RANGES OF pH TOLERANCE OF THE LEMNACEAE.*

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The hydrogen ion concentration is extremely important in many of the chemical reactions which take place within any plant structure and must be taken into consideration in any attempt to understand and explain those factors which may influence the growth, reproduction, abundance and distribution of the Lemnaceae or duckweed family.

Mineral salts in water in which aquatic plants are growing may: (a) furnish raw materials, (b) act as catalytic agents, (c) determine acidity and alkalinity of the medium and (d) provide a balanced solution. In addition, acids and alkalies may have a toxic effect upon living cells by changing their permeability.

The important effect of the extremes of pH concentration upon plant growth in general, being generally accepted, the present paper is a record of various experiments and field observations to determine to what extent the pH concentration ranges commonly found in the waters of the state of Ohio limit or promote the growth and distribution of the various species of Lemnaceae. The major portion of this work was done between October, 1928, and June, 1929, and this report is a revision of a paper prepared at that time.

In all, more than 660 hydrogen ion determinations were made of: (a) the waters in which 7 species of Lemnaceae were growing in 36 Ohio counties, (b) the organic media in which these same species were growing in greenhouse cultures and (c) various buffered inorganic salt solutions with pH values arranged in series.

METHOD OF MAKING DETERMINATIONS.

The colorometric, the quinhydrone and the hydrogen electrode methods of pH determination were compared and the quinhydrone method was found to be superior to the others in every way for the purposes of these experiments. All of the determinations given were made by that method. Samples of

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water to be tested (free from duckweeds or other organic matter) were collected in 40 or 50 cc. stoppered, chemically clean glass bottles and carefully labeled with all necessary data. The actual determinations were made in the laboratory. Samples of greenhouse cultures were tested immediately after collection. Field samples were usually tested within a few hours after collection and at temperatures similar to those at the time of collection. Duplicate samples thus collected would check to within .01 pH or less and often gave identical readings to the third place. Samples redetermined three weeks after collection showed only a very slight variation from the original reading. Thus the method of determination used seemed to be an extremely accurate and practical one.

The pH concentrations of the stream waters of the state of Ohio, as determined from a limited number of samples, range from about pH 5.6 to pH 8.8, although many springs and stagnant bodies of water, especially swamps and bogs, may range down to pH 4. Most of the stream waters of the state are on the alkaline side of the neutral point, especially those of the western half of the state in the limestone areas.

Many of the streams of the acid-forming sandstone regions of the Alleghany Plateau are somewhat acid, especially in the sections covered by the Black Hand formation of the Sugar Grove region. More than 700 Ohio stream water pH determinations by the colorimetric method were made by E. L. Wickliffe, Chief of the Bureau of Scientific Research, Ohio Division of Conservation (unpublished). His results showed that the stream waters of the eastern half of the Lake Erie drainage average pH 7.5, the western half pH 8.0, the unglaciated Ohio drainage pH 7.27 and the glaciated Ohio drainage ph 7.9.

It is very important to remember that the pH concentration of any body of water is not a fixed value, but is highly variable. The extremes of pH values over a period of time are probably of more importance in effecting plant life than just the average pH values of the same period. pH values of water areas supporting duckweed populations are known to vary with (a) dissolved soil constituents, (b) temperature, (c) presence of buffers, (d) time of year or season, (e) amount of rainfall, (f) decay of organic matter and other factors.

Rather large variations in pH values result from temperature changes alone, especially in poorly buffered solutions. Low temperatures tend toward acidity, high temperatures

toward alkalinity. This is largely due to the greater amounts of carbon dioxide dissolved at the lower temperatures. Most of the very acid determinations made of bog waters are partly due to the lower temperatures of the water among bog plants. Samples of water collected only a few feet away from the edge of a sphagnum bog were often distinctly alkaline. A sample of distilled water at 23 degrees C. had a pH of 4.98, but when boiled for a few minutes to drive off the dissolved carbon dioxide, gave a pH value of 6.8. pH-temperature curves, made from readings taken by E. L. Wickliffe at Buckeye Lake over a period of several months, showed a rather distinct correlation between the two.

TABLE I.

Species	Number of Times Occurring in pH Values of									
	4.6 to 5.0	5.0 to 5.4	5.4 to 5.8	5.8 to 6.1	6.1 to 6.4	6.4 to 6.7	6.7 to 7.0	7.0 to 7.3	7.3 to 7.6	7.6 to 7.9
Spirodela polyrhiza	....	....	....	4	11	36	39	46	10	....
Lemna trisulca	....	....	8	5	28	35	7	2	....	....
Lemna minor	....	6	14	9	46	51	28	16	10	....
Lemna cyclostasa	....	....	....	2	6	4	1	....	....	....
Wolffia columbiana	....	....	....	5	14	23	21	12	10	....
Wolffia punctata	....	....	....	....	2	10	11	8	10	....
Wolffiella floridana	....	6	5	5	6	....	....	....	....	....

1. *pH Determinations of Water Media of Wild Plants.*

Table I summarizes the number of times each of the seven species studied occurred in water of the several pH ranges. The results include 212 determinations of water areas in 36 Ohio counties in which one or more species of duckweeds were found growing. Note that *Lemna cyclostasa* and *Wolffiella floridana* have a very restricted distribution in Ohio.

2. *pH Determinations of Greenhouse Cultures.*

A total of 228 pH determinations were made of the water media containing organic matter in which the seven species were growing under greenhouse conditions. Most of these determinations were made from November, 1928, to June, 1929. Cultures tested were of plants growing in tap water or pond water to which had been added a quantity of soil or crushed

stone of all types available, various fertilizers, manures, ground peat or other organic matter. The media produced varied from about pH 4 to pH 8 and made it possible to observe the plants in a wide range of growth conditions. Check cultures were made of each medium type prepared to aid in the interpretation of results. All pH determinations were checked by testing duplicate samples.

The size, general condition and rate of growth of the plants in each case, were carefully noted. Frequent renewals of the

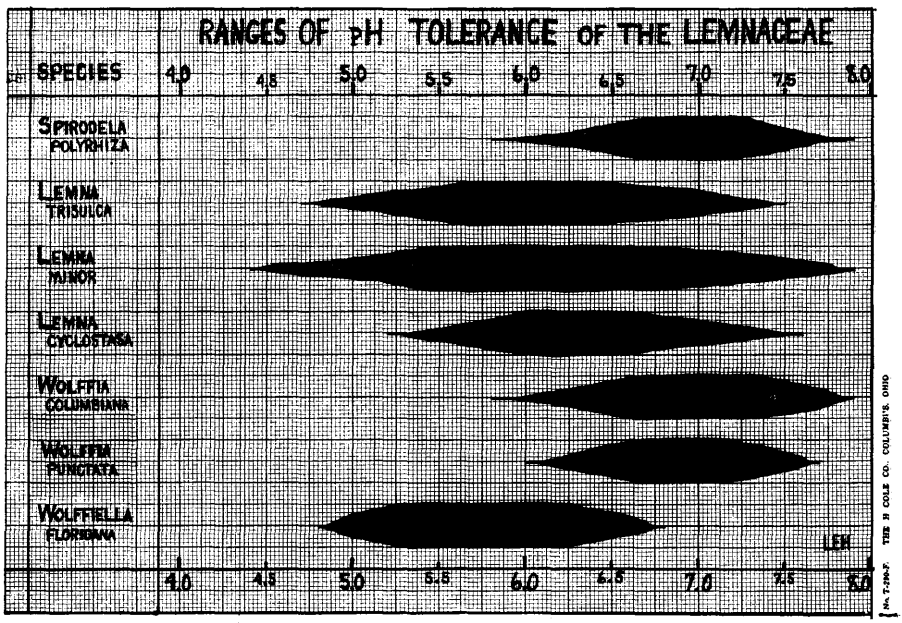
TABLE II.

Species	pH Values Accompanying		
	Good Growth	Fair Growth	Poor Growth
Spirodela polyrhiza.....	6.6 to 7.3	6.4 to 6.6 7.4 to 7.6	5.8 to 6.4 7.6 to 7.9
Lemna trisulca.....	5.6 to 6.5	5.2 to 5.6 6.5 to 7.2	4.6 to 5.2 7.2 to 7.5
Lemna minor.....	5.4 to 6.8	5.0 to 5.4 6.8 to 7.4	4.5 to 5.0 7.4 to 7.8
Lemna cyclostasa.....	6.0 to 6.7	5.7 to 6.0 6.7 to 7.0	5.2 to 5.7 7.0 to 7.6
Wolffia columbiana.....	6.5 to 7.4	6.3 to 6.5 7.4 to 7.7	5.8 to 6.3 7.7 to 7.9
Wolffia punctata.....	6.5 to 7.3	6.3 to 6.5 7.3 to 7.6	6.0 to 6.3 7.6 to 7.7
Wolffiella floridana.....	5.2 to 6.2	5.0 to 5.2 6.2 to 6.5	4.8 to 5.0 6.5 to 6.8

medium to remove bacterial or algal growth, usually resulted in more rapid growth. There was considerable evidence to show that the pH value for best growth varies considerably with the composition of the medium. Plants in media containing an abundance of organic matter were tolerant of somewhat wider extremes of pH plants growing in media entirely or almost lacking in organic matter. As might be expected, plants in culture jars grew in a somewhat wider range of pH than plants of the same species occupy under natural conditions. A summary of growth results is shown in Table II.

3. *Growth of Plants of Seven Lemnaceae Species in Various Buffered Inorganic Salt Solutions with pH Values Arranged in Series.*

In these experiments a Clark (1925) series of buffer solutions was used. Cultures of 10 to 50 plants of each species were placed in 100 cc. wide-mouthed glass bottles with media of pH values as follows: 1.2, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9 and 10. The medium used was made of equal volumes of



Clark's buffer solutions and Knop's solution. Later the experiment was repeated using Crone's and Shive's solutions. Also the experiment was repeated using McIlvaine's buffer solutions, but the results obtained were so nearly identical with those where Clark's solutions were used, that any of the variations in growth of the cultures appeared to be the actual effects of the pH values and were not seriously modified by any toxic or beneficial effects of the salts.

Where the plants remained alive, the old media were replaced with new solutions. In a few cases renewal was made every two or four days, but no differences in results were noted when compared with cultures which were changed at the usual

weekly periods. The pH of the media in each case was checked by the quinhydrone method at the beginning of the experiment and once (sometimes more often) each week thereafter. Where plants remained alive, the cultures were continued for a 12-week period.

All of the species in the pH 1.2 media died in less than an hour, the roots dying first, shriveling commencing at the tip and proceeding upward toward the point of attachment at the node. In the strong acid solutions, the *Wolffias* died first, then *Spirodela* and lastly the *Lemna* species.

In the literature, only two references were found concerning ranges of pH tolerance of any duckweed species. Clark (1926) grew *Spirodela polyrhiza* in solutions ranging from pH 4.4 to 4.8 and reported best growth from pH 4.6 to 4.8. This does not agree with my results or with those of Deuber (1926), who found that the same species made best growth from pH 6.2 to pH 6.8.

A summary of the results for each species is as follows:

1. *Spirodela polyrhiza*.—This species died in all of the media of pH 5 or less. Plants in pH 5.5 and pH 6 made poor growth, a few plants died and many resting plants were produced. Plants from pH 6 to pH 7.5 made vigorous growth and multiplied rapidly. At pH 8 very poor growth took place, but the plants remained alive 6 weeks and produced resting plants abundantly. More alkaline solutions killed the species.

2. *Lemna trisulca* died at pH 5 or below and at pH 8 or above, made poor growth at pH 5.5 and 7.5 and good growth from pH 6 to pH 7.

3. *Lemna minor* became pale in color and soon died at pH 4 or less and at pH 8 or more. Poor growth was made at pH 4.5 and 5.0. Good growth was made from pH 6 to pH 7.5.

4. *Lemna cyclostasa* died at pH 5 or below and at pH 8 or above, made poor growth at pH 5.5 and 7.5 and good growth from pH 6 to pH 7.

5. *Wolffia columbiana* died at pH 5.5 or below, made poor growth at pH 6, vigorous growth at pH 6.5 to pH 8, poor growth again at pH 8.5 and eventually died, but lived a number of days even at pH 9 and 10.

6. *Wolffia punctata* gave results almost identical with those of *W. columbiana* except that the species was slightly less tolerant of the pH extremes.

7. *Wolffiella floridana* grew only from pH 5 to pH 6.5.

SUMMARY AND CONCLUSIONS.

1. The pH concentration, though not always a limiting factor in our waters, is often an important one in affecting growth and distribution.

2. Some factor other than pH must be used to explain the total absence of duckweeds in a number of important bodies of water in the state. Examples: Bonnett Lake, Holmes Co., pH 6.34; West Swamp, Westerville, Franklin Co., pH 6.96; Chippewa Lake, Medina Co., pH 6.31; and Lake Brady, Crystal Lake, Muzzey Lake and Lake Pippen, all in Portage Co., with waters of pH 7.07, 5.77, 6.55 and 6.37, respectively.

3. *Spirodela polyrhiza*, almost without exception, is deeply pigmented wherever found in alkaline waters. *Lemna minor* frequently produced some red pigment in waters of pH 7 to 7.9.

4. *Spirodela polyrhiza* was found in waters ranging from pH 5.9 to 7.9 and was abundant from pH 6.3 to 7.5.

5. *Lemna trisulca* grew in waters ranging from pH 4.9 to 7.3 and abundantly from pH 5.1 to 6.7. This species, however, showed a marked preference for the cool acid waters of bog types.

6. *Lemna minor* showed a marked tolerance for pH variations, being found from pH 4.4 to 7.9 and abundantly from 5.1 to 6.7, a tolerance range including the pH ranges of tolerance of all of the other Ohio species.

7. *Wolffia columbiana*. Range of pH 5.9 to 7.8. Best growth and most abundant from pH 6.4 to 7.4.

8. *Wolffia punctata*. Range of pH 6.0 to 7.7. Best growth and most abundant from pH 6.4 to 7.4.

9. *Wolffiella floridana* tolerated pH ranges of 4.8 to 6.8. This species grows best among the abundant organic matter of bogs or boggy-swamp types.

10. The following graph shows the ranges of pH tolerance of each species as indicated by the results of the three sets of experiments and pH determinations. The width of the bands following each species indicate the frequency of occurrence and the limits of good growth of each species for each pH value.

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Progress in Microbiology.

This is the third book to appear in the "Century of Progress" series published in connection with the Century of Progress Exposition. It is an exceedingly interesting account of the part microbes have played and are playing in man's varied activities. The first thought in the minds of many on hearing the word microbe is "disease." This story of microbes, while taking full account of the important part played by microscopic forms in disease, draws interest as well to the useful and essential activities of such forms in agriculture, industry, sewage disposal and other of man's fundamental problems. The development of the story of microbes and man is so clearly and instructively drawn that the book should appeal to a wide circle of readers among those interested in keeping informed on modern progress in the sciences.—L. H. S.

Man and Microbes, by STANHOPE BAYNE-JONES. x+128 pp. Baltimore, The Williams and Wilkins Co., 1932. $1.00.

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The Story of Living Things, by CHARLES SINGER. xxxv+572 pp. New York, Harper and Bros., 1931. $5.00.