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The Determination of Hydrogen Ion Concentration in Connection With Fresh-Water Biological Studies

BY

VICTOR E. SHELFORD

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ARTICLE IX.—*The Determination of Hydrogen Ion Concentration in Connection with Fresh-Water Biological Studies.* BY VICTOR E. SHELFORD.

INTRODUCTION

Generally speaking, the significance of hydrogen ion concentration and its determination are among the most complicated problems confronting students of fresh-water ecology. Determinations of carbon dioxid, "alkalinity," and dissolved oxygen have long been among the means used for ascertaining the suitability of a water for organisms, as well as its suitability for domestic and industrial purposes, but the relations of these properties of a water to each other chemically or to organisms have never been fully investigated.

There is great confusion as to methods of measuring and expressing so-called "alkalinity." It is expressed (*a*) in terms of a carbonate of a predominating alkaline metal—for example, as $CaCO_3$; (*b*) as bound and half-bound CO_2; (*c*) as alkali reserve; and (*d*) as buffer value. It is from time to time confused with CO_2 tension, total CO_2, and the normal acid used. It is not possible to straighten out this confusion. The investigator can only ascertain the methods used by different writers and carefully translate their records and statements into the terms he himself has elected to use.

There is almost as great confusion in the use of terms and methods in connection with hydrogen ion concentration. This grows out of the fact that the turning points of various standard indicators have been referred to as "neutral," notably in the case of phenolphthalein, the turning point of which is decidedly alkaline. There are now improved methods of determining hydrogen ion concentration, including true neutrality. These have been used in the work here reported, which has consisted chiefly of (1) observations on the hydrogen ion concentration over fish breeding-grounds in several Illinois localities, July to June, 1919-20; (2) studies of the reactions of fishes to hydrogen ion concentration; and (3) studies of the effect of different hydrogen ion concentrations on the survival of fishes in water of low oxygen-content. This work has been supplemented by studies by Fenner Stickney on the resistance and reactions of a dragon-fly nymph to various hydrogen ion concentrations and by experiments by Miss Ada Hall on the effect of hydrogen ions in the development of toad and whitefish eggs, carried on under the auspices of the Department of Zoology and of the Graduate School of the University of Illinois. The author's acquaintance with the questions discussed has also been extended by a series of determinations of hydrogen ions in rivers and lakes in the following drainage systems: Puget Sound, Columbia River, Interior Basin, Colorado River, and Arkansas River. These determinations will be published elsewhere.

These studies have been made by the author largely with a view to ascertaining whether or not hydrogen ion determinations give indications of value in the field of animal ecology. The author's answer is in the affirmative. It is accordingly the purpose of this paper to show (1) that hydrogen ion concentration is one of the factors governing the movement and distribution of fishes, its importance on the whole probably equaling that of well-known factors; (2) that breeding places of fish are characterized by differences in hydrogen ion concentration; (3) that hydrogen ion concentration has marked influence on the survival of fishes under adverse conditions.

Methods of determining H Ion Concentration (pH)

The methods in common use are the colorimetric and the electrometric. The former is based on the colors assumed by solutions of various dyes in the presence of various hydrogen ion concentrations. The latter is based on measurement of the differences of the electric potential between the solution to be tested and a hydrogen electrode. In this process it is necessary to bubble hydrogen through the sample, and as this withdraws CO_2 it is not practicable as a field method. It is desirable to dip the apparatus into the mud or water and measure while *in situ*. This might be possible except for the additional fact that the pressure of the hydrogen (gas) must be maintained at one atmosphere, or corrected to this pressure, which renders the use of the electrometric method impracticable in the field. Colorimetric standards may be color charts for rough work or for work on soils—those published in Clark's book ('20) may be purchased separately—but for use in fresh water greater accuracy is necessary. For this purpose "buffer" solutions of known hydrogen ion concentration are placed in hard glass tubes, a standard amount of indicator added, and the tubes sealed up and used for comparison with water samples with the same amount and kind of indicator added. Several firms make such sets of standards, but the range at present is limited, and others must be added for some waters. These additional buffers may be made by a competent chemist. Standardization of buffers electrometrically is quite essential; also the purchased sets must be checked electrometrically at the beginning and end of each series of readings.

Hydrogen ion concentration is expressed as pH—the logarithm of the reciprocal of the normality of free hydrogen ions. The pH scale and relative amounts of free hydrogen ions are as follows:

	Acid							Neutral	Alkaline						
pH	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	10000000	1000000	100000	10000	1000	100	10	1	0.1	0.01	0.001	0.0001	0.00001	0.000001	0.0000001

The range to be expected in Illinois waters is about 8.5 to 6.7 for most streams and lakes not contaminated. Streams receiving mine seepage may have much higher hydrogen ion concentrations and still support certain species of fish.

THE INFLUENCE OF HYDROGEN ION CONCENTRATION ON FERTILIZATION, DEVELOPMENT, GROWTH, AND SURVIVAL IN WATER DEFICIENT IN DISSOLVED OXYGEN

1. *Fertilization of fish eggs.*—Miss Hall found that when oxygen is 4 c.c. per liter fertilization of the eggs of the whitefish takes place equally well in acid, neutral, and alkaline waters. When oxygen is lower (2.9 c.c. per liter) pH 6.2-6.6 is more favorable to fertilization than 7.0-8.4. This relation is quite inexplicably the reverse of what would be expected by comparison with other relations to oxygen.

2. *Development.*—Miss Hall showed that in the toad and whitefish the early cleavage and gastrulation period is most sensitive to hydrogen ion concentration and low oxygen. She further showed that early death or retarded development occurred in both toad and whitefish embryos with pH 6.2 and O_2 about 1 c. c. per liter. In the case of the whitefish, still lower oxygens gave little difference correlated with pH. The experiments with sufficient oxygen indicate that pH 7.0 is near the maximum H ion concentration which permits best development. Even so high a concentration is probably not common on fish breeding-grounds in Illinois waters. Higher concentrations, to pH 6.2, show interference with development, hatching, or length of life. That acid interferes with development was demonstrated by Loeb in 1898, and this having been found generally true, of marine animals at least, the demonstration has become a class experiment with marine forms.

3. *Survival.*—The influence of hydrogen ion concentration on the survival of fishes in low-oxygen water is shown in Tables I-IV.

TABLE I

Showing the effect of hydrogen ion concentration on the survival of rock bass in low-oxygen water, August, 1918.

Number of fish	Weight gm.	Survival in min.	pH	O_2 in c. c. per liter	Temp., deg. C.
a	10	43	6.0	.42	16
b	10	55	6.5	.22	16
c	11.5	50	6.2	.68	16
d	14.5	1500+	6.5	.79	16
e	16	71	6.1	.70	16
f	18	350	6.5	.79	16
g	18	60	6.2	.68	16
h	21	55	6.5	.42	16
i	28	45	6.3	.28	16
j	138	124	6.0	.72	16

TABLE II

Showing the effect of hydrogen ion concentration on the survival of large-mouthed black bass in low-oxygen water, January, 1921.

Number of fish		Av. weight gm.	Survival in min.	pH	O_2 c. c.	Temp., deg. C.
a	6	346	84	8.6	.18	13.5
	6	287	62	6.5	.18	13.5
b	7	275	64	8.5	.055	13.5
	6	285	47	6.2	.06	13.5
c	2	255	70	8.5	.06	13.5
	12	328	63	7.6	.06	13.5
	10	320	50	6.2	.06	13.5

Comparing individuals *a*, *b*, and *c* (Table I) it is evident that a decrease of .5 on the pH scale is more important than one of .20 c.c. in O_2 and that a decrease of .3 on the pH scale equals in effect a difference of .46 c.c. in oxygen. Furthermore, fish *c* was heavier than *a* or *b*, and the larger fish usually live longest. Fishes *f* and *g* show a striking difference in survival when both pH and O_2 are moved in the unfavorable direction. Fishes *h* and *i* are similar to *f* and *g*, but the 28-gram fish (*i*) should have lived longer considering its larger size.

Turning to Table II, group *a* shows that the average time-survival is greater at pH 8.6 than at 6.5, but the difference in weight would operate to exaggerate this. However, the same result is shown in group *b*, where both oxygen and size of fish would operate to reverse the result. Furthermore, group *c*, with fish-weight standing on the whole so as to reverse the result, shows a progressive decrease in survival-time with increase of hydrogen ion concentration.

TABLE III

Showing the effect of hydrogen ion concentration on the survival of bluegills in low-oxygen water. December, 1920, and January, 1921.

Number of fish	Av. weight gm.	Av. survival in min.	pH	O_2, c. c. per l.	Temp., deg. C.
a 4	11±	*21+	7.25	.12	13
4	11±	†21+	8.85	.12	13
b 4	11±	‡60	8.0	.12	13
4	11±	§47	8.8	.12	13
c 5	14.5	15	6.4	.12	13
6	16	30	8.8	.12	13
d 6	11	31	8.8	.12	13
4	11	23	6.5	.12	13
4	11.5	18	6.2	.12	13
e 13	12.3	8	8.5	.06	13.5
15	11.8	27	6.5	.06	13.5

* O. K. at 21 minutes. † Anaesthetized at 21 minutes.
‡ All anaesthetized at 21 minutes. § Two anaesthetized at 21 minutes.

Table III shows results with bluegills. Groups *a* and *b* are self-explanatory. Group *c* shows survival one-half as long at the low pH as at the high. Group *d* shows a progressive shortening of life as pH is lowered. Group *e* suggests that with very low oxygen, pH 6.5 is more favorable than 8.5. The work of Wells and Hall (Table IV) shows similar results with different species.

TABLE IV

Showing the relation of hydrogen ions (CO_2 added) and oxygen to the survival of fishes. Taken from the work of authors indicated.

Author	Species	Wt. in gm.	Survival in min.	pH	O_2, c. c. per liter	Survival time per gram
Wells	*Ambloplites rupestris*	±2.0	22	6.6*	.1–.15	10.9
		±2.0	46	7.0*	.1–.15	20.2
		±2.0	140	7.7*	.1–.15	62.5
Wells	*Notropis cornutus*	.6–.8	15	6.6*	.1–.15	25
		.6–.8	195	7.8*	.1–.15	246
Hall	*Coregonus clupeiformis* at hatching	....	148	6.3	.1	
		....	225	9.0	.1	
		....	240	6.3	.1	
		....	300	9.0	.1	

* pH calculated.

Powers ('22) has shown that marine fishes require more O_2 at high hydrogen ion concentrations. Thus, in general, the unfavorable effect attributed to low oxygen is partly due to the pH which accompanies it, and this depends largely on the amount of carbonates in the water. The hydrogen ion concentrations which many workers have considered that they were using when acid was added to distilled water have not been attained because of the carbonates present. It has been customary to regard distilled water as essentially free from salts, especially carbonates. Wells ('15) stated that he conducted experiments in distilled water which contained no salts. Later he showed that it had a conductivity of 600×10^{-7} which suggests $\pm.0005$ N solution of carbonates, which is now known to be approximately the carbonate content of distilled water from the source where he obtained his. He did not have anything like the H ion concentration he assumed, either in the killing or gradient experiments. If no salt had been present the pH of his ".000075 H_2SO_4" would have been between 4.0 and 5.0, which would have quickly killed the fish. An unfortunate loose statement ('15, p. 253) that "fresh-water fishes * * * cannot live normally in neutral [he used the term neutrality for the turning point of phenolphthalein, which as he used it is pH 8.0] or alkaline water caused misunderstanding. In fact, most fresh waters of the Eastern United States are alkaline, and most fresh-water animals live in such waters. The facts were that in water with pH 8.0+ the particular species with which he worked became sluggish and were unsuitable for behavior studies, or died in higher hydroxyl ion concentrations. The concentrations in which he killed the fish were probably only titrated with standard acid, and therefore it is not possible to state what the pH may have been.

TABLE V*

Showing the range of hydrogen ion concentrations entered and selected by several species of fish. The fishes were confined in a long narrow tank differing in hydrogen ion concentration at the two ends. Ordinarily they did not swim the entire length of the tank, but moved back and forth, turning at definite points, and finally coming to rest. These turning and resting points differed in different experiments (see column headings) of the same series. The upper figures of each couplet show the pH range within which the fishes came to rest; the lower figures of each couplet give the mean points of turning back when headed toward higher or lower pH, and the mean columns give the average pH content at the resting points selected. The third column from the right shows for all experiments the mean pH of the selections made. The second column from the right gives merely the maximum and minimum of the upper figures of the couplets opposite. Aerated water was deep well-water aerated. (Species nomenclature after Forbes and Richardson.)

Number of experiments and species used	Aerated water H_2SO_4		Aerated water HCl		Boiled water CO_2 Na_2CO_3		Boiled water HCl Na_2CO_3		Rain-water CO_2		Distilled water Na_2CO_3 HCl		Distilled water Na_2CO_3		Distilled water HCl and cadmium and sodium chlorides Fatal		Mean of selections	Extremes selected	Extreme range, mean in parenthesis Summary
	Mean		Mean		Mean		Mean		Mean		Mean		Mean		Mean				
(15) *Lepomis humilis*	7.4	7.8–7.0 7.4–7.3	7.2	7.9–7.0 7.4–6.8	......	....	......	....	7.1	.... 7.6–6.9	7.1	.... 7.3–6.8	......	....	......	....	7.2	7.9–7.0	7.6 (7.2) 6.8
(12) *Micropterus salmoides*	7.2	7.6–7.1 7.3	7.5	7.6–7.5 7.8–7.0	......	....	......	....	......	....	7.1	.... 7.5–6.9	7.5	7.8–7.1 7.9–6.7	......	....	7.3	7.8–7.1	7.9 (7.3) 6.7
(15 *Micropterus dolomieu*	7.2	8.0–6.9 7.8–7.6	7.1	7.1–6.6	8.0	.... 8.1–7.1	7.4	8.2–6.8 7.6–6.6	7.4	.... 7.0	......	.... 7.8–6.8	7.8	.... 8.2–7.5	......	....	7.4	8.2–6.6	8.2 (7.4) 6.6
(17) *Ambloplites rupestris*	7.4	7.8–7.1 7.3–7.2	7.7	7.8–6.8 7.5–7.4	7.5	7.7–7.4 7.0	7.1	.... 8.0–6.5	......	....	......	....	......	.... 7.9–7.3	......	....	7.4	7.8–6.8	8.0 (7.3) 6.5
(12) *Abramis crysoleucas*	7.2	7.3–7.1 7.2–6.8	7.4	7.5–7.3 7.5–7.0	......	....	......	....	......	....	......	....	8.2	.... 8.3–7.2	7.1	.... 7.3–6.8	7.4	8.2–7.1	8.3 (7.5) 6.8
(13) *Lepomis megalotis*	7.3	7.8–7.0 7.4–7.3	......	....	7.3	7.3–7.2 7.8–7.0	......	....	7.6	.... 7.2	......	....	7.4	.... 7.6–6.8	7.2	.... 8.2–7.1	7.4	7.8–7.0	8.2 (7.5) 6.8
(6) *Lepomis cyanellus*	7.2	7.7–7.1 7.7–7.4	......	....	......	....	......	....	7.6	.... 6.8	7.4	.... 8.0–7.1	......	.... 7.6–6.8	......	....	7.4	7.7–7.1	8.0 (7.4) 6.8
(1) *Notropis umbratilis*	......	....	7.5	.... 7.6–7.2	......	....	......	....	......	....	......	....	......	....	......	....	7.5	....	7.6 (7.5) 7.4
(10) *Pimephales notatus*	7.5	7.7–7.5 7.2	7.7	.... 7.6–7.2	......	....	8.2	.... 8.4–8.0	......	....	7.6	.... 7.8–7.1	7.8	.... 8.0–7.4	......	....	7.6	8.2–7.5	8.4 (7.75) 7.1
(13) *Notropis cornutus*	......	....	......	....	8.2	8.4–8.2 8.4–7.1	7.4	.... 8.3–7.0	......	....	......	....	......	....	......	....	7.8	8.4–7.4	8.4 (7.7) 7.0
(12) *Notropis whipplii*	7.8	7.9–7.7 8.0–7.3	......	....	8.2	8.4–7.6 8.4–7.6	......	....	......	....	......	....	......	....	......	....	7.9	8.4–7.6	8.4 (7.85) 7.3

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*The movements on which this table is based were graphed according to a method used by Wells ('15), and by the author in previous papers—one of them being Article VI of Vol. XI (1917) of this series—and in articles cited therein. Various types of irregularity in the figures of the table are illustrated by the following citations: In the case of *Lepomis humilis*, first double column, the discrepancy evident in the couplets is due to the fact that the fishes made some selections without swimming back and forth; in the second double column, 7.2 is evidently not the mean between 7.9 and 7.0, but it is the average of a number of selections not recorded in the table; in thefifth and sixth double columns no range in selection is shown because only one or two experiments were made in the type of water indicated, but the fishes clearly selected 7.1 as the resting point—as shown in the mean column. In the case of *Lepomis cyanellus*, fifth double column, as the fishes went to the end of the tank on the higher numerical end, no selection range could be recorded; in the seventh double column, no selection is indicated because the fishes continued to swim back and forth between the extremes indicated. Inspection of the graphs in articles referred to will facilitate an understanding of this table.

It seems abundantly demonstrated that various fresh-water fishes will live in pH 9.0. This is shown by the work of Miss Hall on whitefish and by the observation of Garrey ('16) in connection with the St. Louis city water. The resistance of forms other than fish, especially stagnant water bottom-forms may be great. Stickney ('22) found that a dragon-fly nymph, *Libellula pulchella,* lived in very high H ion concentrations. It tolerated pH 1.0 for 12 hours or more. Miss Hall found toads' eggs very resistant as compared with those of whitefish.

REACTIONS OF FISH TO DIFFERENCES IN HYDROGEN ION CONCENTRATIONS

A large series of experiments was conducted by the author* with ten species of fish and a number of types of water; aerated well-water, boiled water, distilled water, rain-water, etc. The results are given in Table V and in Figure 1. In nearly all cases the fishes reacted definitely to differences. With any given temperature, salt content, etc., they usually behaved consistently, and with any one set of conditions could be depended on to select a given hydrogen ion concentration; but as conditions were varied and the number of readings increased the results varied and were as represented in Table V and Figure 1. The selections tend to fall in two or three places, which with a larger number of readings would probably be reduced to one maximum. Each species will be seen to have a definite range which differs from every other species—as shown by the polygons. All figures run higher with the Na_2CO_3. An average of approximately twelve experiments were run with each species (the exact number is given in Table V). Fishes accustomed to live in clear open waters, especially the minnows, select the lower hydrogen ion concentrations. It is evident from the range of concentrations selected by them that all these species might be found in the same small stream during non-critical periods. The order in which the species arrange themselves corresponds to the frequency of their occurrence in the bodies of water mentioned (Fig. 1).

The range selected by each species is rather wide, though with a few exceptions the fishes show unmistakable evidences of reacting. Some of the reasons for the broken character of the curves, irregularities, wide range selected, and variation from time to time are as follows: (1) differences in salt content of the water, both experimental differences and those due to differences in aeration in storage reservoirs; (2) difference in steepness of the gradient—a difference of 6.5 to 8.2 would not be encountered in so short a distance in nature.

The following additional reactions have been estimated from published graphs and by calculations by one of the equations of Greenfield and Baker ('20), which are presented on page 388.

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* For methods see Bul. Ill. State Lab. Nat. Hist., Vol. 11 (Art. VI), page 293.

	pH selected*
Bluegills *(Lepomis pallidus)*, Wells ('15)	7.7–7.9
Bullhead *(Ameiurus melas)*, Wells ('15)	7.4–7.7
Crappie *(Pomoxis annularis)*, Wells ('15)	7.3–7.4
Rock bass *(Ambloplites rupestris)*, Shelford and Allee ('11)	7.9
Golden shiner *(Abramis crysoleucas)*, Shelford and Allee ('11)	7.7

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*pH calculated by Greenfield and Baker equation mentioned on p. 385.

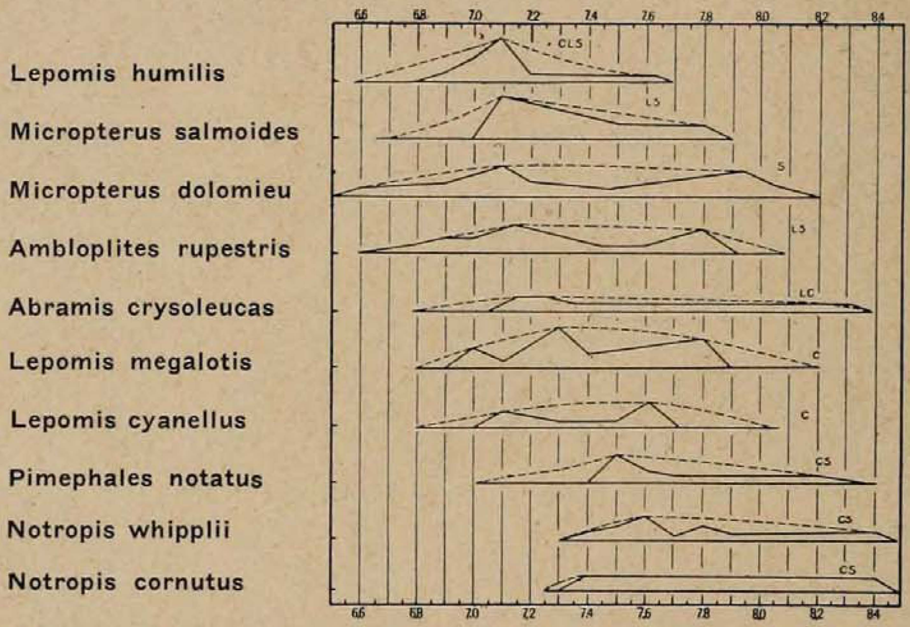

FIG. 1. Showing the range of hydrogen ion concentrations selected by ten species of fish (about 12 experiments per species). The actual selections are plotted, and a broken line indicates the author's impression as to the probable range in which they would be found. The polygons fall approximately in the order of occurrence in swift water. The letters indicate occurrence in creeks (C), small rivers (S), and in lakes and ponds (L). When two or three letters are used in connection with a polygon, the first one indicates the type of water in which the species is most numerous, and a single letter signifies that the species is commonly found only in that one type of water.

AN EXAMINATION OF CERTAIN ILLINOIS WATERS WITH SPECIAL REFERENCE TO HYDROGEN ION CONCENTRATION

A study of fish breeding-grounds was conducted by the author in 1919-20, and the pH values observed are shown in Table VI; the oxygen, in Table VII. There was a marked difference between the carp and bass breeding-grounds; in no case were they the same either at the top or bottom. The bass breeding-grounds were characterized by clean sand bottom; the carp grounds, by dark mud. Table VI shows sharp differences in oxygen content at the bottom at all dates on which they were examined.

TABLE VI

Showing oxygen in c.c. per liter and per cent. of saturation on fish breeding-grounds, 1919-20: Round Prairie, carp breeding-grounds; Warner's Cut, bass breeding-grounds; Hickory Creek, a typical unpolluted stream; ponds at the head of Lake Michigan.

Locality	July 16		Sept. 4		Nov. 28		Apr. 2		June 3		Mean	
	c. c.	%	c. c.	%	c. c.	%	c. c.	%	c. c.	%	c. c.	%
Round Prairie..	9.75	12	dry	dry	5.5	58	4.1	52	4.6*	71*	3.45	44
Warner's Cut...	8.0	150	dry	dry	9.2	95	5.4	70	(†)	....	7.5	105
Illinois River...	3.7	61	2.5	40	...	..	6.0	77	4.8	78	4.2	64
Pond I	....	...	14.7	235	7.8	94	....	....	6.9	110	9.8	146
Pond V	....	...	7.3	135	7.5	88	8.1*	183*	4.5	70	6.4	98
Pond VII, W...	....	...	17.8	330	8.1	96	....	....	7.5	120	11.1	182
Pond XIV	....	...	12.4	230	6.8	81	....	....	6.9	112	8.7	141

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* Not included in the mean.

† High on-shore wind prevented our reaching this station. The strong waves should have given a 100% saturation.

TABLE VII

Showing the hydrogen ion concentration in different fish-waters, July, 1919, to June, 1920.

Locality	Bottom						Top					
	July 16	Sept. 4	Nov. 28	Apr. 2	June 3	Mean	July 16	Sept. 4	Nov. 28	Apr. 2	June 3	Mean
Lake Michigan..	....	....	...	...	....	....	7.9	7.9	7.9	7.8	7.9	7.9
Round Prairie...	7.15	dry	7.3	7.3	7.3	7.27	7.2	dry	7.3	7.4	7.6	7.4
Warner's Cut...	7.95	dry	7.9	7.4	...	7.75	8.2	dry	7.9	7.5	...	7.8
Ill. Riv., Jefferson St., Havana...	7.58	....	...	...	....	....	7.7	7.4	7.6	7.5	7.4	7.5
Spoon River	7.4	....	...	...	....	....	7.5	7.5	7.9	....	7.3	7.55
Hickory Cr......	7.6	7.6	7.5	7.5	7.8	7.6	7.9	7.8	7.5	7.6	7.8	7.7
Pond I	7.55	7.4	7.9	...	8.0	7.7	7.6	7.8	7.9	....	7.9	7.8
Pond V	7.7	7.3	7.5	...	7.8	7.6	7.0	7.7	7.6	8.0*	7.9	7.5
Pond VII, W.....	8.5	7.85	7.9	...	7.4	7.9	8.2	8.2	7.9	....	7.9	8.05
Pond XIV	7.85	7.5	7.6	...	7.3	7.5	7.9	8.2	7.9	....	7.9	7.97
Pond VII, E.....	6.8	6.8	7.3	...	7.6?	7.1	7.2	....	7.3	....	7.7	7.4

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* Not included in the mean.

The series of ponds was quite fully studied in 1909-1911 (see Biological Bulletin, volumes 21 and 22).

Pond I at that time contained bass, sunfish, and perch. Bare sand breeding-bottom.

Pond V contained perch and chub-suckers. Bare sand breeding-bottom.

Pond VII contained chub-suckers, golden shiner, bullhead, and mud minnow. No bare breeding-bottom. Pond VII, E, is much older than VII, W.

Pond XIV contained black bullheads. No bare breeding-bottom.

A few observations were made on pH at bottom under certain types of vegetation: Under white water-lilies, 6.9-7.2; under yellow water-lilies, 7.7-7.9; under duckweed and smartweed, 6.8-7.3.

GENERAL RELATIONS OF HYDROGEN ION CONCENTRATION TO OTHER FACTORS

1. *Relations of pH to dissolved oxygen.*—There are nearly always correlations between CO_2 content and dissolved oxygen, at least with a fairly constant 'alkalinity." A fairly constant alkalinity exists in the sea. Relative to the sea, McClendon ('17a) states that "in so far as the sea is a closed system O_2 varies inversely with CO_2, due to the action of organisms, the possible error being 30 percent." He presents a chart showing the amount of oxygen to be expected in sea water of various "alkalinities," etc. Below the thermocline a lake in summer is a closed system, and in so far as alkalinity remains constant there is an inverse relation of O_2 and CO_2, which is, however, by no means constant. In some cases the sum of O_2 and CO_2 in c.c. per liter is a constant, but the relation is always an inverse one. This stability of the O_2 and CO_2 values probably depends upon circulation, diffusion, changes in alkalinity, etc., but in all the work described herein there is a direct relation between pH and O_2 (cf. Table VII and Table VI). When hydrogen ions content decreased, as indicated by higher pH figures, oxygen increased.

2. *Relations of pH to carbon dioxid and "alkalinity".*—In waters of about the same alkalinity the amount of free CO_2 is as good an index of its suitability for fishes as hydrogen ions (Shelford and Allee, '10). The amount of CO_2 means nothing, however, unless alkalinity be measured. The work of Greenfield and Baker ('20) shows that the H^+ ions may be calculated. The equation is

$$H^+ = \frac{4\ CO_2 \times 10^{-7}}{(HCO_3^-)} + 1 \times 10^{-8}$$

when CO_2 is expressed in p.p.m. and bicarbonate as p.p.m. $CaCO_3$ but when both bicarbonate and free CO_2 are expressed in c.c. per liter* the equation is

$$(H^+) = \frac{3.5 \times 10^{-7}\ CO_2}{(HCO_3^-)} + 1 \times 10^{-8}$$

"To check the accuracy of these calculations, several samples of water, from a variety of sources and varying widely in mineral and organic content, were examined. Bicarbonate and free carbon dioxide

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* Total must be used, i. e. bound and half-bound.

were determined according to 'Standard Methods of Water Analysis'. The free carbon dioxide titrations were continued to a faint pink which was persistent for 3 min. The hydrogen ion concentration was determined colorimetrically, using standard buffer solutions which had been checked by means of the hydrogen electrode. * * *

"In only one case [out of 63] was the difference between the determined and calculated P_h^+ greater than 0.3, and the mean variation is about 0.1. It will also be noted that the wider variations occurred in the cases of low bicarbonate content, which is to be expected from the assumptions made in the development of the equation. * * *

"From their experience with the colorimetric P_h^+ determinations, the authors do not feel that determinations can be made much more accurately than 0.2 P_h^+, using open tubes and ordinary methods of transferring the test sample to the tube. The effect of aeration of such unstable solutions as natural waters should amount to this much or more. It is advisable in all cases to make several determinations.

"The formula cannot be applied to waters which are alkaline to phenolphthalein. An attempt was made to develop such an equation, but no waters naturally alkaline to phenolphthalein were available for checking the calculations. For unusual cases of this kind and cases of low bicarbonate content, it may be better to use some more complete and more complex equation, such as has been developed by Prideaux (Proc. Roy. Soc. London (A) 91, 535)."

"Equations are developed for calculating H^+-ion concentration, in which the carbon dioxide and bicarbonate are expressed in the manner in which they are ordinarily determined. These equations are less accurate with low bicarbonate concentrations, and do not apply to waters alkaline to phenolphthalein."

TABLE VIII

Comparison of calculated H ion concentrations with those determined colorimetrically. (Excerpts from a table by Greenfield and Baker.)*

Res. on evap.	Free CO_2 p. p. m.	Bicarb. as p. p. m. $CaCO_3$	P_h^+ det.	P_h^+ calc.	Error	Source of sample
375	0.0	154	7.9	8.00	—.10	Vermilion River
375	7.0	152	7.5	7.54	.04	Vermilion River
375	15.5	152	7.2	7.28	.08	Vermilion River
375	16.5	152	7.1	7.29	.19	Vermilion River
375	29.5	152	6.9	7.05	.15	Vermilion River
384	6.0	100	7.3	7.47	.17	Kankakee River
384	14.0	100	7.1	7.18	.08	Kankakee River
384	25.0	96	6.7	6.94	.24	Kankakee River
206	15.0	44	6.9	6.83	—.07	Ohio River
206	29.0	44	6.5	6.52	.02	Ohio River
206	30.0	44	6.5	6.52	.02	Ohio River
225	11.0	90	7.3	7.23	—.07	Miss. River

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* Jour. Industr. and Eng. Chem., Vol. 12 (No. 10), p. 991. 1920.

TABLE IX

Values of pH corresponding to varying concentrations of free carbonic acid and bicarbonates calculated by the mass-law equation. (Table prepared by Greenfield and Baker.) Near-neutrality in bold-face figures. V. E. S.

HCO_3 p.p.m. $CaCO_3$	CO_2, parts per million												
	0	1	2	4	6	8	10	12	14	16	20	30	40
10	8.00	7.30	**7.05**	6.77	6.60	6.48	6.39	6.31	6.24	6.19	6.09	5.92	5.79
20	8.00	7.52	7.30	**7 05**	**6 89**	6.77	6.68	6.60	6.54	6.48	6.39	6.21	6.09
40	8.00	7.70	7.52	7.22	7.15	**7 05**	**6 96**	6.89	6.82	6.79	6.68	6.51	6.39
60	8.00	7.78	7.63	7.43	7.30	7.20	7.12	**7 04**	6.98	6.93	6.84	6.68	6.56
70	8.00	7.80	7.67	7.48	7.35	7.25	7.17	7.10	**7 04**	6.99	6.90	6.74	6.62
80	8.00	7.82	7.70	7.52	7.40	7.30	7.22	7.15	7.10	**7 05**	6.96	6.80	6.68
90	8.00	7.84	7.72	7.56	7.43	7.34	7.26	7.20	7.14	7.09	**7 00**	6.84	6.73
100	8.00	7.85	7.74	7.58	7.47	7.38	7.30	7.24	7.18	7.13	7.05	6.89	6.77
120	8.00	7.87	7.78	7.63	7.52	7.44	7.36	7.30	7.25	7.20	7.11	6.96	6.84
140	8.00	7.89	7.80	7.67	7.57	7.48	7.41	7.35	7.30	7.25	7.17	**7.02**	6.91
160	8.00	7.90	7.82	7.70	7.60	7.52	7.46	7.40	7.35	7.30	7.22	7.07	6.96
180	8.00	7.91	7.84	7.72	7.63	7.56	7.49	7.43	7.39	7.34	7.26	7.12	**7 00**

3. *Relations of pH to putrescibility and pollution.*—Under ordinary conditions putrescibility accompanies high free CO_2. This is determined partially, however, by alkalinity, but with a constant alkalinity, CO_2 usually varies directly with the putrescibility. In the Illinois River, with an alkalinity of 100 to 140 p.p.m. of $CaCO_3$, samples show pH values up to 7.2 with CO_2 at 8 p.p.m. several days after being shipped from points above Chillicothe. Here the calculated values were about 3 points too high, which was probably because the formula gives incorrect results in the presence of free ammonia. The low CO_2 values are surprising for the Illinois River.* Farther down the stream, at Chillicothe, September 6, 1919, I found pH 6.8 at the surface, and 6.9 at the bottom with green algae in evidence, and O_2 .9 c.c. per liter at the bottom, 20 feet from the west shore. On September 3, at Liverpool we found pH 6.9 at the bottom and O_2 .96 c.c. per liter, with the bottom fauna destroyed. The fact that CO_2 values appear to be small in this stream makes the calculated pH values too high. In this case, therefore, pH determinations would appear more significant than CO_2 determinations. Calculated values should not be used except where determinations are not practicable—e. g., in working over old data. Too many waters contain ammonia or other disturbing substances.

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* Weston and Turner ('17) found high CO_2 in a polluted stream, but fail to give the alkalinity.

DISCUSSION AND SUMMARY OF RESULTS

Present-day methods of determining hydrogen ion concentration in detailed manner have been developed by biochemists, bacteriologists, and oceanographers; not by physical chemists, as one might expect. Its importance in the study of fishes came to the author's attention in course of experiments performed in 1911 (Shelford and Allee). This work was followed by that of several students. The important papers by Palitzsch and Sorensen—see bibliography in Clark ('20)—were overlooked by the writer until 1916. These important papers appear not to have been appreciated until 1915. The aid and advice of physical chemists was sought, but these investigators regarded differences in pH of less than unit value—e. g. between 8.0 and 9.01—of little importance, though they made important contributions to the subject. The turning-point of phenolphthalein was stated by Washburn ('15) to be pH 9.0; its turning-point in water analysis (faint pink lasting 3 minutes) is pH 8.0; all depends upon the method employed.

The summary of Wells' paper ('15) previously referred to, should read about as follows:

1. Hydrogen ion concentration is an important factor in determining the reaction and resistance of fishes.
2. Most fresh-water fishes select slightly alkaline water in a gradient, but when offered a gradient from the turning-point of phenolphthalein to higher hydroxyl ion concentrations (pH 8.0-9.0) the more alkaline end is selected.
3. The optimum CO_2 varies from 0 (pH 8.0) for bluegills to 6 c.c. per liter in water with about 200 parts per million alkalinity (pH 7.4-7.6) for sunfishes and crappies. Optimum sulphuric acid was not determined because of carbonates in the distilled water used.
4. The distribution of plankton in Wisconsin and New York lakes shows fewest animals in the stratum at the turning-point of phenolphthalein (pH 8.0), suggesting a negative reaction to water with pH 8.0.
5. No good results were obtained with once-distilled water because of carbonates present.

The chief occasion for criticism of Wells' paper is the confusion relative to neutrality. A repetition of his experiments, which was one of the purposes of the present paper, and a close study of his work shows that he regarded the turning-point of phenolphthalein (pH 8.0) as neutral, or "near enough" to it. The avoidance of pH 8.00 when this accompanied higher alkalinity, as found by Wells, has been confirmed by Miss Hall in case of young whitefish. The table constructed by Wells showing the avoidance of pH 8.0 by plankton (New York and Wisconsin lakes) is correct in this respect, but the columns to the right and left of the phenolphthalein neutrality are by no means comparable in hydrogen ion concentration. The statements by Wells relative to CO_2, together with those in the paper of 1911 by Shelford and Allee, are correct only for the "alkalinities" used, which varied around 100 p.p.m. of $CaCO_3$ or 120 p. p. m. of HCO_3. Several statements quoted from Wells ('15) and

Washburn ('15) in my chapter in Ward and Whipple's "Fresh Water Biology" are erroneous. Shull's "Principles of Animal Biology" (page 277) includes statements of a similar nature which should be corrected. These various statements relative to CO_2 optima, etc., are not to be regarded as errors when made; later evidence has merely shown that hydrogen ion concentration is a better index than CO_2.

There are a number of general reasons for determining hydrogen ions, such as the close regulation of their concentration in the animal body and their apparent great physiological importance in vertebrates. The writer does not, however, wish to give the impression that he means to advocate the use of pH determinations to displace any other determinations now in common use unless it be CO_2, and not even in this case until further investigations have been conducted.

CONCLUSIONS

Hydrogen ions should be determined for the following specific reasons:

1. The effect of various concentrations of oxygen are modified by the hydrogen ions present, particularly the ill effect of low oxygen on survival and development, which is increased by the high hydrogen ion concentration which accompanies it in waters of low alkalinity. In a general way the hydrogen ion concentration varies inversely with the oxygen content in bodies of water with stable alkalinity (e. g., see Tables VI and VII and Graph 1).

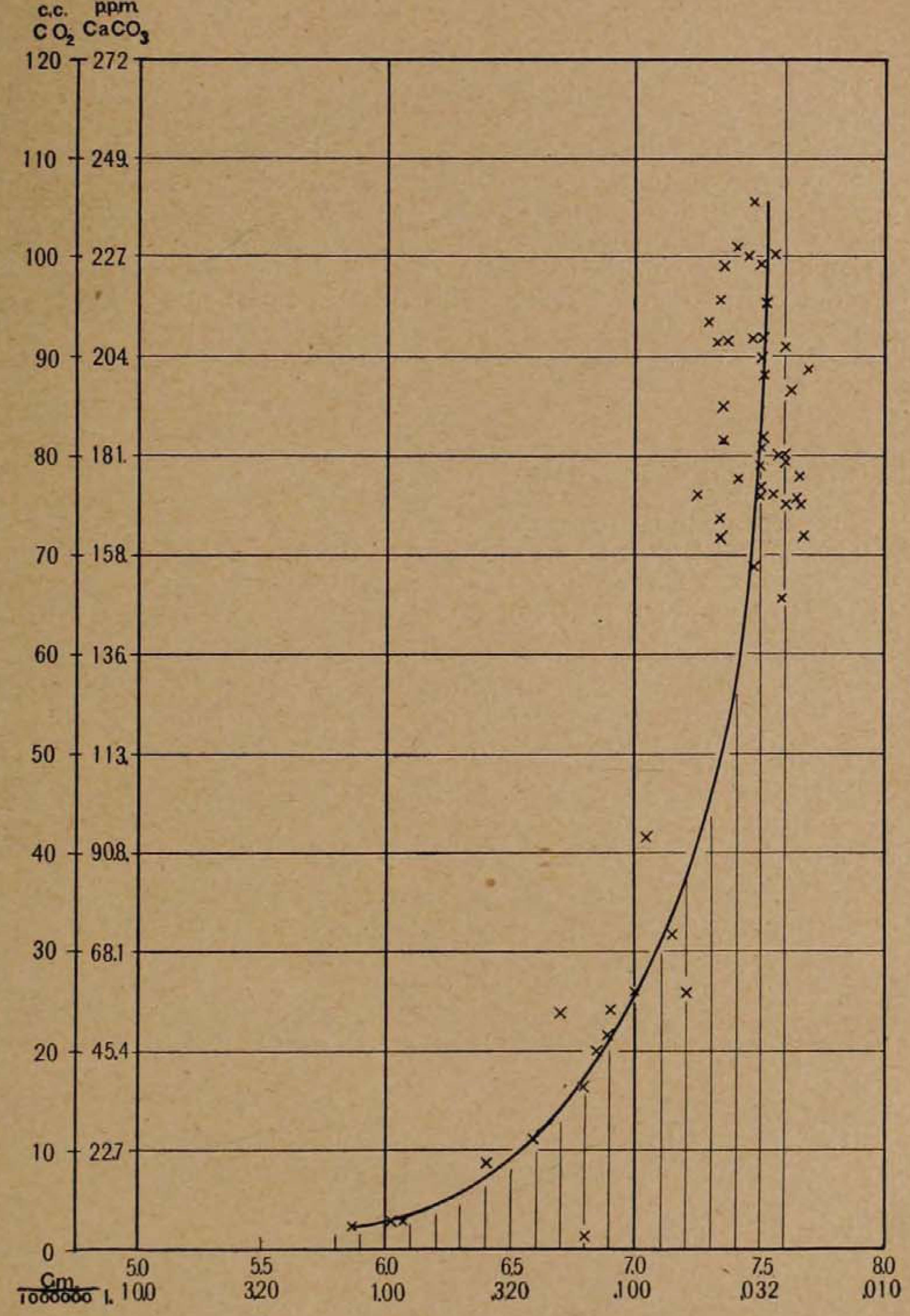
2. With the *same oxygen content,* different hydrogen ion concentrations have definite effects on the rate of development and time of survival of aquatic animals.

3. Many animals react definitely to hydrogen ion concentration (fishes, crayfishes, Entomostraca) in a manner similar to their reactions to temperature. Each species tolerates a rather wide range with a fairly definite optimum.

4. The determination of hydrogen ions is to be preferred to CO_2 determinations because it is the free hydrogen ions which are most effective, and, as shown in Table IX, column 6, for example, while the CO_2 remains constant (6 p.p.m.), hydrogen ions concentration changes by tenfold (pH 6.6 to 7.63) owing to difference in alkalinity.

5. Colorimetric determinations of pH are about as accurate as CO_2 determinations and can be made on the spot very quickly. With some simple device for collecting (see Richardson's Fig 6, page 372, Vol. XIII of this series), aeration can be reduced, and very small quantities of water used as contrasted with the usual 250 c.c. samples.

6. The distribution of plants and animals is correlated with hydrogen ion concentration.



Graph 1. Showing the range of hydrogen ion concentration accompanying a *trace* of oxygen in Wisconsin lakes. Calculated from data obtained by Birge and Juday ('11). At the left, alkalinity is shown as CO_2 and $CaCO_3$; below, hydrogen ions, as pH and as grams per million liters.

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