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ART. VIII. THE REACTIONS AND RESISTANCE OF FISHES TO CARBON DIOXIDE AND CARBON MONOXIDE

BY

MORRIS M. WELLS, PH.D.

ART. IX. <sup>-</sup> EQUIPMENT FOR MAINTAINING A FLOW OF OXYGEN-FREE WATER, AND FOR CONTROLLING GAS CONTENT

 $\mathbf{B}\mathbf{Y}$ 

VICTOR E. SHELFORD, PH.D.

ART. X. A COLLECTING BOTTLE ESPECIALLY ADAPTED FOR THE QUAN-TITATIVE AND QUALITATIVE DETERMINATION OF DISSOLVED GASES, PARTICULARLY VERY SMALL QUANTITIES OF OXYGEN

BY

EDWIN B. POWERS, M. A.

#### ERRATA AND ADDENDA.

Page 50, second column, line 13 from bottom, for Danais archippus read Anosia plexippus; line 8 from bottom, for mellifica read mellifera.

Page 51, line 11 from bottom, for Danais read Anosia.

Page 159, at right of diagram, for Bracon agrilli read Bracon agrili.

Page 289, second column, last line but one, for Scalops real Scalopus.

Page 294, line 3, for catcsbeana read catesbiana.

Pages 327 and 330, line 12, for orcus read oreas.

Page 347, line 4, for Cecidomyidæ read Cecidomyiidæ.

Page 356, line 7, for Anthomyidæ read Anthomyiidæ.

Page 368, line 18, dele second word.

Page 373, after line 10 insert as follows: 53a, subpruinosa Casey, 1884, p. 38.

Page 375, after submucida Le Conte, 48, insert subpruinosa Casey, 53a.

Page 377, after line 7, insert as follows:---

1884. Casey, Thomas L. Contributions to the Descriptive and Systematic Coleopterology of North America. Part I.

Page 379, line 11 from bottom, for sensu lata read sensu lato.

Page 382, line 12, for VII read VIII.

Page 408, line 2, for the next article in read Article VIII of.

Page 410, line 6 from bottom, for = 4 read '11.

Page 412, line 7, for 31 read 30.

Page 421, line 17 from bottom, insert it before grows.

ARTICLE VIII.—The Reactions and Resistance of Fishes to Carbon Dioxide and Carbon Monoxide.\* By MORRIS M. WELLS.

#### INTRODUCTION

Carbon monoxide and carbon dioxide are both present in the waste that is diverted into natural waters by many works where illuminating gas is manufactured and, since the waste as a whole is known to be exceedingly poisonous to aquatic organisms, the rôle played in its toxic action by the two gases in question was investigated at the time that the many other organic substances of which the waste is composed were studied by Shelford.<sup>†</sup> The investigation has shown that both of the gases are poisonous to fresh-water fishes even when present in the water in relatively small proportions, but the monoxide has been found to be by far the more deadly of the two.

Carbon dioxide is present normally in the natural habitats of practically all fresh-water organisms, but its toxicity does not manifest itself unless it occurs in concentrations which are high as compared with lethal concentrations of carbon monoxide. At a concentration of 10 c.c. per liter, carbon dioxide will quickly prove fatal to the more sensitive fishes; and it is doubtful if there are any freshwater fishes that could continue to live in water where the carbondioxide content averaged as high as 6 c.c. per liter throughout the year. On the other hand, there is evidence that a certain small concentration of carbon dioxide, that is, a certain degree of acidity, is beneficial, if not actually essential to the continued existence of some, and perhaps many, fresh-water fishes. It would not be at all safe to assume that all fresh-water organisms require an environment whose reaction (to phenolphthalein) is slightly acid, for it is known from investigation that certain organisms, as the plankton in freshwater lakes, seem actually to prefer alkalinity to acidity. Furthermore, there are many cases on record where fishes that normally live in water that is slightly acid from the presence of CO<sub>2</sub> have continued

<sup>\*</sup>Contributions from the Zoological Laboratory of the University of Illinois, No. 107.

<sup>&</sup>lt;sup>†</sup>An Experimental Study of the Effects of Gas Waste upon Fishes, with Especial Reference to Stream Pollution. By V. E. Shelford. Bull. Ill. State Lab. Nat. Hist., Vol. XI, Art. VI, pp. 381-410. 1917.

to live more or less normally in water that had become alkaline either from treatment or from the using up of the  $CO_2$ , both free and half bound, by the algae growing in the water. However, it is still to be demonstrated that there are any species of truly fresh-water fishes that can reproduce successfully in water that is decidedly alkaline to phenolphthalein throughout the year.

It should not be concluded that, since a certain small amount of  $CO_2$  seems to make the water more acceptable to certain fresh-water organisms, it will be well to add this gas to natural waters, for all the carbon dioxide that is necessary to organisms living in nature is produced in the natural waters by the decomposition of organic materials contained therein; and, in fact, the processes of decay often raise the concentration of carbon dioxide to a point where it is detrimental, and even fatal, to the aquatic inhabitants of the water. The addition of any substance, therefore, which will increase the amount of carbon dioxide the only toxic substance contained in gas-house waste, the effect of this waste upon the aquatic organisms would still need to be regarded with suspicion, for while the more hardy organisms might survive its presence the less resistant species would be sure to fare badly, at least near the point of introduction.

Carbon monoxide—which differs from the dioxide in that the carbon atom in the monoxide is holding in combination but one oxygen atom instead of two and is, therefore, chemically speaking, unsaturated—is a well-known poisonous gas, and its frightfully deadly effect when present in the atmosphere in even exceedingly small quantities has been vividly demonstrated by many investigators. Two to three per cent. of carbon monoxide in the air breathed by a mouse will cause the death of the animal in from one to two minutes. The familiar poisonous effects of illuminating gas are largely due to the comparatively large per cent. of carbon monoxide which it contains.

The investigation of the toxic properties of the two gases in question was carried on as follows. The work with carbon dioxide as summarily presented here has been carried on since 1912, partly at the University of Chicago and partly at the University of Illinois. The carbon-monoxide investigations have all been carried on in the laboratory of the State Laboratory of Natural History at the University of Illinois, and with facilities which constitute a part of the equipment of the Vivarium. The results show that both gases are toxic to fresh-water fishes in the concentrations which would result from the introduction of gas-house wastes into natural waters, and that carbon monoxide is by far the more deadly, killing the most resistant fishes in concentrations that would be negligible or beneficial in the case of carbon dioxide.

#### PROPERTIES OF THE GASES

Carbon monoxide is lighter than air, having a specific gravity of 0.967. It is odorless, tasteless, and colorless, burns with a characteristic pale blue flame, forming carbon dioxide, and is only slightly soluble in water, 23.1 c.c. dissolving in a liter at 20°C.

Carbon dioxide is heavier than air (specific gravity 1.519), is odorless, has a decidedly acid taste, is colorless at ordinary temperatures, and will not burn, since the carbon atom already holds in combination all the oxygen with which it has the power to combine. It is very soluble in water, a liter of water at ordinary temperatures holding in combination almost a liter of the gas. The carbon dioxide does not simply dissolve in the water, but unites with it to form carbonic acid  $(CO_2+H_2O=H_2CO_3)$ .

### METHODS AND MATERIALS

Two types of experiments have been made, one to determine the resistance of the fishes to the gases in various concentrations, another to determine the reactions of the fishes to the gases in the gradient. The latter type gives the fishes an opportunity to select or reject the water containing the gas. The fishes were collected in the small streams of northern Illinois, fifteen to twenty different species having been tested more or less fully.

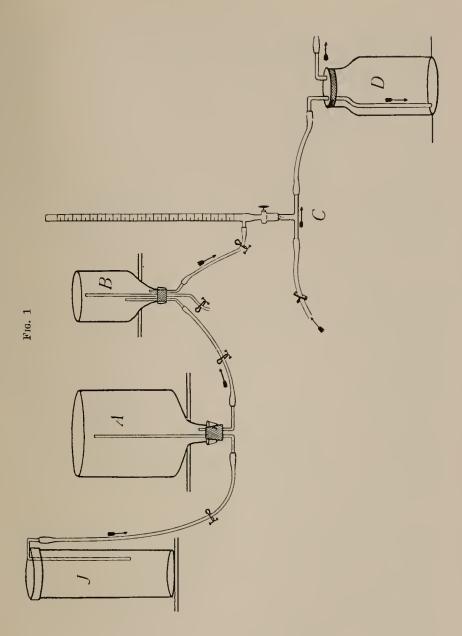
Resistance Experiments.—A paper (Wells, '13) has already been published which gives the detailed methods and observations concerning the resistance of fresh-water fishes to carbon dioxide. Briefly, the experiments were made as follows:—A stream of water flowing at a rate of from 500—600 c.c. per minute was passed through two experimental bottles having a capacity of seven and three liters respectively. The gas was introduced into the flow at a point far enough away to allow it to dissolve before it reached the bottles. The exact concentrations of  $CO_2$  in the experiments was determined by titration of samples collected as the water flowed out of the experimental bottles. These determinations were made at regular intervals throughout each experiment.

The resistance experiments with carbon monoxide were made much as were those just described for carbon dioxide. The method of determining the concentration of the gas in the collected samples was that described in Hempel's "Gas Analysis".\* The gas was boiled off and absorbed with a hydrochloric acid solution of cuprous chloride.

<sup>\*</sup>Hempel's 'Gas Analysis,'' 1910 edition, p. 203.

Carbon dioxide can be bought in tanks and used directly from them, but it is necessary to make the monoxide. This was done by the common method of heating oxalic acid with five to six times its weight of concentrated sulphuric acid. In the reaction both the dioxide and the monoxide are formed; the dioxide is removed by passing the gas through two wash bottles containing concentrated NaOH, and the gas remaining was also led through two wash bottles containing distilled water before it was collected in large 20-liter bottles over water. Analysis of the gas showed it to be 95 per cent. CO and 5 per cent. atmospheric gases in the proportion in which they dissolve in water from the atmosphere. These latter gases must have come out of the water over which the CO was collected. The generation of the gas and the experiments were performed with a canary bird at hand, the gas being particularly poisonous to birds.

Figure 1 illustrates the method used in introducing the CO into the water that flowed through the experimental bottles. The method may be useful wherever the introduction of small amounts of any substance, especially a volatile one, into a stream of running water is desired. It reduces the exposure to the atmosphere to a minimum, and the concentration can thus be kept relatively constant. The steps in introducing the CO were as follows:—(I) Water from J was siphoned into A, which was already full of CO. The clamp between A and B was kept closed, and thus the water in A was subjected to some pressure. After some hours the water was found to be saturated with CO. (2) B was now lowered, and the pinch clamp between A and B was loosened. The water in A now ran into B, displacing the air through the glass tube leading to the top of B. More water from J flowed into A at the same time; but if the exchange was made rather slowly, practically no mixing took place and analysis showed the water in B to be saturated or even slightly supersaturated with CO. (3) The clamp between A and B was closed, B was raised to the position shown in the figure, and the clamp between B and the burette was opened. The water now ascended in the burette till it reached the level of the water in B. (4) By opening the burette cock the saturated water was now run into the glass tee at C, where it mixed with the tap water. The rate of flow from the burette was determined by counting the drops per minute, the number of drops per c.c. having previously been determined. (5) The mixture then flowed through the experimental bottles of which D is the first. (6) Finally, as has been stated before, the actual concentration of gas in the water in the experiment was determined by analysis.



The fishes were left in the experimental bottles until dead, and the time between introduction and death (dying time) is the basis of a comparison of the relative resistances of the different species. Table I is a summary of nine experiments in which four different concentrations of CO were used.

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Showing the relative resistance of several species of fishes to different concentrations of carbon monoxide. The concentration of the CO solution which flowed through the experimental bottles is expressed in c.c. per liter.

Species of fish	Weight of fish in grams	CO 1.2 c.c. per liter	CO 3.8 c.c. per liter	CO 6 c.c. per liter	CO 11.7 c.c. per liter
Moxostoma aureolum (Red-horse)	10-15			28 min.	10 min.
Notropis blennius (Straw-colored minnow)	24	1 hr.	45 min.	28 min.	
Pimcphales notatus (Blunt-nosed minnow)	1-1.5	1 hr., 55 min.	1 hr., 20 min.		
Lepomis humilis (Orange-spotted sunfish)	2-6	5 hr., 40 min.	4 hr., 5 min.	1 hr., 5 min.	45 min.
Lepomis cyanellus (Green sunfish)	2-4	6 hr., 10 min.	5 hr., 25 min.	5 hr., 51 min.	4 hr., 5 min.
Ameiurus melas (Black bullhead)	20.5				9 hr., 55 min.

This table shows the high toxicity of water which contains even small amounts of carbon monoxide in solution, and that stronger concentrations are proportionately more deadly. From the results of a large number of similar experiments with  $CO_2$  (Wells, '13) it is evident that a concentration of from 75–100 c.c. per liter of carbon dioxide is required to equal the killing effectiveness of 1 c.c. per liter of carbon monoxide.

Water which contains lethal amounts of  $CO_2$  in solution will soon lose its toxicity if exposed to the atmosphere for a comparatively short time. The  $CO_2$  passes into the atmosphere until there is equilibrium between the gas in the atmosphere and in the water. Since the atmosphere ordinarily contains but minute amounts of  $CO_2$ , practically all of the gas will pass from the water; a solution containing 100 c.c.  $CO_2$  per liter will lose all but 1 to 2 c.c. per liter within two or three hours.

Normally, the atmosphere does not contain even a trace of CO, and it would appear, therefore, that water containing small quantities

of this gas would rapidly lose its toxicity when exposed in open dishes. This was found not to be the case, however, for a saturated solution of CO did not lose its toxic properties even after two weeks' exposure. A liter of the saturated solution from A (Fig. 1) was placed in each of four 5 in.  $\times$  8 in. battery jars and the jars were set in a stream of running water to keep the temperature constant at 18° C. A liter of tap water was placed in a fifth jar and set beside the other jars. Two small fish (Lepomis humilis) weighing between 3.5 and 8 grams each were placed in each jar. In the CO solution the fishes died very quickly, while those in the tap water continued to swim about normally. The dead fishes were removed at once and other individuals of the same size and species (except as noted) were placed in the jars at intervals during the next two weeks. All of the fishes placed in the CO solutions died, while the control pair was normal throughout the entire time and for two weeks afterward, when they were removed.

Table II shows the procedure in one of the experiments.

TABLE II

Showing the rate at which a liter of a saturated solution of carbon monoxide loses its toxic properties when exposed to the atmosphere in a  $5 \times 8$  inch battery-jar. Fishes used, Lepomis humilis. Two individuals placed in the jar each time.

Time	Weight of fish in grams	Dying time	Age of solution at beginning of experiment	
Nov. 22, 11:30 a.m.	4.0 6.7	$\begin{array}{c} 16 \text{ min.} \\ 25 \end{array}$	Fresh	
Nov. 22, 12:05 p. m.	5.4 7.3	45 min. 45 ''	<b>35 minutes</b> 35 ''	
Nov. 22, 3:34 p. m.	4.6 4.8	1 hr., 1 min. 1 · · 1 · ·	4 hr., 4 min. 4'' 4''	
Nov. 22, 7:58 p.m.	5.0 5.3	1 hr., 42 min. 1 '' 42 ''	8 hr., 28 min. 8 '' 28 ''	
Nov. 23	4.1 7.2	$\frac{1}{1} \frac{hr.}{} \frac{1}{27} \frac{min.}{}$	1 d <b>ay</b> 1 ''	
Nov. 24	$\begin{array}{c} 3.5\\ 4.0\end{array}$	4 hr., 40 min. 5 '' 15 ''	2 days 2 ''	
Nov. 25	5.6 6.0	5 hr., 45 min. 6 days*	3 days 3 ''	
Nov. 26	5.0 5.9	5 da., 8 hr. 5 '' 18 ''	4 days 4 ''	
Dec. 5	4.0	1 hr., 30 min.	13 day <b>s</b> ,	

\*This fish was Lepomis cyanellus, which is a more hardy fish than L. humilis.

It will be noted that the solution became less deadly as time passed, yet the fishes placed in it on the fourth day were dead on the ninth day. The explanation for the rapid death of the fish on the thirteenth day is not clear. The last of the other fishes was removed on the ninth day and the water was then not disturbed till the thirteenth day. During these four intervening days the water seems to have gained in toxicity. The tests were not carried further because the stock of fishes was nearly exhausted. The solutions in the other jars all showed the same remarkable retention of toxicity, but none of them was left undisturbed for several days and then retested, as it was not thought that such treatment would have any effect other than a further gradual diminishing of the toxicity.

It is quite evident that solutions of CO do not behave as one might expect were the gas simply in solution in the water, and it is hard to account for the tenacity with which these solutions maintain their toxicity except by supposing that the gas forms some irreversible or slowly reversible compound with the water itself, or with some substance in solution or suspension in it. In any event, it is certain that the addition of even minute amounts of CO to natural waters introduces a serious menace to the life of the organisms therein. The extremely toxic effect of very small concentrations of the gas, together with the fact that water once poisoned with it is slow to resume its normal condition, makes this gas a source of grave danger to aquatic life wherever introduced into natural waters.

Reaction Experiments.—Shelford and Allee ('14) showed that fresh-water fishes are very sensitive to carbon dioxide in a gradient and that they will turn back quite definitely from small concentrations of the gas. I have shown further (Wells, '15) that fresh-water fishes tend to select a concentration of carbon dioxide that for most species varies between I and 6 c.c.  $CO_2$  per liter. Shelford ('14) has pointed out that carbon dioxide may be used as an index to the suitability of bodies of water for fishes.

To determine whether or not fishes detect carbon monoxide and react to it in a gradient, a series of thirty-five experiments was run in which ten species of fishes were tried out. The amount of CO introduced into the treated end of the gradient varied from .5 to I c.c. per liter. Higher concentrations were tried, but they killed the fishes so rapidly that no results could be obtained with them. With the lower concentrations some very interesting results were obtained. There was no indication upon the part of the fishes that they detected the presence of the CO with any precision, and most of the records (made by graphing the movements of the fishes to a time scale) show a decided preference for the CO or treated water. (See Chart I) This does not mean that the fishes were overcome in this end and thus showed an apparent preference only, for the graphs show that in many cases they swam quite regularly back and forth from one end of the tank to the other but spent the greater part of the time in the CO water. In some instances they actually turned back from the tap water.

This preference for treated water over the tap water was noted in a series of experiments made later with salts, and at that time it was found that the acidity of the tap water, due to the presence in it of 18 c.c. per liter of carbon dioxide, was the cause of the negative reaction of the fishes (see Wells, '15a). When the salt experiments were made in aerated tap water in which the CO<sub>2</sub> was diminished to 5 c.c. per liter, normal results were obtained. In the carbon-monoxide experiments the fishes were evidently negative to the tap water because of its acidity, which, though comparatively low, was more stimulating to them than was the much more highly fatal concentration of CO. Furthermore, it would seem that the CO antagonized to some extent the action of the CO<sub>2</sub>, for otherwise the fishes would not have shown a preference for the CO end of the gradient unless they are actually positive to CO solutions in spite of their fatal effect. That such selection of fatal environments may actually occur is not impossible, but it would not be safe so to conclude until the acid factor, referred to above, has been eliminated, and this has not vet been done. However, it is safe to say that CO in solution produces no avoiding reaction upon the part of the fishes used, and for this reason its introduction into natural waters would be doubly dangerous to fishes inhabiting them.

## GENERAL RESISTANCE OF FISHES

Whether or not a particular species can persist in a given environment depends, so far as the organism is concerned, upon its ability to detect detrimental changes in the environment and to react to them, and upon its power of resisting such hurtful factors as can not be avoided by the proper reaction. These factors of reaction and resistance can never be entirely separated, but their relative importance varies widely with different species. Attached or sluggish organisms must depend to a great extent upon their powers of resistance to tide them over a period of unfavorable conditions; highly active organisms, on the other hand, may seldom find it necessary to put their resistance powers to the test, for they can move away from the disturbing conditions if these do not cover too large an area. It is probable that the reactions of most fishes have more to do with their persistence in natural environments than does their power of resistance, for the appearance of adverse conditions in natural waters is seldom so general or so sudden that fishes can not escape, by the proper reactions, at least sufficiently for survival, and observation and experiment indicate that most fishes will so react.

Although the exact relation between reaction and resistance in organisms is not clear, as a general rule, those organisms which show but little power of resistance to adverse environmental changes are for the most part quite sensitive and quick to react to such changes, while the more resistant species frequently show little or no signs of definite reaction to the detrimental factor.

The resistance of fishes to hurtful conditions varies with the species, with age (or size and weight), with the individual (that is with physiological state), and with the season. Practically all of the fishes worked with are least resistant just after the breeding season, or in the months of June, July, and August (see Wells, '16). In September the curve of resistance begins to run up, and it continues to rise throughout the winter months, reaching its maximum in March, April, and May-that is, at the beginning of the breeding season or just before. The relative resistance of species does not seem to vary greatly with the season. Just how much species vary in their relative resistance to different harmful factors is a matter for further investigation. The work so far, however, indicates that if species I is more resistant than species 2 to factor a, it is fairly safe to conclude that it will show a greater resistance to factor b also. Table III an attempt has been made to arrange the more common species of northern Illinois fishes according to their powers of resistance to detrimental environmental factors in general. Such an arrangement must at this time be considered more or less tentative because of the large number of unsolved questions concerning fish resistance in general, but the list as given will prove suggestive.

In the table the least resistant species is placed at the head of the list and given an arbitrary resistance value of 1. The succeeding species show an increasing resistance to lethal factors and their relative resistance is indicated by the figure in the middle column. The third column indicates the environments where each species is most likely to be found.

#### TABLE III

Indicating the relative resistance of the more common species of fishes to be taken in the waters of Northern Illinois, together with data as to the best type of ecological environment for each species. In column 2 the resistance of the least resistant species is arbitrarily taken to be unity.

Relative resistance	Best place to collect
1	Small rivers and clear shallow lakes. Prefers sandy bottom
2	Among the stones in the ripples of creeks and small rivers
2.3	Sandy-bottomed pools in creeks and small rivers
2.4	Lake Michigan and pools in creeks and small rivers. Prefers bottoms contain- ing some sand.
3.0	Common in Lake Michigan. Other larger lakes and rivers.
4.0	The headwaters of small creeks. In vegeta- tion along bank.
5.0	Small clear ereeks. Along with horned dace but does not go up stream as far. Often in vegetation along bank.
5.0	Swift streams; clean bottom. Small deep lakes; cold water.
6.0	Sluggish rivers and small shallow lakes with mud bottom.
6.0	Pools, mud bottom, in creeks and small rivers.
7.0	Creeks and small rivers. Swifter parts of pools.
7.0	Creeks and small rivers. Swifter parts of pools.
8.0	Wide distribution. Abundant in ponds, lagoons, and all sluggish water.
8.0	Practically same location as for white erappic.
10.0	Clean-bottomed pools with rocks. Creeks and small rivers.
10.0	Abundant in Lake Mich. Also in larger rivers but not in creeks.
15.0	Pools in creeks. Often with mud bottom.
45.0	Ponds; pools in small creeks. Mud bottom among vegetation.
	resistance

While in Table III only eighteen species of fishes are listed, the comparative resistance of other species may be estimated by comparing their resistance with that of some one of the listed species. By

placing a species of unknown resistance in an experiment with one of the species given in Table III one may obtain results that will make it possible for him to compare the resistance of the unknown species with that of any of the species listed. It should be pointed out also, that fishes of the same large taxonomic group have in general a similar power of resisting detrimental factors. Thus, the darters are a group possessing for the most part a low ability to resist untoward conditions. The minnows (Cyprinidae) are fairly resistant as a group; the sunfishes are more resistant than the minnows; and the catfishes are notably our most resistant group of fresh-water fishes. The place of an untried species in the resistance table can be reckoned more or less accurately by placing it with the listed representatives of the taxonomic group to which it belongs.

From column 3 (Table III) it will be seen that the resistance of the fishes is rather closely correlated with the type of environment which they inhabit. The more resistant species are found in ponds, shallow, muddy-bottomed lakes, or in the stagnant pools of streams. These are the fishes which one sees in aquaria. They are able to withstand increased temperature and wide fluctuation in the oxygen and carbon-dioxide content of the water, and to some extent are able to live in the presence of the excretory products of their own metabolism. The stream fishes proper can not do this, and therefore die when placed for any length of time in standing water.

#### SUMMARY

1. The introduction of either carbon dioxide or carbon monoxide into fish waters is certain to prove detrimental to the aquatic organisms, and especially to the fishes present in the water.

2. Both carbon dioxide and carbon monoxide are poisonous to fishes. Of the two gases, the monoxide is by far the more deadly.

3. Fishes are very sensitive to small changes in the carbon-dioxide content of the water, and tend to avoid detrimental concentrations of this gas by a very definite turning back from them. Fishes do not appear to detect the presence of carbon monoxide in the water, and will swim into concentrations of this gas that kill them in a few minutes.

4. In general, the resistance of fishes is correlated with the environment in which they are found. The more resistant species are found in ponds and shallow lakes while the least resistant fishes occur in the swift streams and in cold, deep lakes.

### BIBLIOGRAPHY

Shelford, V. E.

'14. Suggestions as to indices of the suitability of bodies of water for fishes. Trans. Am. Fisheries Soc., 1914:1-14.

- Shelford, V. E., and Allee, W. C.
  - '14. Rapid modification of behavior of fishes by contact with modified water. Jour. An. Behav., 4:1-30.
- Wells, M. M.
  - <sup>'13.</sup> The resistance of fishes to different concentrations and combinations of carbon dioxide and oxygen. Biol. Bull., 25: 323-347.
  - <sup>'15.</sup> Reactions and resistance of fishes in their natural environment, to acidity, alkalinity and neutrality. Biol. Bull., 29: 221-257.
  - '15a. The reactions and resistance of fishes in their natural environment to salts. Jour. Exper. Zool., 19: 243-283.
  - '16. Starvation and the resistance of fishes to lack of oxygen and to KCN. Biol. Bull., 31:441-452.

#### CHART I

Graph 1 shows the reaction of a small-mouthed black bass to water containing 35 c.c. per liter of carbon dioxide introduced at the right-hand end. The fish avoided it sharply, staying very close to the left end, where the carbon dioxide was only 3 c.c. per liter.

Graph 2 shows the reaction of an individual of the tadpole-cat (Schilbeodes gyrinus) to carbon dioxide of the same concentration as in the case of Graph 1. It will be noted that while the fish was negative to the higher concentration, more excursions were made into the higher concentration, and more time was spent there than in the case of the small-mouthed black bass. The tadpole-cat ranks with the rest of the bullhead group in having a high resistance to adverse conditions.

Graph 3 shows the reaction of a small-mouthed black bass to 0.5 c.c. of carbon monoxide per liter in the right-hand end of the gradient tank. The reaction is reversed as compared with that to carbon dioxide. The avoidance of the pure water is striking.

Graph 4 shows the reaction of the black bullhead to 0.5 c.e. per liter of carbon monoxide. The fish becomes slightly positive at the end of three minutes, and is increasingly so as time goes on, indicating that the preference for the monoxide increases with time.

Graph 5 shows the movement of a specimen of a black bullhead when there is no difference between the two ends of the tank.

