THE PHYTOPLANKTON OF A SOLUTION POND WITH SPECIAL REFERENCE TO THE PERIODICITY OF CERTAIN ALGAE

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Review of Literature. Numerous contributions have been made to the knowledge of algal periodicity in England and, from time to time, in this country. The pioneer work in this field was done by Fritsch. In a paper of his (15) published in 1906, he suggested problems for investigation and later, in collaboration with Rich, carried out observations on the "Occurrence and Reproduction of Algae in Nature" (16). They found that Spirogyra exhibits a purely vernal or both vernal and autumnal phase with an intervening space of scarcity or complete disappearance. Reproduction takes place in the vernal phase, and is most probably the result of certain periodically recurring combinations of factors, which vary for different species. They concluded that dilution of the water to its ordinary degree of concentration is necessary to an autumnal phase of Spirogyra.

West (45) worked on the Desmid plankton and their distribution and periodicity in some British Lakes. Later Pearsal contributed several valuable papers. He found that Diatoms were more abundant or dominant during the winter, that a deficiency of oxygen, nitrates, silica or calcium was usually a limiting factor and that diatom maximum follows a flood period. Later he made four hundred complete and several thousand partial analyses of surface water which tend to confirm his Diatom Theory (33).

Griffiths (19) found that deep pools contained little or no Chlorophyceae while in shallow pools, they become more numerous. A great abundance of Myxophyceae is not usual in shallow pools. He also found the desmid constituent associated with a high K-Na/Ca-Mg salts ratio and low organic content of the water. According to him, the dominance of the Myxophyceae may be ascribed to the increasing organic enrichment of the anaerobic type. Many of the so-called "water blooms" occur in the fall when the seasonal vertical circulation is bringing up to the surface layers the fermentation products from the bottom of the lakes. Certain algae, namely the Protococcales, are associated with the occurence of sediments lying in shallow water where the oxygen content of the water is relatively high. Other algae, the Volvocales, are not only associated with the organic enrichment in general but also with the aeration of the water beyond that due to absorption from the atmosphere.

Delf (11) found a well marked periodicity in the occurence of the majority of the algae in the ponds of Hampstead Heath, where the period of greatest diversity and abundance was from February to April or May, and corresponded to a period of variable rainfall, gradually ascending temperatures, increasing light intensity and of comparatively slight development of animal life. Delf's observations of Hampstead Heath ponds tend to confirm Fritsch's views on the seasonal activity of Spirogyra.

Whipple and Parker (49) made a study of the amount of dissolved oxygen and carbonic acid in natural waters and the effect of these upon the occurence of microscopic organisms. Special attention was given to the effect of decomposing

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organic matter, sewage pollution, stagnation of deep lakes, etc., upon the gaseous content of the waters. In water with a high organic content, bacterial decomposition may take place. The result is a reduction of the oxygen content of the water which is frequently true of shallow ponds in the summer. Bodies of surface water have what may be called a process of gaseous exchange like that which takes place in respiration, taking in oxygen and giving out carbon dioxide and again a taking in of carbon dioxide and giving off of oxygen and the effect of this process upon the presence of the microscopic organism was noted by these authors. They also point out the relation between the high carbonic acid in ground waters and the peculiar tendency of such waters to support growths of diatoms. Carbonic acid and oxygen both are necessary to the life of the plankton and the presence or absence of one or both of these gases helps to explain certain problems of vertical and seasonal distribution.

American investigators of this problem have been Walker, Brown, Copeland, Chambers, Platt, Transeau, and Anderson.

Brown (5) made a study of the periodicity of algae in certain ponds and streams in and near Bloomington, one of which was the pond upon which this study is based. The temperature of the water and air was recorded for each visit to the pond but the hydrogen-ion concentration of the water was not taken. The solid matter in the water was estimated but the organic content was not determined.

Copeland (9) made a study of periodicity of Spirogyra in nature and attempted to parallel natural conditions in laboratory cultures. He concluded that conjugation in Spirogyra results not so much from external as from internal conditions.

Anderson and Walker (1) studied the seasonal distribution of algae in some Sandhill Lakes. They found the desmids most abundant in July. Other green algae were found throughout the period of observation but were most conspicuous in the month of July. These authors describe a situation in the Sandhill Lakes which coincides with a condition of the pond in this study, that is, the effect of pasturing upon the occurence of the Cyanophyceae. They mention Anabaena and Nostoc as dominant algae occurring with Spirogyra, Scytonema, Oedogonium and Mougeotia.

Transeau (41) classified algae into winter, spring, summer, and autumn annuals, perennials, ephemerals and irregulars. He discovered that continued high water was attended by increased fruiting of algae. In his study of the plankton of the Illinois River, he found no marked evidence in the presence of nitrates and diatom periodicity (40). The diatom pulses do not show any constant relation to the movement in nitrates either in amount or direction. He suggests this may be due to the sewage contamination, which is far in excess of the demand which the diatoms make. The distribution of the diatom pulses throughout the year seems to preclude the factor of temperature as the immediate cause of pulse except as it may effect the growth of the individual species. He suggests a correlation between the plankton pulses and the lunar cycle.

Chambers (7) found an intimate and mutual relation between the algae and the submerged aquatics in a body of water and the gases dissolved in that water. They fluctuate together. Stagnant water, on account of the large amount of carbon dioxide and the small amount of oxygen favors the formation of colonies and filamentous rather than free individual cells. Colonies and filamentous forms may be produced artificially with some plants, by increasing the amount of carbon dioxide or diminishing the amount of oxygen in the culture solutions. Narrow, much branched filaments are adapted to and produced by poorly aerated water. The periodicity of spore formation is not readily influenced by aeration or gas content of the water. It seems to be more a matter of heredity.

The Pond. The body of water studied was a small fresh water pond about one and a quarter miles northeast of Bloomington. The pond is situated on the top of Roger's Hill overlooking Bloomington and surrounding country and is one of the highest points in Monroe County with an elevation of 940 feet. It is oval in shape. Scattered throughout a large part of the pond is *Typha latifolia* L. Associated with Typha in the shallower portion of the pond, is *Echinochloa crusgalli* (L.) Beauv. which forms a margin in some parts. *Ricciocarpus natans* (L.) Corca may be found more or less in abundance floating among the Typha stems. On the east side of the pond is a zone of *Cephalanthus occidentalis* L., *Polygonum pennsylvanicum* L., *Acalypha virginica* L., *Ambrosia artemisiifolia* L., and *Bidens frondosa* L. are common plants along the margin of the pond.

In the spring of 1926, the pond measured about fifty feet across and was thirty inches deep in the middle. In October 1929, the pond measured forty-two feet from shore to shore at its narrowest part and sixty feet in length. Measurements were taken again January 25th when the pond was covered with a layer of ice two inches thick. The measurements at that time were fifty-seven feet wide, and seventy feet long with a depth of twenty-one inches in the middle.

The geology of this pond was explained fully by Scott in 1910 (36) and more recently reviewed by Welch in 1928 (44). Let it suffice here to say that the pond is the result of the clogging of an old sink hole due to an acculmuation of silt and plant debris over the bottom. It is underlain by Mitchell limestone. At the beginning of this study, there was no outlet to the pond and the greatest loss of water was due to evaporation and probably to some seepage through the rock beneath. The pond is fed by a small spring running into it from the north side. While the pond seemingly remains stagnant, at no time have I found it to become foul. This fact was also noted by Brown (5). The pond is rapidly filling up, both from natural causes by the accumulation of silt and plant debris and by artificial means. This fact is evident when considering the difference in measurements recorded by those who have studied the pond in 1906 and 1910. About a year ago the owner of the land made an unsuccessful attempt to drain the pond by making a ditch on the south side so the hill top might be available for cultivation. It is only a matter of a few seasons until this destruction will have reached completion. Therefore, it was with much surprise that I discovered in the pond forty-two species of algae representing thirty-two different genera. Of these, by far the greatest number of species were Desmids, although the individual species were not particularly conspicuous in numbers.

Methods of Collection and Preservation. Observations began in Apri 1926 and continued at an average of twice a week until in August when they were discontinued and the study was not resumed until September 1929. As may be expected, changes occurred in the pond during the three years which elapsed. At one time it was known to have become completely dry in October, 1928. Naturally this would affect the occurrence and abundance of the algae in the pond, reducing some, and giving others a chance of flourishing when the pond again filled with water. That striking changes have occurred in the algal flora of the pond in the twenty-three years since Brown's study may be seen by an examination of the following list.

Species	Brown 1906	Scott 1910	White 1926	White 1929
Closterium strigosum Breb.	×			
C. Dianae Ehrb.	×	×		
C. Ehrenbergii	×			
Cosmarium zygospores	×			
C. Botrytis Menegh.		×	×	×
C. tetraopthalmum Kuetz.		×		
Docidium crenulatum Raben.		×		
Spirogyra majuscula Kuetz.		×		
Zygnema cruciatum (Vauch.) Ag.	×			
Z. stellum Ag.		×		
Oedogonium crassiusculum Wittr.	×			
O. undulatum Breb.		×		
O. Sp.			×	×
Chaetophora pisiformis (Roth) Ag.	×	×		
Bulbochaete crenulata Prings.	×			
Ulothrix aequalis Kutz.	×			
Coleochaete scutata Breb.	×			
Herposteiron on Oe. crassiusculum	×			
Gloecystis gigas (Kuetz.) Lagerh.	×			
Ophiocytium bicuspidatum (Borge) Lemm.	×			
Anabaena sp.	×		×	

Samples of water containing algae were taken from the pond and examined under the microscope in the laboratory. The relative abundance of the organisms was based upon the examination of a number of microscopic fields for each sample. The charts (Fig. 2-3) illustrate in a diagramatic way the relative abundance of the more dominant species. A stoppered bottle fastened securely to a long pole was lowered in the pond, the stopper removed to allow the bottle to fill with water, replaced and drawn to the surface. Water samples were taken in this manner for the determination of the hydrogen-ion concentration of the water at the different points in the pond. No very striking results were obtained and it is sufficient to say that the water was very nearly normal at each of the tests. Surface water of lakes is usually about normal. The depth of the pond may not have been great enough to give any marked difference in the tests. While tests of the water were made at different depths, namely, the surface, four and six inches below the surface and on the bottom, the results ranged from 6.8 before the heavy rains of November and 7.2 after the rains. For the determination of the acidity of the water the colorometric method was used. At first, results were verified with the electrometric method but, since the results compared very well, the former was used thereafter.

After identifications were made, the specimens were fixed in formalin acetic alcohol, later washed and preserved in seventy percent alcohol, after which they were sealed and placed in the Indiana University herbarium. For each specimen preserved, the location, date of collection and other necessary data were recorded in an alphabetized card index. A list of the algae identified during the course of

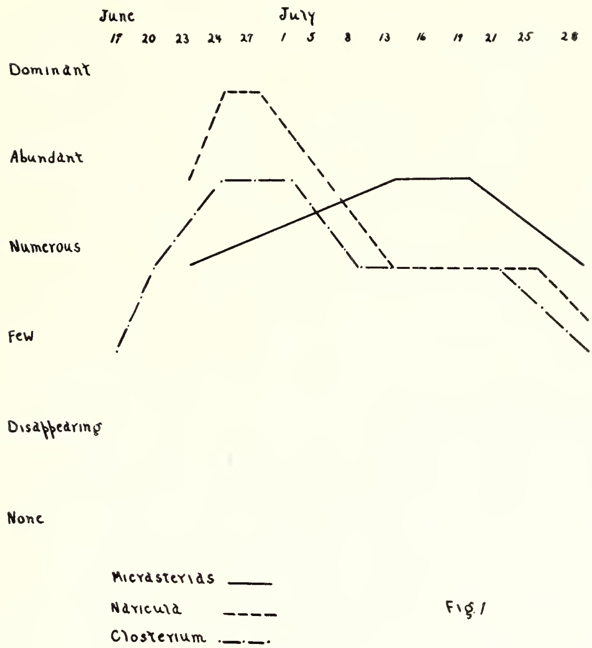
June
July
17 20 23 24 27 1 5 8 13 16 19 21 25 28
Dominant
Abundant
Numerous
Few
Disappearing
None
Micrasterias
Navicula
Closterium
Fig. 1

Fig. 1

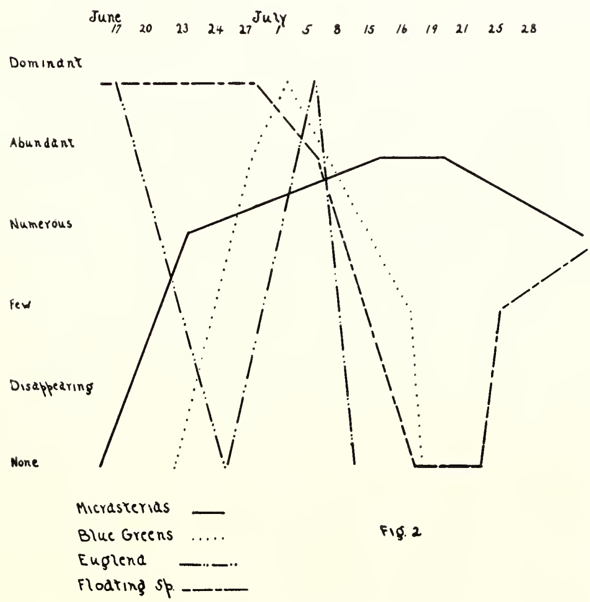
June
July
17 20 23 24 27 1 5 8 15 16 19 21 25 28
Dominant
Abundant
Numerous
Few
Disappearing
None
Micrasterias
Blue Greens
Euglena
Floating Sp.
Fig. 2

Fig. 2

this study is entered in an accession book. Many species of algae from sources other than the hill pond were identified and placed in the herbarium. These, with the species identified from the solution pond are included in the list accompanying this paper. Only the specimens collected and identified in the fall and winter of 1929 and 1930 were preserved. The species were checked by Palmer's check list (27) and a list of algae identified during this study which had not been recorded for the state of Indiana was made.

Climatological Data. In order to interpret the following pages, a discussion of some climatological factors will be helpful. The maximum and minimum daily temperatures and the mean for Bloomington are tabulated in Table 2. The number of days clear, partly cloudy and cloudy for each month in question is given in Table 3 and the daily precipitation is shown on Table 4.

In February 1926 the average precipitation for the month was somewhat above normal. Following the comparatively warm weather of February with a mean temperature of 34.5 degrees for the State, March was colder throughout, having a mean temperature of 34.3 degrees. Only twice in the past forty years has March been so cold. Precipitation averaged below the normal and there was little sunshine during the month. Development of vegetation was slow and the season was a week or ten days behind the normal. April was a very cold month. The mean temperature was the lowest for April since 1907 and with that exception the lowest on official record. The temperature for May was somewhat above normal. June was cooler. With the exception of June 1902, it was the coldest June on official record. The only warm spell extended from the 11th to the fourteenth and then the temperature was only of moderate degree. The month of July was nearly normal in temperature and cloudiness, however Bloomington had a deficiency of two inches in precipitation. The greatest precipitation for Bloomington occurring at any one time during the month was 0.94 inches on the third. That for the remainder of the month was very minute in quantity.

September 1929 was the fifth month with a mean temperature below normal. The precipitation for Bloomington for this month was interpolated from surrounding stations and estimated to be 4.17 inches. The warmest part of the month occurred from the first to the fourth, during which time a maxima of 90 degrees and above were quite general. The lowest temperatures in all parts of the state were on the eighteenth and nineteenth.

October averaged below normal in temperature, however there were no record breaking extremes. The precipitation was 1.72 inches in excess for Bloomington. November was the coldest November since 1911. The beginning of the month was mild but zero weather prevailed at the close. The precipitation for Bloomington was slightly in excess of the average for the month. The below zero temperatures recorded for the second and third of December were followed by two weeks of mild weather. Colder weather extended from the nineteenth to the twenty-fourth inclusive. In spite of the extremely low temperatures, the average was slightly above the normal for the month. The precipitation for this month, like November, was slightly in excess.

January was the ninth successive month with an average temperature for the state below normal and the temperature for Bloomington was 1.4 degrees lower than the average. The precipitation for Bloomington was 5.23 inches in excess, with a total precipitation of 8.87 inches for the month. Flood conditions prevailed in many parts of the state.

DAILY TEMPERATURE
TABLE NO. 2

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Mean
February	41 33	47 33	45 34	42 28	36 28	53 27	46 25	44 34	39 29	30 29	32 14	33 22	54 33	50 35	38 28	42 19	58 30	50 43	48 24	54 20	60 34	56 31	32 26	41 23	52 39	43 31	33 26	54 22	... ...	... ...	... ...	44.8 28.3
March	51 29	31 16	33 12	32 16	34 14	42 22	40 24	25 10	45 15	47 26	41 32	38 24	27 16	32 12	37 23	35 19	48 24	57 36	57 40	57 39	55 34	49 38	63 38	75 37	69 40	42 30	35 22	44 21	53 25	47 35	52 27	44.9 25.7
April	42 26	52 22	53 31	53 23	59 40	52 34	68 47	49 36	53 29	63 29	56 43	49 31	65 30	58 38	52 30	65 31	63 39	54 34	50 26	61 27	75 44	73 54	65 53	64 49	50 37	56 32	59 32	57 39	65 32	81 50	... ...	59.1 35.9
May	85 56	82 51	74 47	64 30	78 38	85 48	85 58	84 56	80 54	76 53	64 42	69 43	70 45	68 49	68 44	80 52	81 54	81 57	77 52	72 41	78 47	74 52	66 35	89 47	90 58	91 61	84 56	81 52	87 60	85 63	82 60	78.4 50.4
June	76 62	82 57	78 54	69 50	73 40	76 47	79 54	80 50	83 50	85 33	89 61	83 67	88 61	81 71	75 54	73 55	87 62	80 57	76 58	80 53	85 59	80 52	81 57	81 52	81 59	75 55	76 44	82 48	91 55	93 68	... ...	80.6 55.5
July	92 60	98 69	97 72	89 ...	86 70	91 74	91 66	91 67	91 71	88 69	78 53	81 55	79 61	71 50	80 50	83 52	88 52	94 62	95 63	96 65	98 62	92 71	85 67	89 62	90 65	85 67	83 67	89 61	93 61	93 69	86 73	88.5 63.5
August	84 71	84 68	81 67	89 68	90 72	90 73	79 60	80 56	82 61	91 64	93 70	85 67	83 68	86 72	84 70	84 70	79 69	82 70	79 69	83 70	87 67	89 63	88 64	79 66	79 57	82 55	86 63	88 64	87 67	84 65	82 64	84.5 66.1
September	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	77.9 55.9
October	65 42	66 40	67 42	58 39	69 49	72 51	74 53	70 40	61 45	62 47	69 48	66 56	70 50	71 47	73 43	79 55	73 40	68 36	78 42	67 53	59 49	51 40	42 33	41 32	59 33	69 40	70 52	60 39	61 44	62 49	65 59	65.1 44.8
November	68 54	53 44	59 38	41 35	48 28	59 28	47 35	55 31	57 30	61 31	62 37	70 37	70 48	51 47	48 41	50 34	51 37	44 39	43 36	37 30	35 21	30 20	35 15	37 16	44 26	49 26	44 37	27 24	12 7	21 1	... ...	46.9 31.0
December	31 11	17 −3	18 −1	32 10	52 32	54 42	53 33	50 28	37 29	46 35	48 39	62 38	63 59	65 46	52 46	49 41	58 44	51 19	22 9	14 6	30 7	20 9	22 9	32 8	34 26	50 20	47 37	38 30	38 30	55 35	58 34	41.9 26.1
January	63 51	61 38	41 25	40 17	53 28	57 42	55 36	55 34	43 35	37 21	34 24	49 33	58 45	56 39	39 19	26 15	22 15	17 −15	19 −2	27 11	28 20	22 −3	14 −8	23 7	28 7	29 8	35 26	33 23	24 2	32 5	33 17	37.2 19.8
February	42 35	45 32	50 31	46 37	41 31	49 30	46 33	41 26	48 37	39 29	47 22	51 40	50 31	43 24	37 20	33 12	52 23	60 38	70 41	68 50	70 43	67 53	62 52	70 53	69 55	58 39	51 34	52 32	... ...	... ...	... ...	52.0 34.8
March	51 33	35 18	28 16	43 23	53 33	58 29	56 40	53 32	54 28	64 36	60 34	54 30	67 46	55 33	64 33	72 43	73 51	66 36	47 29	60 33	60 32	45 25	61 29	59 42	53 27	34 23	35 26	47 26	49 30	46 27	60 29	53.0 31.4

TABLE No. 3

	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Clear	6	8	13	17	16	17	10	....	12	9	4	8	10	18
Partly Cloudy	6	5	4	9	11	11	9	....	10	4	10	8	8	3
Cloudy	16	18	13	5	3	3	12	....	9	17	17	15	10	10

DAILY PRECIPITATION

TABLE No. 4

MONTH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
February	.25	...	.09	.01	...	...	...	...	.39	...	...	.11	...	1.40	T	...	...	.07	.24	...	...	.16	.02	...	1.04	T	T	...	...	...	...	3.78
March	T	T	...	...	...	.36	.20	...	...	T	.20	.08	T	T	.12	.01	...	...	...	.45	...	1.21	.05	...	.02	T	...	...	...	.34	.55	3.59
April	.55	.04	.39	...	...	.53	.50	1.64	.05	...	.21	.31	...	...	...	...	...	.33	...	...	...	.02	.13	.09	.05	...	...	.24	...	...	...	5.08
May	...	...	.71	...	...	...	...	...	.02	.15	.40	...	...	...	...	...	...	...	.41	...	...	...	...	...	...	...	.04	...	...	...	.02	1.75
June	1.88	...	...	.22	...	...	T	...	...	...	...	.77	.06	.09	.03	...	T	...	...	...	...	T	...	.08	.07	.04	...	...	...	T	...	3.24
July	...	...	...	.94	.11	.03	.04	...	.04	.55	...	...	.12	...	...	...	...	...	...	...	...	...	.23	...	...	...	.06	...	...	...	.06	2.18
August	.96	1.59	.06	.42	...	...	.94	...	...	...	...	1.49	.21	...	.11	T	.01	.15	.42	1.50	.01	...	...	.36	...	...	...	...	...	...	...	8.23
September	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4.17
October	...	...	...	...	...	...	...	...	...	...	.39	.14	...	...	...	...	...	...	...	.47	.99	1.37	.23	...	...	...	...	.02	.15	.02	1.14	4.92
November	1.64	.02	...	...	...	...	...	...	...	.63	.17	.04	.60	.03	...	.03	.01	...	...	...	...	.03	...	...	...	...	...	.20	...	.49	...	3.89
December	...	...	...	...	...	...	.72	...	.03	.03	.04	.42	.16	.08	.08	.37	1.35	.78	.09	.02	...	.06	.12	...	...	...	...	...	.01	.03	...	4.41
January	.17	.86	...	...	...	.02	1.30	1.32	1.54	.26	...	.70	1.04	1.08	...	.03	.08	.08	...	.06	.14	.03	...	...	...	.07	.09	...	...	...	...	8.87
February	.01	T	...	.32	...	.01	...	...	T	...	...	1.58	.17	...	.02	...	...	...	...	...	...	.07	.42	...	.84	.30	...	...	...	...	...	3.74
March	.04	.04	.01	...	...	...	.33	...	...	...	.10	.02	...	...	...	...	...	.29	.01	...	.03	...	...	.49	.49	.16	.02	...	...	...	...	2.03

February was the warmest February on official record for the state. The temperature for Bloomington was 13 degrees higher than the average for the month. The precipitation for Bloomington was 0.74 inches in excess but the average precipitation of the entire state had a deficiency of 0.05 inches.

The Flagellates. The only member of the flagellates observed in any quantity was *Euglena viridis* Ehrb. In the fall of 1929, a few specimens of *Euglena spirogyra* Ehrb. were collected. The former was first collected in June 1926 growing abundantly in tiny pools along the water's edge made by the foot tracks of cattle as they came for water. The flagellate was found on the northeast side near the spring entrance and at that time formed a thin light green scum over the surface of the water. The water level was very low due to a deficiency in precipitation for the state for the month of May and June of 1.12 inches and 0.90 inch respectively. By June twentieth *Euglena viridis* had disappeared.

July third, the pond was replenished by rains and the water had risen several inches. The water in the pond had been considerably stirred up, however *Euglena viridis* was abundant around the edge of the pond. It was present as a pale green scum in some points, in others, it was distributed throughout the water imparting a greenish tint to the water. Three days later, *Euglena viridis* had disappeared entirely. On my visit to the pond July sixteenth, I found the water level much lower and the depth at the center only eighteen inches. The ground, which was drying up, caking in some places, had a soft green, film-like covering. In some places, the green gave place to a brownish color. Upon examination it was learned that this was *Euglena viridis* which had encysted as the water began to dry up. This, then, accounted for the sudden disappearance of the flagellate by June twentieth and its reappearance after the rain July third. It disappeared permanently after July eighth.

Although *Euglena viridis* was found in great abundance in 1926, only an occasional specimen was found in the fall of 1929. In fact, the flagellate was so scarce as to be considered a negligible constituent of the plankton. Although it appears sparingly at all times of the year, it may increase more rapidly in the summer. Bracher (4) states that Euglena is less sensitive to low temperatures than diatoms. Since diatoms were found more or less abundantly in the pond during the fall, the absence of *Euglena viridis* at this time must have some other explanation. Temperature evidently is not the limiting factor in this case. *Euglena viridis* is especially sensitive to light and its abundance in June 1926 may be partly accounted for in this way, for, during the month of June, sixteen days were clear, eleven only partly cloudy and three cloudy.

Fritsch states that the amount of dissolved substances in the water acts as a limiting factor. Probably in this lies the explanation for the abundance of *Euglena viridis* in June, and early July 1926 and its almost total absence in September and October 1929. Cattle came to the spring to drink and at the same time supplied the water with nitrogenous waste, thus increasing the amount of organic matter in the pond. During the time which elapsed between my periods of observation, the field was under cultivation, thus cutting off this source of organic material.

The Cyanophyceae. Representatives of five genera of Cyanophyceae were collected in the pond during the time the pond was under my observation. They were *Nostoc verrucosum* (Linn.) Vauch., Anabaena sp., Rivularia sp., Chroccoccus sp., and *Cylindrospermum limnicola* Kg. Of these, all except Cylindrospermum were found in the summer of 1926. *Nostoc verrucosum*, Anabaena and Rivularia were found in a similar place in the pond. Of these three, Anabaena was most abundant, occurring with Nostoc among decaying Oedogonium and plant tissue, all of which formed a dark brown scum floating near the center of the pond. A luxuriant growth of Spirogyra preceded the development of the Cyanophyceae and, as the Spirogyra broke down, the blue greens reached their maximum which was about the last of June. Cylindrospermum was found on moist soil on the south side of the pond September 30, 1929. While it was collected frequently with Vaucheria in the weeks immediately following, at no time was it observed in any great quantity. Not a trace of the genera of blue greens observed in 1926 was found in any samples taken in the fall of 1929.

Since most investigators have found that Cyanophyceae reach their maximum during the cooler months, usually in September and October, and since none were observed at this time of the year, it may be that they were absent in the summer of 1929 and an explanation for their absence may be sought in the composition of the water. Cyanophyceae grow readily in water with a high organic content, the presence of nitrates being highly favorable. (32). There is a great deal of decaying plant fibers, grasses, Typha, etc., in the pond but evidently these decaying plant tissues do not supply enough organic material for the flourishing of the Cyanophyceae. The deficiency may lie in the nitrate content of the water and must have been supplied from some other source. As has been previously stated, in 1926 the hillside was pastured and cattle visited the pond for water and also supplied the water with nitrogenous waste. As has been mentioned, *Euglena viridis* was abundant at that time and only seldom collected recently, it seems reasonable to assume that a lack of nitrates in the water may be responsible for the absence of both the blue greens and *Euglena viridis*.

Acting upon this hypothesis, two samples of water were taken from the pond on the north and east sides respectively and were tested for organic matter on the twenty-eighth of February, 1930. The method and determination is given as follows: Place in a porcelain casserole 200 cc. of the water under examination and add 10 cc. of dilute sulphuric acid. It was then heated rapidly to incipient boiling and a standard permanganate solution from a burette run in until the water had a marked pink color. The solution was boiled thirty minutes, more permanganate being added from time to time, if necessary, in order to maintain approximately the intensity of red color observed at the start. A little distilled water was added from time to time to replace the loss due to evaporation. After removing from the fire, 10 cc. or more oxalic acid was added to destroy the color and then permanganate added till a faint pink tinge again appeared. From the total permanganate used the amount corresponding to 10 cc. or more of oxalic acid was deducted and from the remainder the milligrams of required oxygen consumed by the organic matter present in the water was calculated. Correction must be made for nitrates, ferrous salts or hyrdogen sulphide, if any of them be present.

Determination of iron: A standard iron solution was prepared by dissolving 0.1 gram pure iron in a little HCl to which a few drops of HNO_3 have been added, evaporating to dryness, moistening with HCl and then diluting to one litre. One cc. of this solution will contain 0.1 milligramme of iron.

For the determination 100 c.c. water was evaporated to dryness, ignited at low redness sufficient to decompose the organic matter and 5 cc. concentrated HCl added. It was warmed slightly, filtered, washed and diluted to 100 cc. in a Nessler tube and few drops of potassium permanganate solution added to make pink color persist five minutes. Five c.c. of potassium sulphocyanide solution wa added and the depth of color produced was compared with those of known amount of the standard iron solution which have been diluted and treated in the same way with similar quantities of HCl, $KMnO_4$ and KCnS.

Determination of nitrates: One hundred cc. of water was placed in a Nessler tube, 2 cc. sulphanilic acid solution and 2 cc. of solution of hydrochloride of napthylamine added, mixed and allowed to stand for thirty minutes. At the same time other Nessler tubes were prepared containing known amounts of standard solution of sodium nitrate and diluted to the mark with distilled water and reagents added as in the other sample. When the time had expired the depth of pink color of the unknown was compared with the known and accurate determination of the amount of nitrogen present was made. The steps in the process and the results are expressed as follows:

Sample I.

Permanganate solution..26.95 cc.

Oxalic acid..12.70 cc

10.5 CC. Oxalic acid—10 cc. permanganate.

$$1 \text{ cc.} = \frac{1}{10.05} \text{ of } 10 \text{ or } \frac{10}{10.05}$$

$\frac{10}{10.05}$ of 12.7 = 12.63 cc. of oxalic acid required to react with the permanganate solution.

26.95 cc. − 12.63 cc. = 14.32 cc. of permanganate required to oxidize the organic matter.

1 cc. permanganate = 0.1 mg. available oxygen. Therefore 14.32 times 0.1 = 1.432 mg. oxygen used.

1.432 times 5 = 7.16 mg. oxygen required per milion parts.

Sample II.

Permanganate solution..27.00 cc.

Oxalic acid required...13.25 cc.

10.05 cc. of oxalic = 10 cc. permanganate.

$1 \text{ cc. oxalic acid} = \frac{1}{10.05} \text{ times } 10 \text{ or } \frac{10}{10.05} = 13.18$ cc. oxalic acid required by the permanganate.

27.00 cc. − 13.18 cc. = 13.82 cc. permanganate required for oxidation.

1 cc. permanganate = 0.1 mg. oxidizing power.

13.82 times 0.1 mg. = 1.382 mg. of 0.

1.382 mg. times 5 = 6.91 mg. 0 per million parts.

In each sample the ferrous iron and nitrites were present in such minute quantities that they were disregarded entirely. The organic material is expressed as milligrammes of oxygen required to oxidize the organic matter present in the water. The results of the tests show that the pond water has a very high organic content.

Chlorophyceae. As is true in many shallow ponds, the green algae occurring in the pond are by far the most conspicuous and abundant at all times of the year. The Conjugatae were observed fruiting in April and were most abundant during April and May with the exception of *Spirogyra crassa* which may be found locally in the pond at all times of the year. It was observed in abundance from April to June in 1926 and was collected at each visit during 1929 and was the dominant species of the east side of the pond. The alga was observed fruiting the twenty-third of September and in October 1929. No mention of *Spirogyra crassa* in this pond was made by any previous investigator. In fact, no species of Spirogyra was recorded by Brown (5) from the pond. The species referred to here as *Spirogyra crassa* corresponds to the *Spirogyra crassa* (Hass) Wittrock except in its extremely large size. The cells of the vegetative filaments are (an average of four) 111 microns by 148 microns. The fruiting filaments measured 148 by 213 microns. The zygotes measured 195 by 130 microns. The last was an average of seven measurements taken. Many specimens were found with slightly greater measurements. It is possible that the specimen here is not crassa but some other species, however, the author was not able to find a species in which the measurements corresponded with those found and throughout this paper the specimen is referred to as *Spirogyra crassa*. Spirogyra disappeared temporarily after the rains of early November. At no time was it observed floating.

Other species of Spirogyra were collected from time to time in 1929 mixed with *Spirogyra crassa*, however, they exhibited no great abundance and were not found fruiting in the fall of 1929. No marked autumnal phase in Spirogyra was noted. This may have been due to the lack of rainfall. According to Fritsch and Rich, an autumnal phase depends upon the proper dilution of the water.

The genus Zygnema was represented in the pond by *Zygnema cruciatum* (Vauch.) Ag. and was found to be rather abundant in 1906 in March, occurring in the edge of the pond in shallow water. (Brown 5). It disappeared in June. *Zygnema insigne* (Hass) Kutz. was observed by the author in April 1926 associated with *Vaucheria geminata racemosa* (Vauch.) Walz. Both were found fruiting April thirteenth. By the middle of May they had entirely disappeared and no trace of either was found throughout the summer. They occurred in the northeast side of the pond near the entrance of the spring.

October twenty-fourth, 1929 a few scattered unhealthy looking filaments of Zygnema were collected on the northeast side of the pond where in 1926 the species had occurred in such abundance. It was not expected to occur in any abundance at this time of the year but the author thought it might have died out during the time elapsing since the study began. November thirteenth, a few unhealthy filaments of Zygnema were found on the southeast side of the pond, the first specimens to be collected from this section of the pond. This would indicate that Zygnema probably will occur in the spring in its usual abundance and be found in a larger area of the pond than previously.

Oedogonium which occurs with *Spirogyra crassa* and Tribonema on the east side of the pond attached to rails extending into the water and to dead stems, at no time during my observation reached any marked degree of dominance. It is possible that the species suffers from competition with the two filamentous algae with which it occurs (11). *Spirogyra crassa* occurs at all times of the year and may be important in keeping Oedogonium from becoming dominant. Tribonema tends to become dominant during lower temperatures and *Spirogyra crassa* in the spring. Hence a constant struggle for Oedogonium. The alga was observed at all times of the year but was more abundant in the spring months.

Brown (5) refers to *Oedogonium crassiusculum* Wittr. as abundant and the dominant alga of the pond in September 1906 found attached to dead Typha stems. He also found Bulbochaete in November 1906 persisting throughout the winter. While Bulbochaete was collected in June 1926, no trace of the genus was found in the fall of 1929. According to Scott (36) in 1910, Oedogonium *undulatum* Breb. was the most abundant alga in the pond and was present throughout the year.

Tribonema tends to become dominant during the lower temperatures of the fall and winter. It was found abundant on the east side in November and December. According to Fritsch, it would seem that bright sunshine is not essential to its growth because of its abundance at a time of the year when sunshine is lowest and also its location in the only shady portion of the pond near the only woody shrub. The shade loving habit of this plant was observed by Delf (11) and later by Hodgetts (21). Delf found Tribonema to have a well marked maximum in February and March with a less distinctive secondary maximum in October and November.

Only one species of Vaucheria, namely *V. geminata racemosa* (Vauch.) Walz. was collected from the pond. It was first observed by Brown fruiting in October 1906 but is not recorded as occurring in the pond by Scott (36) in 1910. The alga was associated with *Zygnema insigne* (Hass.) Kutz. and was quite abundant in April and May 1926 when it was observed fruiting. At that time it was collected at only one point in the pond, the northeast side. At the first visit to the pond in September 1929, the water was very low. Vaucheria grew on moist earth forming a felt-like covering near the water's edge all around the pond. It was in a vegetative state. It seems to prefer the cooler months covering the moist ground around the edge of the pond in October and November. In April 1926, the alga was observed only at one side of the pond. This suggests a possible fall maximum in October and November. There was a large amount of precipitation between the visits to the pond of October 24th and November 7th. On the latter date, the pond was three feet wider on each side. The Vaucheria which previously formed a covering on the moist earth was now submerged and was first observed fruiting at this visit. Thereafter, from time to time, filaments were found in fruit but soon loosened themselves from the substratum, took on an unhealthy appearance and died. Very few species of algae were collected near the edge of the pond at this time. During the latter part of November and the first of December, all algal growth seemed suspended and little or no material was collected. Only Tribonema was conspicuous.

Throughout the ensuing month, algae were grown in the laboratory on agar-agar and in Knop's nutritive solution. The latter was made of four parts calcium nitrate and one part each of magnesium sulphate, potassium nitrate and potassium phosphate. The last three were dissolved in distilled water and added to the solution of Calcium Nitrate and the whole diluted to from 0.2 to 0.5 percent. A trace of iron sulphate was added to the solution.

The mild weather of the month of February was favorable to the growth of Vaucheria and the alga flourished in unusual abundance near the edge of the pond. Large quantities of splendid fruiting material was collected and preserved.

Desmids. Of the forty-two species of algae identified from the hill pond seventeen belonged to the Desmidiaceae. They were present in the pond at all times of the year. *Hyalotheca dissiliens* (Smith) Breb. and *Closterium Venus* Kg. were collected in May. Both continued throughout June and the latter extended into July. *Micrasterias radiata* Hass., by far the most abundant of any of the desmids in the pond throughout the summer, *Staurastrum alternans* Breb., *St. Minnesotense* Wolle, *Xanthidium fasciculatum* (Ehrb.) Ralfs., and *Closterium subtile* Breb. were also found in June. While *Micrasterias radiata* Hass. was the dominant desmid, Staurastrum, Hyalotheca, Cosmarium, and Closterium may be considered numerous, *Docidium Trabecula* (Ehrb.) Naeg., *Desmidium aptogonium* Breb., scattered and Gumnozyga, Gonatozygon, and Cylindrocystis, rare.

During the fall of 1929, *Micrasterias radiata* Hass. was most abundant. It was found in the south side near the edge of the pond during September, October, and early part of November among sediment and decaying plants. After the heavy rains of late October and the first of November, the desmid disappeared and has not been collected since. *Arthrodesmus convergens* (Ehrb.) Ralfs., *Desmidium aptogonium* Breb., *Cosmarium ovale* Ralfs., and *Micrasterias Crux-melitensis* Ehrb. were observed once during the fall. *Cosmarium Botrytis* Menegh., *Docidium Trabecula* (Ehrb.) Naeg. were of frequent occurrence and *Closterium moniliferum* Ehrb. was found occasionally.

The accompanying lists will illustrate the occurrence of these algae. By far the greatest number of species were found in June.

Closterium Dianae Ehrb., *Cosmarium tetraophthalmum* Kuet., *Docidium crenulatum* Raben., reported by Scott (36) and *Closterium Ehrenbergii* by Brown (5) in 1906 were not found during this study. No Hyalotheca or Straurastrum were observed by Brown in 1906. Closterium and Docidium seem to thrive best in shallow water among filaments of *Vaucheria geminata racemosa* (Vauch.) Walz. Hodgetts (21) found that monthly mean temperatures of 10 to 15.5 degrees Centigrade were most favorable to desmid growth. Only a moderate amount of bright sunshine is required for vegetative growth but abundant bright sunshine is necessary to conjugation. According to this study, Closterium exhibits a decided vernal phase which confirms the view of Hodgetts. However a few specimens of *Closterium moniliferum* Ehrb. were collected on two occasions in September and October but they seem to prefer the higher temperatures. No conjugating specimens were observed at any time during this study.

Summary. Refore reaching any particular conclusions, it is understood that a more detailed study of several phases of this problem is necessary, one of which is the determination of nitrates of the water. However, the following are significant.

1. While Euglena is sensitive to light, and less so to temperature, the limiting factor in this case, is probably a deficiency in nitrates.
2. While Cyanophyceae usually do not occur in great abundance in shallow ponds, their absence in 1929 and 1930 is likely due to a lack of nitrates. Cyanophyceae and Euglena may be considered as indicators of the relative amount of nitrogenous matter in the water.
3. Green algae predominate in the pond. Desmids make up a large part of the algal flora and reach their maximum in the summer.
4. Vaucheria showed a very decided maximum in February.
5. Tribonema was the most important alga of the pond in November and December.
6. *Micrasterias radiata* was conspicuous in the early fall of 1929.

Algae From the Pond.

Flagellates

Euglena viridis Ehrb., June-July 1926.
Euglena spirogyra Ehrb., Sept.-Oct. 1929.

Cyanophyceae

Nostocaceae:

Nostoc verrucosum (L.) Vauch., June 1926.
Anabaena sp., June 1926.
Cylindrospermum limnicola Kg., Sept.-Oct. 1929.

Rivulariaceae:

Rivularia sp., June 1926.

Chroococceae:

Chrococcus sp., July 1926.

Chlorophyceae

Zygnemaceae:

Zygnema insigne (Hass.) Kutzing., April-May 1926.
Spirogyra portcalis (Muller) Cleve, April-May 1926.
Spirogyra crassa (Hass.) Wittrock, 1926-1929.

Spirogyra neglecta (Hass.) Kutz., April 1926.

Spirogyra mirablis (Hass.) Kutz., Nov. 1929.
Spirogyra fluviatilis (Hilse) Raben., Oct. 1929.

Desmidiaceae:

Desmidium aptogonium Breb., May-June 1926, Sept. 1929.
Docidium Trabecula (Ehrb.) Naeg., July 1926, Sept. 1929.
Micrasterias radiata Hass., June-Aug. 1926, Sept.-Oct.-Nov. 1929.
Strarastrum alternans Breb., June 1926.
Staurastrum Minnesotense Wolle, June-July 1926.
Xanthidium fasiculatum (Ehrb.) Ralfs., June 1926.
Hyalotheca dissiliens (Smith) Breb., May-June 1926.
Gymnozyga sp., July 1926.
Cosmarium Botrytis Menegh., July 1926, Sept. 1929.
Closterium Venus Kg., May-June 1926.
Closterium moniliferum Ehrb., Sept. 1929.
Closterium subtile Breb., June 1926.
Gonatozygon sp., July 1926.
Cylindrocystis sp.
Micrasterias Crux-melitensis Ehrb., Oct. 1929.
Cosmarium ovale Ralfs., Oct. 1929.
Arthrodesmus convergens (Ehrb.) Ralfs., Sept. 1929.

Oedogoniaceae:

Oedogonium sp., April-July 1926, Sept.
Bulbochaete sp., June 1926.

Vaucheriaceae:

Vaucheria geminata racemosa (Vauch.) Walz., April-May 1926, Sept. 1929.

Volvocaceae:

Chalamydomonas pulviusculus Ehrb., Sept.-Oct.-Nov. 1929.

Palmellaceae:

Tetraspora lubrica (Roth.) Ag., April-July 1926.
Gloecystis sp., July 1926.

Confervales:

Tribonema sp., 1926-1929.

Cylindrocapsa sp., 1926.
Microspora sp., 1926.

Characium Pringsheimii Braun., June 1826, fall 1929.
Botryococcus Braunii Kutz., July 1926.

Bacillaricae:

Navicula viridis Kg., June-July 1926, fall 1929.

Algae Not Previously Reported for Indiana.

Staurastrum Minnesotense Wolle, Spirulina teniussima Kutz. Spirogyra portcalis (Muller) Cleve., Spirogyra fluviatilis (Hilse) Raben., Spirogyra insignis var. Rraunii Raben., Cylindrospermum limnicola Kg., Oscillatoria splendida Brev., Micrasterias radiata Hassal., Closterium subtile Breb., Herposterion confervicola Nageli., (The genus was reported for the state in 1908 in Monroe County). Myxonema glomeratum Hasen., Cladophora callicoma Kutzing., Stigeoclonium radiana Kg., (Myxonema radians Kutzing reported for Marshall County). Cladophora uberrima Lambert., Cladophora insignis (Ag.) Kutzing., Oscillaria aerugineo-coerulea Kg., Conferva utriculosa Kutzing., Gloecapsa quarternata Kutzing., Navicula mesogongyla Kg., Lyngbya naveanum Grun., Gloecapsa gelatinosa Kuetzing., Cosmarium ovale Ralfs., Chlamydomonas pulvisculus Ehrb.

Species of Algae not Previously Reported for Monroe County.

Stigeoclonium radians Kg., Cladophora uberrima Lambert., Cladophora insignis (Ag.) Kutzing., Oscillaria aerugineo-coerulea Kg., Conferva utriculosa Kutzing., Diatoma vulgare Bory., Gloecapsa quarternata Kutzing., Navicula mesogongyla Kg., Lyngbya naveanum Grun., Gloecapsa gelatinosa Kuetzing., Cosmarium ovale Ralfs., Staurastrum Minnesotense Woelle., Spirogyra portcalis (Muller) Cleve., Spirogyra fluviatilis (Hilse) Raben., Spirogyra mirabilis (Hass.) Kutz., Spirogyra insigne var. Braunii Raben., Cylindrospermum limnicola Kg., Oscillatoria splendida Grev., Micrasterias radiata Hassal., Micrasterias Crux-melitensis Ehrb., Closterium subtile Breb., Herposterion confervicola Nageli., Myxonema glomeratum Hasen.

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