.

The first series of experiments tested the tolerance of salmon to high carbon dioxide concentrations in water having an initial dissolved oxygen content near 6 mg/l. Since fish were transferred directly from river water to the test vessels, they had no opportunity to acclimatize themselves to the test carbon dioxide concentrations. Also, no attempt was made to control pH.

A similar method was used in a second series of experiments to determine the maximum carbon dioxide concentration salmon could tolerate when the initial dissolved oxygen content was reduced to 3 mg/l. As before, the pH was uncontrolled.

The third series of experiments was designed to establish whether or not alkalinity influenced the minimum dissolved oxygen requirements of salmon which were suddenly exposed to high concentrations of carbon dioxide.

Three bottles were used in each test. In the first bottle, the alkalinity present in the river water was increased by adding sodium bicarbonate; in the second bottle, the alkalinity was reduced by adding sulfuric acid and aerating the water in order to remove liberated carbon dioxide; and in the third bottle, the alkalinity was left unaltered. Carbon dioxide was then added, and the free carbon dioxide content was adjusted to the same value in all three bottles. Following the addition of carbon dioxide, five salmon were placed in each bottle, and the concentration of dissolved oxygen remaining in the test water at the time of death of each fish was determined.

A fourth series of experiments dealt with the influence of pH alone at low carbon dioxide concentrations. Here sulfuric acid was added to the test water in varying proportions to create differences in the hydrogen-ion concentration. The test water was thoroughly aerated after introducing the acid to drive off any carbon dioxide that was liberated from the bicarbonate present in the water.

In sealed-bottle experiments the only meaningful observations were the dissolved oxygen, carbon dioxide, and hydrogen-ion concentrations of the water at the time a fish showed distress, turned over, or expired. In reporting all experiments, the concentrations of dissolved gases and the pH at the time of death are reported.

The number of fish used per bottle varied among the different experiments. For 2 series of tests 3 coho salmon were placed in each vessel and the pH, carbon dioxide content, and dissolved oxygen concentration existing after all had died were recorded as an observation. In one test, each bottle contained two bluegills, and the pH and dissolved oxygen content remaining after both had succumbed were recorded. However, in other experiments five salmon were placed in each bottle, and the pH, the dissolved oxygen concentration and, when necessary, the carbon dioxide content existing at the time of death of each individual were recorded. In this way it was possible to obtain five observations per jar.

The length of survival time varied with test conditions, including the number of fish employed. In some instances mortality occurred within minutes, while in others up to 16 hours elapsed before the fish removed enough oxygen to cause suffocation.

Water Analysis

Methods of water analysis prescribed by the American Public Health Association and the American Water Works Association were used. All of the methods in question were taken from the ninth and tenth editions (1949 and

1955 respectively) of Standard Methods for the Examination of Water and Sewage (1 and 2).

The dissolved oxygen was analyzed by the Alsterberg (azide) modification of the Winkler method. Solutions of 0.025 N sodium thiosulfate were prepared bi-weekly and standardized with 0.01 N potassium iodate.

A Beckman pH meter, Model N, was used for determining hydrogen-ion concentration. Alkalinity was evaluated by titrating with 0.02 N sulfuric acid to an electrometric end-point, as prescribed in the tenth edition of Standard Methods.

Carbonate alkalinity was found to be negligible. Therefore, the total and bicarbonate alkalinity are considered to be equal.

Whenever possible, carbon dioxide was calculated from a nomograph (Standard Methods, tenth edition) after first determining pH, bicarbonate alkalinity, temperature, and residue upon evaporation. In instances where the pH values were too low for calculation of carbon dioxide concentration from the nomograph, a water sample was titrated to a phenolphthalein end-point with N/44 sodium hydroxide.

RESULTS

The tested fish demonstrated that they could tolerate abnormally high carbon dioxide and hydrogen-ion concentrations. In most cases, carbon dioxide concentrations which are considered to be extreme in nature caused little increase in the minimum oxygen requirements. However, at certain higher carbon dioxide concentrations the ability of fish to tolerate low dissolved oxygen concentrations was considerably reduced, the magnitude of this effect apparently being influenced somewhat by acclimatization and by the pH or the total alkalinity of the water.

Running-water Experiments

Preliminary running-water experiments were run with coho salmon at 12.0° C. and with steelhead trout at 15.5° to 21.5° C. This early work was started in February, 1955, and terminated in June, 1955. Although the results of this work are inconclusive, the experimental data (tables II and III) indicate that carbon dioxide at concentrations of 55 mg/l or less caused little increase of the minimum dissolved oxygen requirements of steelhead trout and coho salmon.

Definitive experiments with wild coho salmon were initiated in June, 1955, and were concluded in November,

TABLE II

RESISTANCE OF STEELHEAD TROUT TO LOW DISSOLVED OXYGEN CONTENT AT VARIOUS CARBON DIOXIDE CONCENTRATIONS IN RUNNING WATER

Carbon dioxide (mg/l) Mean	Carbon dioxide (mg/l) Range	Dissolved Oxygen (mg/l) Mean	Dissolved Oxygen (mg/l) Range	pH Mean	pH Range	Mean total alkalinity as $CaCO_3$ (mg/l)	Mean Temp. (°C.)	Percent Mortality (of 10 fish)	Date (1955)
3	3	1.6	1.5-1.8	7.25	7.20-7.35	30	19.5	60	6/17
5	5	1.6	1.6-1.8	7.25	7.25	40	17.5	70	5/6
5	5	1.7	1.5-1.8	7.25	7.25	40	17.5	50	5/6
8	8	1.6	1.5-1.8	6.95	6.95	35	15.5	70	4/29
8	8	1.7	1.6-1.8	6.95	6.95	35	15.5	60	4/29
37	32-45	1.8	1.6-2.1	6.25	6.15-6.45	30	17.5	80	5/27
38	36-40	1.7	1.6-1.8	6.30	6.30-6.35	35	15.5	40	4/29
40	37-47	1.7	1.6-1.8	7.15	7.10-7.20	280	17.5	70	5/27
47	45-50	1.7	1.6-1.8	7.10	7.10-7.15	270	15.5	70	4/29
50	46-52	2.0	1.9-2.3	6.10	6.10-6.15	30	19.5	50	6/17
50	40-60	2.0	1.6-2.3	6.95	6.90-7.10	230	21.0	60	6/7
55	48-58	1.9	1.8-2.1	6.90	6.85-6.95	220	19.5	60	6/17

TABLE III

RESISTANCE OF COHO SALMON TO LOW DISSOLVED OXYGEN CONTENT AT VARIOUS CARBON DIOXIDE CONCENTRATIONS IN RUNNING WATER (PRELIMINARY EXPERIMENTS)

Carbon dioxide (mg/l)		Dissolved Oxygen (mg/l)		pH		Mean total alkalinity as $CaCO_3$ (mg/l)	Mean Temp. (°C.)	Percent Mortality (of 10 fish)	Date (1955)
Mean	Range	Mean	Range	Mean	Range				
6	6	1.5	1.4-1.9	7.10	7.10	32	12.0	50	4/1
6	6	1.7	1.5-2.0	7.10	7.10	32	12.0	20	4/1
6	6	1.6	1.5-1.7	7.05	7.05	30	12.0	40	3/22
45	38-50	1.8	1.6-1.9	6.20	6.15-6.30	30	12.0	60	3/22
55	40-58	1.7	1.7-1.9	6.15	6.10-6.25	32	12.0	70	4/1
55	50-58	1.7	1.5-1.9	7.00	6.95-7.05	240	12.0	40	3/22

1955. Temperatures ranging from 19.5° to 21.5° C. were maintained throughout these tests. The experimental data have been divided into three groups for the purpose of showing the minimum dissolved oxygen requirements of salmon in the presence of low carbon dioxide concentrations, in the presence of high carbon dioxide concentrations and low pH, and in the presence of high carbon dioxide concentrations and pH near neutrality.

The results of the definitive experiments are presented graphically in figures 2, 3, and 4. Figures 2 and 3 show the percentages of mortality within 24 hours at various combinations of dissolved oxygen and carbon dioxide concentrations in running-water of low and high alkalinity, respectively. Figure 4 illustrates the relation between dissolved oxygen and carbon dioxide concentrations in running-water in which 20 to 80 percent mortality of coho salmon occurred within 24 hours.

Tolerance of Low Dissolved Oxygen

In the control tests fish were subjected to lowered dissolved oxygen without alteration of the bicarbonate alkalinity or the carbon dioxide content of the river water. The bicarbonate alkalinity did not exceed 40 mg/l as $CaCO_3$, the pH values ranged from 7.15 to 7.40, and the carbon dioxide did not exceed 5 mg/l in these experiments.

The average dissolved oxygen concentrations tested and found to be fatal to some but not all of the test animals varied from 1.7 to 2.0 mg/l. Additional information from this series of tests is summarized in table I V, and the results are shown graphically in figures 2 and 4.

Tolerance of High Carbon Dioxide and Low pH

In these experiments the carbon dioxide content was increased and the bicarbonate alkalinity of the river water was unaltered. Since the alkalinity remained unaltered, reduced pH values resulted from the addition of carbon dioxide.

For all tests the bicarbonate alkalinity ranged from 30 to 80 mg/l as $CaCO_3$; the pH varied from 5.55 to 6.70; the carbon dioxide concentrations varied between 13 mg/l and 180 mg/l; and the dissolved oxygen content varied from 1.9 mg/l to 7.5 mg/l. The tests results, together with additional details, are summarized in table V. The results are also presented graphically in figures 2 and 4.

It can be seen from figure 4 that noticeable increases in the minimum dissolved oxygen requirements of coho salmon occurred at about 50 mg/l of carbon dioxide. When the carbon dioxide reached 125 mg/l, the minimum dissolved oxygen requirement was approximately doubled.

TABLE IV

RESISTANCE OF COHO SALMON TO LOW DISSOLVED OXYGEN CONTENT IN RUNNING WATER
(No carbon dioxide added to test water)

Carbon dioxide (mg/l)		Dissolved Oxygen (mg/l)		pH		Mean total alkalinity as $CaCO_3$ (mg/l)	Mean Temp. (°C.)	Percent Mortality (of 10 fish)	Date (1955)
Mean	Range	Mean	Range	Mean	Range				
3	2-3	1.7	1.6-1.9	7.40	7.35-7.45	33	21.5	90	7/18
3	3-4	1.8	1.8-2.1	7.25	7.20-7.35	30	20.0	60	7/5
3	3-4	1.8	1.7-2.1	7.40	7.20-7.45	35	21.5	80	7/12
3	3-4	1.9	1.8-2.1	7.30	7.20-7.40	32	20.5	20	7/16
3	2-4	2.0	1.9-2.3	7.25	7.20-7.35	30	20.0	0	7/5
4	3-4	1.7	1.5-1.9	7.30	7.20-7.50	40	20.5	80	7/9
4	3-4	1.8	1.7-2.0	7.30	7.20-7.50	40	20.5	80	7/9
4	3-4	1.9	1.8-2.1	7.20	7.20-7.25	30	19.5	10	6/30
4	4-5	1.9	1.9-2.1	7.20	7.05-7.30	40	21.0	10	7/30
4	4	1.9	1.7-2.2	7.20	7.15-7.20	30	19.5	30	7/2
4	4	2.0	1.8-2.2	7.20	7.15-7.20	30	19.5	30	7/2
4	4	2.0	2.0-2.2	7.20	7.20-7.25	30	19.5	50	6/30
5	5-6	1.9	1.8-2.0	7.20	7.15-7.30	38	21.0	50	7/26

TABLE V

RESISTANCE OF COHO SALMON TO LOW DISSOLVED OXYGEN CONTENT AT VARIOUS CARBON DIOXIDE CONCENTRATIONS IN RUNNING WATER
(No $NaHCO_3$ added to test water)

Carbon dioxide (mg/1)		Dissolved Oxygen (mg/1)		pH		Mean total alkalinity as $CaCO_3$ (mg/1)	Mean Temp. (°C.)	Percent Mortality (of 10 fish)	Date (1955)
Mean	Range	Mean	Range	Mean	Range				
13	9-17	1.9	1.8-2.2	6.70	6.60-6.85	33	21.5	80	7/18
13	9-15	2.0	1.9-2.2	6.70	6.60-6.80	32	20.5	70	7/16
14	11-16	1.9	1.8-2.2	6.65	6.60-6.75	30	19.5	30	6/30
20	17-25	1.9	1.8-2.2	6.50	6.40-6.60	30	20.0	80	7/5
22	13-32	2.1	1.9-2.4	6.55	6.40-6.80	40	21.5	70	8/9
24	17-27	1.9	1.8-2.1	6.50	6.45-6.65	38	21.0	50	7/26
24	14-27	1.9	1.8-2.3	6.50	6.45-6.75	38	21.0	20	7/30
26	22-26	1.9	1.8-2.0	6.40	6.40-6.45	30	19.5	60	6/30
33	20-40	1.9	1.8-2.2	6.35	6.25-6.60	38	21.0	60	7/30
34	34-40	2.0	1.9-2.2	6.25	6.20-6.25	30	19.5	50	7/2
37	24-40	1.9	1.8-2.2	6.30	6.25-6.50	37	21.5	60	8/4
38	24-45	1.9	1.8-2.3	6.30	6.20-6.50	37	21.0	70	8/2
40	32-44	2.0	1.7-2.3	6.20	6.15-6.30	30	20.0	50	7/5
42	27-48	2.0	2.0-2.3	6.25	6.20-6.45	37	21.0	60	8/2
47	44-60	2.1	2.0-2.4	6.20	6.10-6.25	37	21.0	50	7/28
48	44-70	2.3	2.2-2.3	6.15	5.95-6.20	35	21.5	60	7/12
48	42-52	3.1	2.0-3.4	6.15	6.10-6.20	33	20.0	0	10/28
50	45-60	2.0	1.8-2.1	6.20	6.10-6.25	38	20.5	100	7/9

TABLE V (continued)

Carbon dioxide (mg/l) Mean	Range	Dissolved Oxygen (mg/l) Mean	Range	pH Mean	Range	Mean total alkalinity as $CaCO_3$ (mg/l)	Mean Temp. (°C.)	Percent Mortality (of 10 fish)	Date (1955)
65	45-80	2.8	2.5-2.9	6.20	6.10-6.35	50	20.5	60	8/29
73	70-75	2.9	2.8-3.2	5.80	5.80-6.00	29	20.0	0	11/4
73	65-85	3.7	3.2-3.9	5.95	5.90-6.00	33	20.0	0	10/28
75	75	2.6	2.6	6.20	6.20	60	21.5	100	9/19
80	65-95	2.8	2.5-3.0	6.20	6.15-6.30	65	20.5	10	9/21
85	60-140	3.2	2.8-3.8	6.10	5.90-6.30	60	21.0	80	9/8
100	90-110	2.5	2.4-2.6	6.15	6.10-6.25	68	21.0	100	9/25
100	94-110	3.2	2.8-3.7	5.75	5.65-5.90	29	20.0	0	11/4
100	58-115	3.2	2.7-4.2	6.05	6.00-6.25	58	21.0	80	9/19
113	90-135	3.4	3.1-3.6	6.00	5.90-6.10	55	21.0	60	8/31
115	105-115	3.5	3.3-3.7	6.05	6.05-6.10	63	21.0	40	9/12
130	110-150	6.4	5.9-6.8	5.95	5.90-6.05	60	21.0	10	9/19
138	135-140	8.2	7.7-8.6	5.75	5.70-5.75	33	20.0	10	10/28
155	130-160	7.5	7.2-7.8	6.00	6.00-6.10	80	21.0	0	9/23
160	140-170	7.5	7.1-7.8	6.00	5.95-6.05	80	21.0	40	9/23
165	130-200	6.9	5.6-7.8	5.90	5.80-6.00	65	20.5	0	9/21
165	130-180	6.8	5.9-7.3	5.95	5.85-6.05	72	21.0	10	9/16
180	150-200	6.4	6.2-7.0	5.90	5.85-6.00	72	21.0	0	9/16

At a carbon dioxide concentration of 160 mg/1, the salmon could not survive in water nearly saturated with atmospheric oxygen.

Tolerance of High Carbon Dioxide and pH near Neutrality

The carbon dioxide content and the bicarbonate alkalinity of the river water were increased in these tests. Sodium bicarbonate solution was added to maintain the pH of the test water as near the pH of the river water as practicable. However, the total alkalinity never was made to exceed 320 mg/1 as $CaCO_3$ so that it would not be far in excess of the total alkalinity of ordinary alkaline natural waters. For this reason, pH values dropped below those normal for river water when large quantities of carbon dioxide were added, but pH values never fell lower than pH 6.35 in the presence of the highest test concentrations of carbon dioxide employed.

Test concentration of ions and gases fell within the following limits: (1) bicarbonate alkalinity - 240 mg/1 to 320 mg/1 as $CaCO_3$; (2) pH - 6.35 to 7.25; (3) carbon dioxide content - 30 mg/1 to 175 mg/1; and (4) dissolved oxygen concentration - 1.9 mg/1 to 7.5 mg/1. Data from these experiments are summarized in table VI, and presented graphically in figures 3 and 4.

Figure 4 shows that the oxygen requirements of coho

TABLE VI

RESISTANCE OF COHO SALMON TO LOW DISSOLVED OXYGEN CONTENT IN VARIOUS CARBON DIOXIDE CONCENTRATIONS IN RUNNING WATER
($NaHCO_3$ added to test water)

Carbon dioxide (mg/l)		Dissolved Oxygen (mg/l)		pH		Mean total alkalinity as $CaCO_3$ (mg/l)	Mean Temp. (°C.)	Percent Mortality (of 10 fish)	Date (1955)
Mean	Range	Mean	Range	Mean	Range				
32	26-36	1.9	1.7-2.1	7.25	7.20-7.35	295	21.5	70	7/18
32	30-40	2.0	1.9-2.1	7.25	7.15-7.30	295	20.5	30	7/16
36	32-40	1.9	1.8-2.1	7.15	7.10-7.20	257	21.0	20	7/26
50	48-59	2.0	2.0-2.5	7.05	7.00-7.10	305	21.0	60	7/28
55	53-57	2.0	1.9-2.2	7.05	7.05	300	21.5	100	7/12
58	43-75	1.9	1.7-2.1	7.00	6.90-7.15	290	20.5	90	7/9
60	44-75	1.9	1.8-2.0	7.00	6.90-7.15	315	21.0	40	7/30
75	73-77	2.1	1.9-2.3	6.85	6.85-6.90	285	21.0	100	9/8
80	70-100	2.0	1.7-2.5	6.90	6.80-6.95	320	21.0	20	9/10
85	70-93	2.2	2.1-2.5	6.80	6.75-6.85	265	21.0	40	8/2
85	73-95	2.2	2.0-2.4	6.80	6.75-6.85	265	21.5	50	8/4
93	85-117	2.5	2.4-2.5	6.75	6.65-6.80	265	20.5	100	8/29

TABLE VI (continued)

Carbon dioxide (mg/l)		Dissolved Oxygen (mg/l)		pH		Mean total alkalinity as $CaCO_3$ (mg/l)	Mean Temp. (°C.)	Percent Mortality (of 10 fish)	Date (1955)
Mean	Range	Mean	Range	Mean	Range				
95	75-100	2.4	2.2-2.5	6.75	6.75-6.85	280	21.5	80	8/9
95	60-105	2.5	2.3-2.7	6.80	6.75-7.00	300	21.5	60	8/16
100	90-120	2.7	2.4-3.0	6.75	6.75-6.80	280	20.5	60	9/14
120	100-125	2.7	2.6-2.8	6.70	6.65-6.75	290	21.0	50	9/12
120	75-130	2.8	2.8-3.2	6.70	6.65-6.90	300	21.5	80	8/16
120	95-130	3.0	2.9-3.2	6.70	6.65-6.80	295	21.0	70	8/31
130	115-145	3.7	3.6-3.7	6.65	6.60-6.70	295	21.0	60	8/31
140	115-155	3.2	3.1-3.2	6.60	6.55-6.70	280	20.5	30	9/14
145	145-150	3.2	3.1-3.4	6.60	6.55-6.60	290	21.0	30	9/12
150	110-170	3.0	2.9-3.2	6.55	6.50-6.70	265	20.5	60	8/29
160	130-170	5.1	4.6-5.9	6.55	6.50-6.65	290	21.0	40	9/8
170	160-180	5.0	4.7-5.2	6.45	6.40-6.45	235	21.0	10	10/8
170	135-175	7.5	6.9-7.9	6.45	6.45-6.55	245	20.5	50	9/25
175	160-210	6.5	6.0-6.7	6.55	6.45-6.60	320	21.0	20	9/10
190	180-200	7.3	7.0-7.5	6.40	6.35-6.45	255	20.5	90	9/25

salmon began to increase at a carbon dioxide concentration near 80 mg/l. Furthermore, their minimum oxygen requirements became doubled at about 150 mg/l of carbon dioxide. At carbon dioxide concentrations exceeding 175 mg/l, the salmon could not survive in water almost saturated with atmospheric oxygen.

Standing-water Experiments

Tests were undertaken to determine the resistance of salmon to high carbon dioxide concentrations when they had no chance to acclimatize; to establish whether or not there is any influence of pH or of alkalinity upon the ability of salmon to tolerate sudden increases in the carbon dioxide content; and to determine the effect of low pH alone on the lethal levels of dissolved oxygen of salmon and sunfish.

Tolerance of High Carbon Dioxide Content

The test fish were subjected to high concentrations of carbon dioxide without benefit of acclimatization. In one series of experiments the initial dissolved oxygen content of the water ranged from 5.8 to 6.9 mg/l. However, in a succeeding test series the initial dissolved oxygen content was reduced to near 3 mg/l (ranging from 3.0 to 3.4 mg/l).

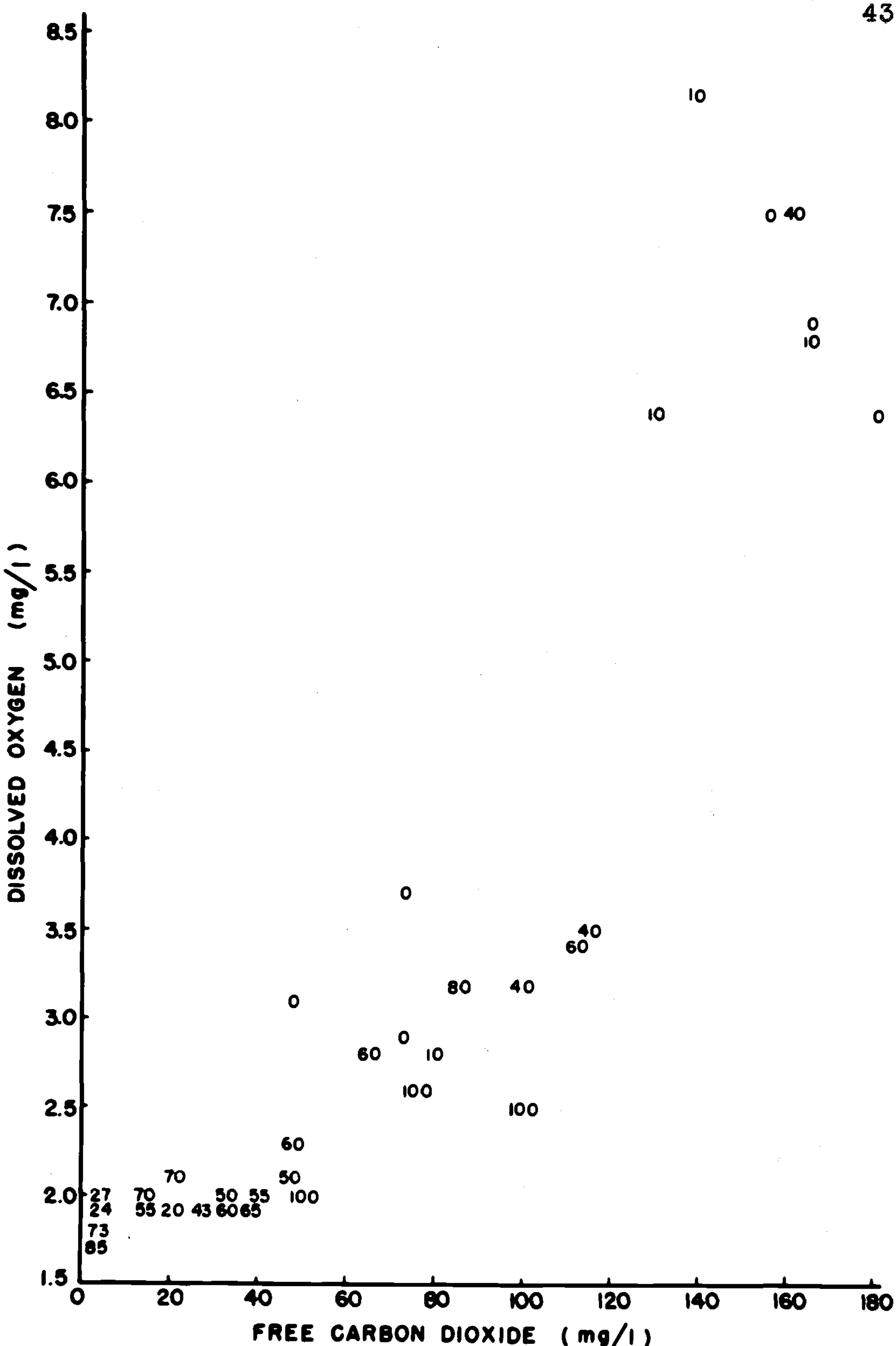

Figure 2. Percentages of mortality of coho salmon within 24 hours at various combinations of dissolved oxygen and carbon dioxide concentrations in running water of low alkalinity.

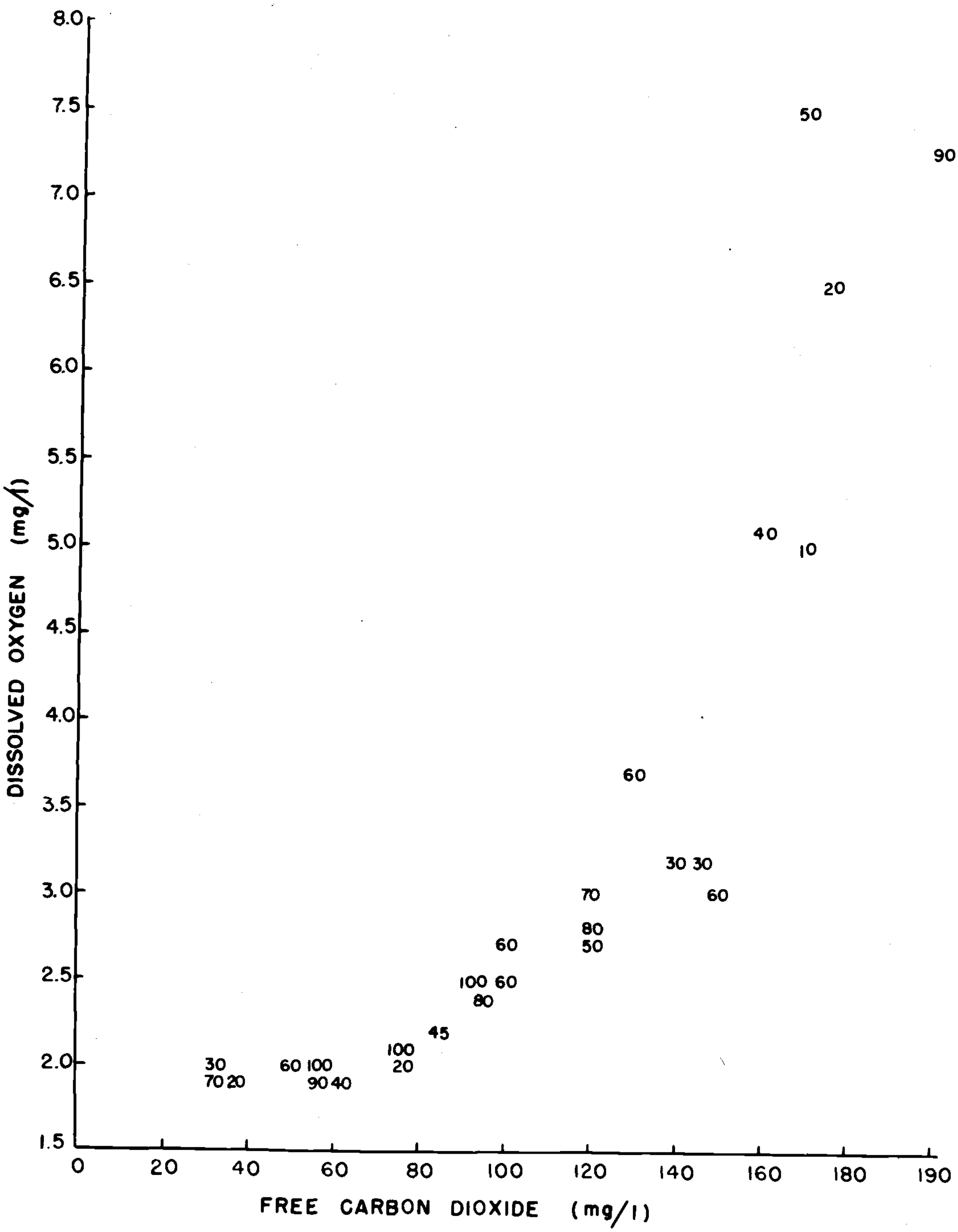

Figure 3. Percentages of mortality of coho salmon within 24 hours at various combinations of dissolved oxygen and carbon dioxide concentrations in running water of high alkalinity.

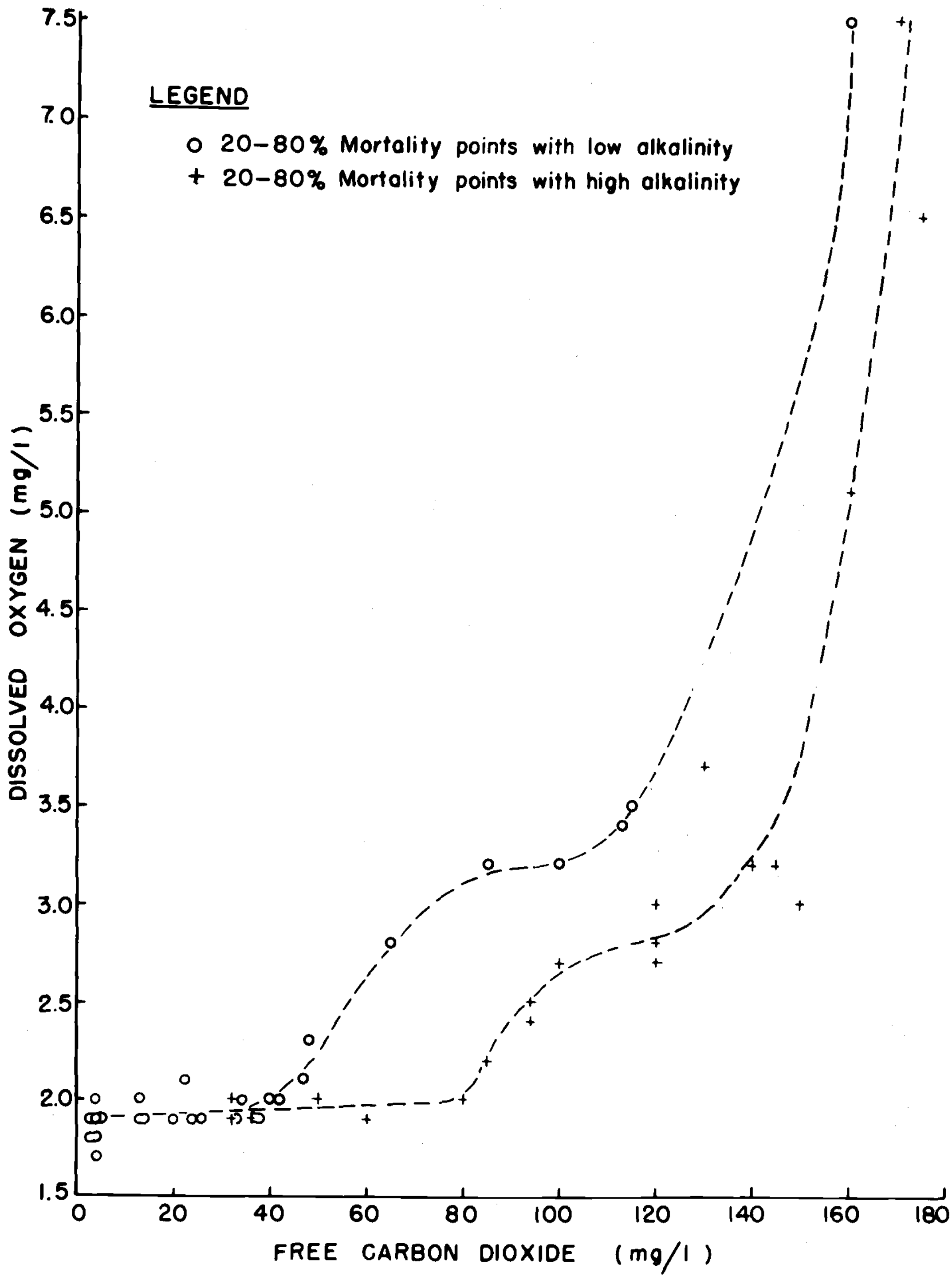

Figure 4. Relation between dissolved oxygen and carbon dioxide concentrations in running water in which 20 to 80 percent mortality of coho salmon occurred within 24 hours.

The data from tests in which the initial dissolved oxygen was 5.8 mg/l or greater is summarized in table VII and presented graphically in figure 5. For these tests the alkalinity of the river water was left unaltered. The pH dropped to 6.10 at the highest reported test concentration of carbon dioxide (nearly 100 mg/l). Each observation is based on the analysis of the water after the last of three test animals had died.

The data of table VII and figure 5 indicate that the lethal dissolved oxygen concentration began to increase sharply when the carbon dioxide content was between 75 and 100 mg/l. This conclusion is supported by data from other experiments which are presented later.

After the salmon had demonstrated that they could remove the oxygen down to 1.6 mg/l when placed in water with dissolved oxygen and carbon dioxide concentrations near 6.0 mg/l and 70 mg/l, respectively, tests were run to see if they could reduce the dissolved oxygen to an equally low level when the respective initial dissolved oxygen and carbon dioxide concentrations were 3.0 to 3.4 mg/l and 60 mg/l or less. The data from this experiment appear in table VIII and figure 5. Each recorded determination of the quality of the water was made after the last of three fish had died. It was found that salmon were unable to reduce the dissolved oxygen in test water

TABLE VII

TOLERANCE OF COHO SALMON TO HIGH CARBON DIOXIDE CONCENTRATIONS IN SEALED VESSELS WITH INITIAL DISSOLVED OXYGEN CONTENT OF 5.8 TO 6.9 mg/l

Carbon dioxide content after death of 3 fish (mg/l)	Dissolved oxygen content after death of 3 fish (mg/l)	pH	Total Alkalinity as $CaCO_3$ (mg/l)	Date (1955)
12	1.2	7.00	60	9/7
12	1.4	7.00	57	9/4
38	1.2	6.50	60	9/6
40	1.6	6.45	57	9/4
60	1.6	6.30	60	9/7
60	1.6	6.30	60	9/7
67	1.3	6.25	60	9/8
75	1.4	6.20	60	9/8
75	2.3	6.20	60	9/7
85	1.5	6.20	65	9/10
90	1.7	6.15	65	9/10
100	7.2	6.05	57	9/4
105	1.8	6.10	65	9/10
105	5.2	6.10	65	9/13
105	5.8	6.10	65	9/13
140	7.1	5.90	57	9/4

TABLE VIII

TOLERANCE OF COHO SALMON TO HIGH CARBON DIOXIDE CONCENTRATIONS IN SEALED VESSELS WITH INITIAL DISSOLVED OXYGEN CONTENT OF 3.0 TO 3.4 mg/1

Carbon dioxide content after death of 3 fish (mg/1)	Dissolved oxygen content after death of 3 fish (mg/1)	pH	Total Alkalinity as $CaCO_3$ (mg/1)	Date (1955)
5	2.0	7.40	60	9/5
6	1.5	7.30	60	9/6
8	1.7	7.25	70	9/19
9	1.4	7.20	70	9/14
10	1.7	7.20	75	9/16
11	2.2	7.05	60	9/5
17	1.5	6.95	75	9/16
24	1.7	6.80	75	9/16
24	2.4	6.70	60	9/5
27	1.5	6.65	60	9/6
28	2.8	6.70	70	9/19
30	2.7	6.60	60	9/5
31	1.8	6.65	70	9/14
33	1.6	6.65	75	9/16
35	3.0	6.60	70	9/19
37	2.6	6.50	60	9/6
44	2.8	6.50	70	9/14
45	3.1	6.50	70	9/19
63	3.0	6.35	70	9/14

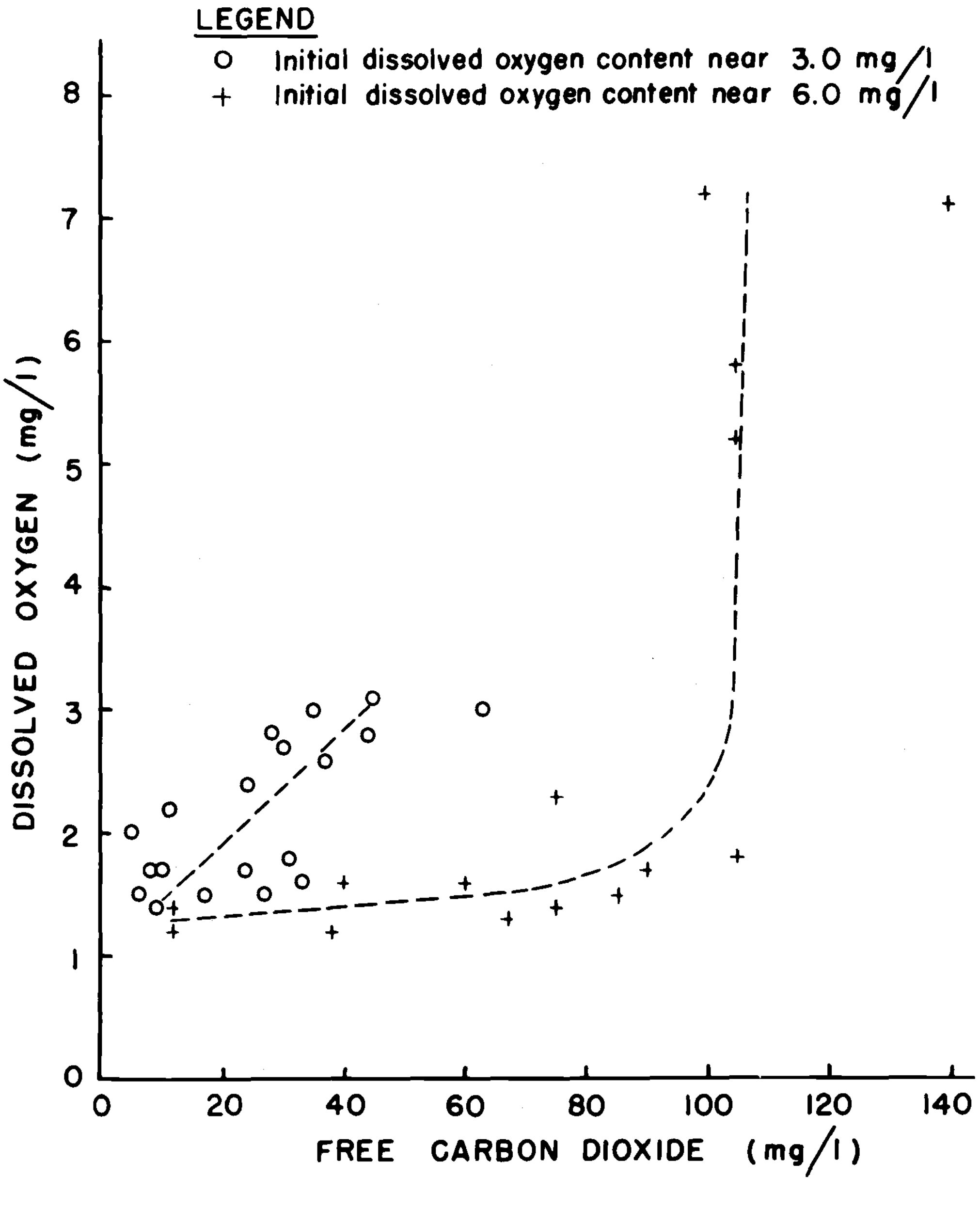

Figure 5. Relation between dissolved oxygen and carbon dioxide concentrations of water in sealed vessels in which total mortality of coho salmon had occurred (standing-water experiments).

below 3.0 mg/l when the carbon dioxide content exceeded 45 mg/l.

Effect of pH or Alkalinity on Resistance to Carbon Dioxide

In these tests five coho salmon were placed in each bottle, and the conditions existing after the death of each individual were recorded as an observation.

The initial dissolved oxygen content of the test water ranged from 7.7 to 8.5 mg/l. The test concentrations of carbon dioxide varied from 5 to 105 mg/l. The test pH values ranged from pH 5.3 to pH 7.8, and the alkalinity of the test waters ranged from 5 to 186 mg/l as $CaCO_3$.

Three bottles were used for each concentration of carbon dioxide tested. Alkalinity was decreased in the first, left unaltered in the second, and increased in the third. The fish were introduced without being acclimatized to the test conditions.

The experimental data are summarized in two tables. The dissolved oxygen concentration at which each fish died is tabulated in table IX along with the corresponding carbon dioxide, hydrogen-ion, and bicarbonate-ion concentrations. The 95 percent confidence interval for the mean dissolved oxygen concentration at which the five fish in each bottle died was estimated. This information

TABLE IX (a)

EFFECT OF pH OR ALKALINITY ON ABILITY OF COHO SALMON TO TOLERATE LOW DISSOLVED OXYGEN IN THE PRESENCE OF VARYING CARBON DIOXIDE CONCENTRATIONS

Group number 1 - 20° C - October 8, 1955 - all values at time of death

Jar	Fish Number	Carbon dioxide content (mg/l)	Dissolved Oxygen content (mg/l)	pH	Total Alkalinity as $CaCO_3$ (mg/l)
	1	8	1.9	6.75	22
	2	8	1.8	6.75	22
I	3	8	1.8	6.75	22
	4	8	1.8	6.75	22
	5	8	1.7	6.75	22
	1	8	1.9	7.10	48
	2	8	1.7	7.10	48
II	3	8	1.7	7.10	48
	4	8	1.7	7.10	48
	5	8	1.6	7.10	48
	1	5	1.8	7.85	186
	2	5	1.8	7.85	186
III	3	6	1.7	7.80	186
	4	6	1.7	7.80	186
	5	6	1.7	7.80	186

TABLE IX (b)

EFFECT OF pH OR ALKALINITY ON ABILITY OF COHO SALMON TO TOLERATE LOW DISSOLVED OXYGEN IN THE PRESENCE OF VARYING CARBON DIOXIDE CONCENTRATIONS

Group number 2 - 20° C - October 8, 1955 - all values at time of death

Jar	Fish Number	Carbon dioxide content (mg/l)	Dissolved oxygen content (mg/l)	pH	Total Alkalinity as $CaCO_3$ (mg/l)
	1	100	7.0	5.80	22
	2	100	6.9	5.80	22
I	3	100	6.9	5.80	22
	4	100	6.8	5.80	22
	5	100	1.9	5.80	22
	1	100	3.0	6.00	48
	2	100	3.0	6.00	48
II	3	100	2.9	6.00	48
	4	100	2.7	6.00	48
	5	100	2.1	6.00	48
	1	105	6.7	6.55	186
	2	105	6.4	6.55	186
III	3	105	1.9	6.55	186
	4	105	1.8	6.55	186
	5	105	1.7	6.55	186

TABLE IX (c)

EFFECT OF pH OR ALKALINITY ON ABILITY OF COHO SALMON TO TOLERATE LOW DISSOLVED OXYGEN IN THE PRESENCE OF VARYING CARBON DIOXIDE CONCENTRATIONS

Group number 3 - 20^{o} C - October 15, 1955 - all values at time of death

Jar	Fish Number	Carbon Dioxide content (mg/l)	Dissolved oxygen content (mg/l)	pH	Total Alkalinity as $CaCO_3$ (mg/l)
	1	70	7.2	5.35	5
	2	70	7.2	5.35	5
I	3	70	6.8	5.35	5
	4	70	1.5	5.35	5
	5	70	1.3	5.35	5
	1	70	1.7	6.05	39
	2	70	1.7	6.05	39
II	3	70	1.7	6.05	39
	4	70	1.6	6.05	39
	5	70	1.6	6.05	39
	1	75	1.5	6.65	170
	2	75	1.5	6.65	170
III	3	75	1.4	6.65	170
	4	75	1.4	6.65	170
	5	75	1.4	6.65	170

TABLE IX (d)

EFFECT OF pH OR ALKALINITY ON ABILITY OF COHO SALMON TO TOLERATE LOW DISSOLVED OXYGEN IN THE PRESENCE OF VARYING CARBON DIOXIDE CONCENTRATIONS

Group number 4 - 20° C - October 15, 1955 - all values at time of death

Jar	Fish Number	Carbon dioxide content (mg/l)	Dissolved oxygen content (mg/l)	pH	Total Alkalinity as $CaCO_3$ (mg/l)
	1	100	7.4	5.30	5
	2	100	7.4	5.30	5
I	3	100	6.0	5.30	5
	4	100	1.6	5.30	5
	5	100	1.6	5.30	5
	1	100	6.6	5.95	42
	2	100	4.5	5.95	42
II	3	100	3.2	5.95	42
	4	100	3.1	5.95	42
	5	100	3.1	5.95	42
	1	100	6.5	6.55	170
	2	100	1.5	6.55	170
III	3	100	1.5	6.55	170
	4	100	1.5	6.55	170
	5	100	1.5	6.55	170

is presented in table X.

From these statistical estimates it appears that coho salmon were generally more tolerant to carbon dioxide in water with high pH and alkalinity than in water with low pH and alkalinity. However, in several experiments too few fish were used to obtain meaningful results, because of the large variance in the dissolved oxygen concentration at which the fish died.

In group number one (table IX a) no carbon dioxide was added to the water. Here the 95 percent confidence limits of the mean dissolved oxygen at which the salmon died were 1.6 and 1.8 mg/l when the alkalinity was unaltered and when it was increased, but in the case where the alkalinity was reduced, these limits were 1.7 and 1.9 mg/l. In group number three (table IX c) where approximately 70 mg/l of carbon dioxide was added to the water, the corresponding confidence limits were as follows:

1. 2.0 and 9.6 mg/l with alkalinity decreased,
2. 1.6 and 1.7 mg/l with alkalinity unaltered,

and 3. 1.4 and 1.5 mg/l with alkalinity increased.

Unfortunately the results of groups two and four were too varied for meaningful interpretation, and no significance is attached to them.

By examining the data of table IX from jar II in

TABLE X

95 PERCENT CONFIDENCE INTERVAL OF MEAN DISSOLVED OXYGEN CONTENT AT THE TIME OF DEATH OF COHO SALMON

Group No.	Jar	Mean carbon dioxide content (mg/l)	Mean pH	Mean total alkalinity as $CaCO_3$ (mg/l)	95% confidence interval for oxygen (mg/l)
1	I	8	6.75	22	$1.7 < u < 1.9$
	II	8	7.10	48	$1.6 < u < 1.8$
	III	6	7.80	186	$1.6 < u < 1.8$
2	I	100	5.80	22	$3.1 < u < 8.7$
	II	100	6.00	48	$2.3 < u < 3.2$
	III	105	6.55	186	$0.5 < u < 7.1$
3	I	70	5.35	5	$2.0 < u < 9.6$
	II	70	6.05	39	$1.6 < u < 1.7$
	III	75	6.65	170	$1.4 < u < 1.5$
4	I	100	5.30	5	$1.1 < u < 8.5$
	II	100	5.95	39	$2.2 < u < 6.0$
	III	100	6.55	170	$0 < u < 7.8$

which the alkalinity was left unaltered, it can be seen that the test fish exhibited an increase in the lethal oxygen concentration at a carbon dioxide concentration between 70 and 100 mg/1. This corresponds to the results of the tests reported earlier in table VII and presented graphically in figure 5 where coho salmon were subjected to rapid increases in carbon dioxide under similar test conditions.

Tolerance to Low pH

No carbon dioxide other than that produced by the respiring test fish was added to any test container where the influence of pH alone was tested. Two species of fish were used. In one case five coho salmon were placed in each test vessel. In the other, two bluegills were sealed in each bottle.

The bluegills were placed in test water having an initial dissolved oxygen content of 3.0 mg/1. The tested pH values ranged from 3.55 to 7.45, and, as table XI and figure 6 show, a slight increase in their minimum oxygen requirements occurred at pH 4.0. There was no indication, however, that the oxygen requirements of bluegills vary significantly with pH within the pH range between 4.7 and 7.5.

The test water used for salmon had an initial

TABLE XI

EFFECT OF pH ON LETHAL DISSOLVED OXYGEN CONCENTRATION OF BLUEGILL SUNFISH

pH	Dissolved oxygen content remaining after death of second of 2 fish (initial dissolved oxygen content - 3.0 mg/l) (mg/l)	Date
7.45	0.48	8/29
7.20	0.52	9/1
6.50	0.44	8/29
4.70	0.46	8/29
4.00	0.60	9/1
4.00	0.55	8/29
3.70	1.09	9/1
3.55	2.79	8/31

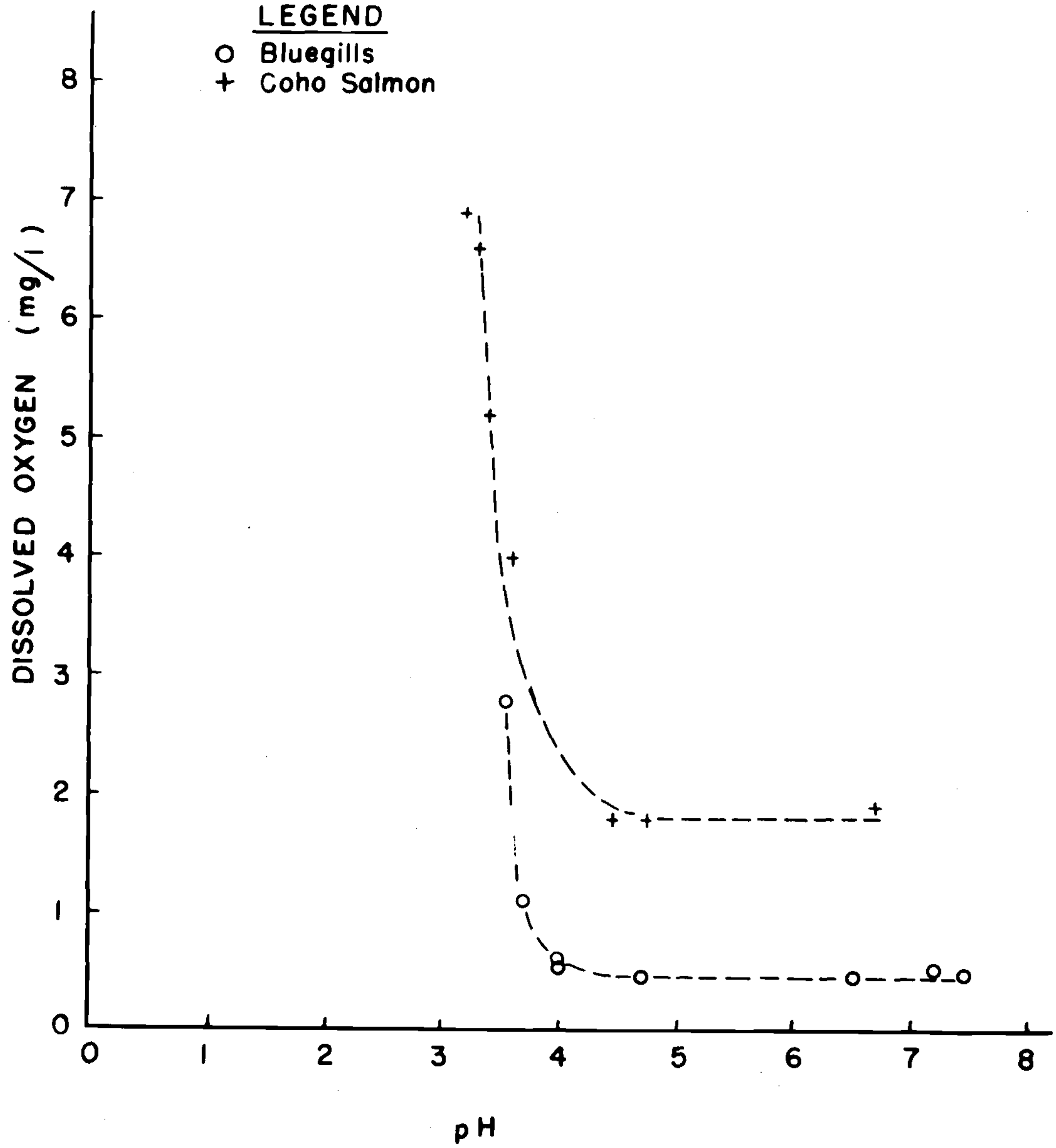

Figure 6. Relation of pH to the lethal dissolved oxygen concentration of bluegills and coho salmon.

dissolved oxygen content of 8.6 to 8.8 mg/1. The pH values tested ranged from pH 3.2 to pH 6.7. From the summary of test data given in table XII, it can be seen that the minimum oxygen requirements of coho salmon did not increase with pH decreasing to pH 4.45, but increased markedly at pH 3.6 and lower values.

At the extreme low pH values where the lethal dissolved oxygen content increased sharply for both salmon and sunfish, it was observed that the epidermal mucus coagulated. The coagulation was accompanied by irregularity of respiratory movements.

DISCUSSION

The experimental results show that the minimum dissolved oxygen requirements of coho salmon increased in the presence of high free carbon dioxide concentrations. In running-water tests wherein salmon were subjected to gradual increases of the carbon dioxide content of the water, marked increases of the minimum dissolved oxygen concentration tolerated occurred at carbon dioxide concentrations near or exceeding 50 mg/1 in water of low alkalinity. In water of high alkalinity, a corresponding increase in the minimum dissolved oxygen requirements of salmon occurred at approximately 80 mg/1 of carbon

TABLE XII

EFFECT OF pH ON LETHAL DISSOLVED OXYGEN CONCENTRATION OF COHO SALMON

pH	Mean dissolved oxygen content at time of death (5 fish) (mg/l)	Range of dissolved oxygen content at time of death (mg/l)	Date
6.70	1.9	1.7 - 2.0	11/5
4.75	1.8	1.7 - 2.0	11/5
4.45	1.8	1.7 - 1.9	11/5
3.60	4.0	2.4 - 4.5	11/5
3.40	5.2	5.1 - 5.2	11/5
3.30	6.6	6.6 - 6.6	10/28
3.20	6.9	6.7 - 7.0	10/28

dioxide. The dissolved oxygen requirements increased markedly with further increase of carbon dioxide concentration beyond these critical values.

There is apparently little justification for the early assertion of Wells (45, p.557) who claimed that carbon dioxide concentrations of 20 mg/l could prove quickly fatal to sensitive fishes. On the other hand, King (23, p.633) may have been wrong when he stated that 200 mg/l of free carbon dioxide had no influence on dissolved oxygen requirements of trout. However, Black, Fry, and Black (4, p.410) found that brook trout could remove oxygen from water saturated with dissolved oxygen as long as the carbon dioxide tension did not exceed 132 m.m. Hg (306 mg/l at the approximate test temperature of 18.5° C.). This is about double the concentration of carbon dioxide that will kill coho salmon under similar conditions.

The results reported by Hart (19, p.226), concerning the influence of carbon dioxide on dissolved oxygen requirements of certain warm-water fish in sealed containers, essentially agree with the present findings. The most sensitive fish that Hart tested was the gizzard shad which was unable to remove oxygen from water one-half saturated with dissolved atmospheric oxygen in the presence of 88 mg/l of carbon dioxide. A less sensitive fish, the largemouth bass, was shown by Hart to require 175 mg/1 of free carbon dioxide before it failed to remove oxygen from water one-half saturated with dissolved atmospheric oxygen. Figure 4 indicates that coho salmon lose their resistance to low dissolved oxygen concentrations equal to one-half saturation (about 4.5 mg/1 at 20° C.) when the carbon dioxide concentration is less than 160 mg/1 but greater than 115 mg/1, depending on whether or not the water is highly alkaline.

The observations by Fish (13, p.27) on the effects of high free carbon dioxide concentrations on king salmon and steelhead trout essentially agree with the present work. Fish stated that 200 mg/1 of free carbon dioxide rapidly anesthetizes these two species. The present findings with running-water experiments indicate that coho salmon may lose their tolerance of high values of reduced dissolved oxygen when carbon dioxide concentrations in excess of 180 mg/1 are present.

There is evidence to indicate that acclimatization to increased carbon dioxide concentration modifies the influence of free carbon dioxide on the minimum dissolved oxygen requirements of coho salmon. A general comparison of results from running-water and standing-water tests seems to indicate this phenomenon. Figures 4 and 5 show that at dissolved oxygen concentrations near 6 mg/1 salmon which had no opportunity to become acclimatized to test concentrations of carbon dioxide (standing-water tests) died in the presence of about two-thirds of the content of carbon dioxide required to kill fish that had the opportunity to acclimatize themselves over a six-hour period (running-water tests). When the initial dissolved oxygen content in the standing-water tests was reduced from 6 mg/l to 3 mg/l, the unacclimatized fish died at once at this dissolved oxygen concentration in the presence of carbon dioxide concentrations approximating one-half of the concentration required to cause death of fish at the same dissolved oxygen value in the running-water tests.

It should be noted that a definitive comparison between the results of running-water and standing-water tests cannot be made since experimental procedures differed. Nevertheless, a general comparison is warranted because the differences in test results between the two kinds of experiments are striking and suggest that acclimatization is an important factor. Additional experiments are needed to demonstrate more directly and to better evaluate this influence.

Additional and perhaps more convincing evidence concerning the effect of acclimatization is provided by the two kinds of sealed-bottle tests alone. As figure 5 shows, the fish started at 6 mg/1 of dissolved oxygen generally survived at a lower dissolved oxygen content than those started at 3 mg/1 of dissolved oxygen at the same concentration of free carbon dioxide. However, acclimatization to low dissolved oxygen content as well as to high carbon dioxide concentration could have been involved here.

The influence of carbon dioxide on the minimum dissolved oxygen requirements of salmon cannot be ascribed to reduced pH alone. In running-water experiments where the alkalinity was low, marked increases in the minimum dissolved oxygen requirements of salmon occurred at pH near 6.2 (50 mg/1 of free carbon dioxide); but when the alkalinity was high, similar increases in the minimum dissolved oxygen requirements of salmon occurred near pH 6.8 (80 mg/1 of free carbon dioxide). Also, the results of standing-water experiments (figure 6) indicate that, down to pH 5 or less, pH alone has no influence on the lethal dissolved oxygen content for coho salmon and bluegills. These data indicate that the influence of free carbon dioxide on the minimum dissolved oxygen tolerance of salmon may be independent of pH.

Among the investigators who have claimed that reduced pH values near pH 5 or greater influence the dissolved oxygen requirements of fish, Shelford (39, p.383) and

Wiebe, <u>et al</u>. (47, pp.442-465) apparently failed to distinguish between the effects of pH and free carbon dioxide. The present findings show that Wiebe's conclusions concerning the bluegill are incorrect, and they indicate that Doudoroff and Katz (10, pp.1440-1441) are correct in assuming that the effects attributed to pH by Wiebe were due, instead, to free carbon dioxide.

The conclusions of Townsend and Cheyne (42, pp.462-465) that pH and not free carbon dioxide increases the minimum dissolved oxygen requirements of coho salmon cannot be attributed to a failure to distinguish between pH and free carbon dioxide. They state that their test water was "low in bicarbonates" and had a natural pH ranging from 7.6 to 8.6. In addition, Townsend and Cheyne say, "it is certain that the tension of carbon dioxide in the water necessary to change the pH from 7.80 to 6.75 is 'far greater' than the carbon dioxide tension produced, on the addition of strong acid, by the release of carbon dioxide from trace amounts of bicarbonates in this water". This information, however, is not very meaningful. They fail to indicate the concentration of bicarbonate considered to be "low" and to present some measure of relative carbon dioxide concentrations so as to clarify the meaning of "far greater".

At the initial pH values reported by these authors (near 8.0), free carbon dioxide, hydroxyl alkalinity, and carbonate alkalinity apparently were virtually absent from the water. The experimental pH value that caused the greatest mortality among salmon at low dissolved oxygen concentrations was pH 6.75. Townsend and Cheyne added 15.1 mg/l of sulfuric acid to their test water in one series of experiments to lower the pH from about 8.0 to 6.75. The addition of 15.1 mg/l of sulfuric acid to water containing adequate bicarbonate theoretically releases 13.5 mg/l of free carbon dioxide. The quantity of free carbon dioxide present in the test water employed by Townsend and Cheyne can be estimated from a nomograph for the evaluation of free carbon dioxide (2, p.57), after making certain assumptions. Let it be assumed that their test water had an initial bicarbonate alkalinity of 50 mg/l as $CaCO_3$, a total solids content of 50 mg/l, and a temperature of 15° C. The addition of 15.1 mg/l of sulfuric acid to water under the assumed conditions would lower the initial bicarbonate alkalinity from 50 mg/l to 35 mg/l as $CaCO_3$. From the nomograph it can be determined that approximately 13.5 mg/l of free carbon dioxide would have been present in the test water at pH 6.75 with the bicarbonate alkalinity equal to 35 mg/l as $CaCO_3$. This agreement confirms the assumption that the initial bicarbonate alkalinity of the water was in the neighborhood of 50 mg/l as $CaCO_3$. Now, with an initial bicarbonate alkalinity of 50 mg/l as $CaCO_3$, it can similarly be determined that 20 mg/l of free carbon dioxide alone would lower the pH to 6.75. Thus, Townsend and Cheyne possibly failed to recognize that they may have obtained carbon dioxide concentrations of about the same order of magnitude by adding either acid or carbon dioxide to their test water to lower the pH.

Assuming that Townsend and Cheyne had 13 to 20 mg/l of free carbon dioxide present in their test water at pH 6.75, the reported finding that this pH value influences the tolerance of coho salmon at a dissolved oxygen content of 2.25 mg/l does not agree with the results of the present work which revealed no influence of free carbon dioxide concentrations as low as 20 mg/l at a dissolved oxygen content of 2.0 and less. Also, these authors report that pH influenced the minimum dissolved oxygen requirements of salmon when lowered from pH 7.5 to pH 7.0. Again no such effect was noted in the present study, and there is no apparent reason for the discrepancy.

Although free carbon dioxide and not a change in pH has been shown to be primarily responsible for the increased minimum dissolved oxygen requirements of salmon, there are indications that some factors other than acclimatization modifies this effect. In running water of low alkalinity the limit of dissolved oxygen tolerance of coho salmon was near 4 mg/l when the carbon dioxide concentration was near 125 mg/l. Under similar test conditions, the salmon in water of high alkalinity had a corresponding dissolved oxygen tolerance limit in the presence of 150 mg/l of carbon dioxide. At carbon dioxide concentrations less than 50 mg/l, salmon exhibited little difference in their ability to tolerate low dissolved oxygen in running water, irrespective of the carbon dioxide content and the alkalinity.

When the carbon dioxide concentrations were high and nearly equal, salmon generally reduced the dissolved oxygen content of standing water of high alkalinity to a lower level than the level to which the dissolved oxygen content of standing water of low alkalinity had been reduced at the time of death. Although these results corroborate the findings from running-water experiments, more experimental work is required to measure more accurately the degree to which low alkalinity reduces the ability of fish to tolerate high carbon dioxide concentrations.

It is not known whether changes in alkalinity modify the ability of salmon to tolerate low dissolved oxygen concentrations in the presence of high carbon dioxide content because of the consequent change of pH, the altered bicarbonate-ion concentration, or some other factor. This would be a difficult problem to solve by oxygen tolerance studies since pH cannot be altered without creating a corresponding change in alkalinity.

The present findings closely correspond to the known facts and theories of respiratory and blood physiology of fishes. Earlier workers have shown that free carbon dioxide has a greater influence on physiological processes of living fish than pH. Powers, et al. (31, pp.243-244) contended that fish were unable to maintain a low carbon dioxide tension of their blood when the environmental concentration was increased. Also, Jobes and Jewell (22, p.185) and Powers, Hopkins, and Hickman (29, p.466) found that environmental pH alone had no influence on blood pH. The present findings support these conclusions, since carbon dioxide had a pronounced effect while reduced pH alone (pH 5.0 or greater) had no effect on the minimum dissolved oxygen requirements of coho salmon.

Jacobs (21, pp.329-331) demonstrated that undissociated molecules of carbon dioxide readily pass through living cell membranes. He found the reverse to be true of strong mineral acids, which exert their influence on external structures. The observations reported by Jacobs explain why free carbon dioxide and not pH influenced the minimum dissolved oxygen requirements of salmon. Carbon dioxide molecules probably penetrated the gill membrane and became absorbed by the blood stream. The hydrogen-ions, on the other hand, probably were unable to effect such an entry and exerted their influence upon external membranes only.

It was observed that the action of sulfuric acid was external. At pH values in the neighborhood of 3.5 a coagulation of the mucus on the gill surfaces occurred. This was accompanied by a marked increase in the minimum dissolved oxygen requirements of the affected fish, probably due to a decrease in the respiratory efficiency of the affected gills postulated by Westfall (46, p.286). However, within the range of pH ordinarily occurring in natural waters and water receiving organic wastes, pH alone did not increase the minimum dissolved oxygen requirements of the test species.

Carbon dioxide entering the blood stream may cause the blood to lose its affinity for oxygen. At the lower carbon dioxide concentrations this loss of affinity is probably referable to the Bohr effect first reported in fish by Krogh and Leitch (24, p.299). There is a strong possibility, however, that an inactivation of the oxygen-combining groups of the blood, as suggested by Root and Irving (35, pp.307-323), occurs at high carbon dioxide concentrations. This inactivation may explain the tendency for the two curves shown in figure 4 to rise steeply at the high carbon dioxide test concentrations, indicating that respiration becomes impossible irrespective of the dissolved oxygen content.

The reported results provide an insight into some conditions which abet the harmful effects of carbon dioxide on fish life. Fully developed fresh-water fishes are less likely to succumb to low dissolved oxygen in the presence of high carbon dioxide content in waters of high alkalinity than in waters of low alkalinity. Also, there is less likelihood of the minimum dissolved oxygen tolerance of developed fishes being influenced by free carbon dioxide if they are afforded the opportunity to acclimatize themselves gradually to increasing concentrations.

Under most natural stream conditions it is unlikely that free carbon dioxide reaches concentrations required to influence significantly the tolerance of fully developed fishes to low dissolved oxygen content. Ellis (11, pp.388-391) found that the free carbon dioxide content of various American rivers generally did not exceed 14 mg/1. Since his determinations were made in polluted as well as unpolluted areas, it appears that free carbon dioxide is of little practical importance in establishing criteria for the minimum dissolved oxygen content of organically polluted streams.

There are indications that ponds and lakes may attain higher carbon dioxide concentrations than streams. Shelford (38, p.10) reported an instance where the free carbon dioxide content of a midwest pond approached 40 mg/l. Such extreme conditions, however, probably occur rarely, and it is doubtful if the minimum dissolved oxygen tolerance of fish would be significantly increased by carbon dioxide concentrations occurring in most standing-water environments.

Although free carbon dioxide rarely attains concentrations in nature which could cause a significant increase in the minimum dissolved oxygen requirements of fish, it is possible for free carbon dioxide to be harmful where fish are suddenly exposed to high carbon dioxide and low dissolved oxygen concentrations simultaneously. This could possible occur where fish swim into the hypolimnion of a lake or water heavily polluted with organic wastes and where fish are present when a strong acid is suddenly discharged into alkaline water.

SUMMARY

1. Two experimental methods were used to evaluate the influence of high concentrations of carbon dioxide and

low pH values on the minimum dissolved oxygen requirements of fresh-water fish. One method was to place fish in bottles having a continuous flow of water, the dissolved oxygen, carbon dioxide, and hydrogen-ion concentrations of which could be controlled. The other method was to seal fish in bottles filled with water having its quality previously adjusted.

2. High concentrations of carbon dioxide caused pronounced increases in the minimum dissolved oxygen requirements of coho salmon. In both running-water and standing-water experiments, noticeable increases in the minimum dissolved oxygen requirements of salmon occurred at free carbon dioxide concentrations between 50 and 100 mg/l, depending on the period of acclimatization and the bicarbonate alkalinity of the test water.

3. Coho salmon that had some opportunity to become acclimatized to high concentrations of carbon dioxide tolerated between 160 and 175 mg/l of free carbon dioxide when dissolved oxygen was near the saturation value and the temperature 20° C.

4. The test results indicate that acclimatization influenced the effect of free carbon dioxide on the minimum dissolved oxygen requirements of coho salmon. Salmon placed directly into water containing 100 mg/l or more of free carbon dioxide (unacclimatized fish) died

at higher dissolved oxygen concentrations than salmon having the opportunity to become gradually acclimatized to similar test conditions.

5. There were also indications that the bicarbonate alkalinity (or possibly the pH) of the test water influenced the effect of free carbon dioxide on the minimum dissolved oxygen requirements of coho salmon to some extent. In running-water experiments with carbon dioxide concentrations exceeding 50 mg/l, salmon in water of high alkalinity and pH near neutrality generally tolerated lower dissolved oxygen concentrations than salmon in water of low alkalinity and low pH.

6. Salmon placed in water having a low pH and containing no initial free carbon dioxide increased their lethal dissolved oxygen concentration at pH values less than 4.45, while bluegills increased their lethal dissolved oxygen concentration markedly at pH values less than 4.0. At extremely low pH values, a noticeable coagulation of the external mucus occurred.

7. The carbon dioxide concentration of most waters polluted with putrescible organic wastes probably does not attain concentrations harmful to fully developed fresh-water fishes. Also, instances of high carbon dioxide concentrations resulting from the addition of strong acids to highly alkaline water are probably rare

and generally of little significance. It may be possible, however, for relatively low concentrations of free carbon dioxide to be harmful to fish suddenly exposed to high carbon dioxide and low dissolved oxygen concentrations simultaneously.

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