QUANTITATIVE AND QUALITATIVE EVALUATION OF PLANKTON FROM FERTILIZED AND NON-FERTILIZED HATCHERY PONDS, WITH AN APPRAISAL OF METHODS USED

Thesis for the Degree of M. S.

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Roswell DeWitt VanDeusen

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This is to certify that the

thesis entitled

QUANTITATIVE AND QUALITATIVE EVALUATION OF PLANKTON FROM FERTILIZED AND NON-FERTILIZED HATCHERY PONDS, WITH AN APPRAISAL OF METHODS USED.

presented by

ROSWELL DEWITT VANDEUSEN

has been accepted towards fulfillment
of the requirements for

M. S. degree in Zoology

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Major professor

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QUANTITATIVE AND QUALITATIVE EVALUATION OF PLANKTON FROM FERTILIZED AND NON-FERTILIZED HATCHERY PONDS, WITH AN APPRAISAL OF METHODS USED.

By

Roswell DeWitt VanDeusen

A THESIS

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INTRODUCTION

This paper has a three-fold purpose; (1) to determine the kinds of plankton organisms produced in fertilized and non-fertilized hatchery ponds, (2) to measure the effect of fertilizers on quantity of plankton produced, and (3) to compare the results of methods commonly used to appraise the weekly standing crops of plankton. The series of experiments were carried out on eight ponds at the Wolf Lake State Fish Hatchery and were designed to measure the effect of fertilizers on the production of bait minnows and on closely related production of the pond flora and other fauna.

During the Spring of 1946, plans were formulated to carry out a cooperative project between the Institute for Fisheries Research of the Michigan Department of Conservation and Michigan State College. This study is only a phase of the over-all enterprise to determine the effects of fertilization upon the plant and animal life in ponds and lakes, in a glaciated region such as Michigan. A survey of the kinds and quantities of plankton produced in these altered environments was offered as a graduate problem to be sponsored as a fellowship by the Institute for Fisheries Research. The institute furnished the salary and field expenses and the college supplied equipment for work in the field and laboratory. The field work was done under the supervision of Dr. P. I. Tack of the Zoology Department of Michigan State College.

The study was commenced on April 11, 1946, when the first samples of water were brought to the campus greenhouse for testing. Field studies began on June 17, 1946, and continued until September 16, 1946, when the ponds were drained and the fish removed. During the following six months the plankton collections and data were analyzed in the laboratory.

This work is concerned primarily with plankton organisms, therefore, a definition of plankton is deemed in order. Aquatic plants and animals are divided into three groups: "1. Plankton organisms of relatively small size, mostly microscopic, which have either relatively small powers of locomotion or none at all and which drift in the water subject to the action of waves, currents, and other forms of water motion." 2. Nekton, and 3. Benthos, Welch (1935). It has been found that in most natural lakes and ponds the greatest crop of plankton occurs in the spring and autumn with corresponding lowest crop during the late summer and winter. This study was carried on during the summer period when environmental conditions are not best suited to promote maximum growth of plankton. Since the ponds are filled in the spring and drained in the fall of the year, certain factors must become present during this time so that the micro-organisms may increase and contribute to the basic food chains. Fertilizer substances containing nitrogen, phosphorus, and potash are added to water areas to increase the "secondary producers" or bacteria, and phytoplankton which will in turn increase the "primary consumers" or zooplankton and aquatic insects. To complete the cycle, larger fish will feed directly upon the zooplankters and insects, especially during their early development, or may feed indirectly upon them by consuming forage fish which are believed to feed upon the minute organisms.

DESCRIPTION OF WATER AREAS

Location of study

The Wolf Lake State Fish Hatchery is the largest hatchery in the state and is located seven miles west of Kalamazoo, on M-43; (R-13-W., T-2-S., Sections 13 and 14), Almena Township, VanBuren County, Michigan. The hatchery lies in a gently rolling section. Soils of this region are predominantly sandy and support such specialized crops as grapes, raspberries, and asparagus.

Ponds of the system are maintained by three springs supplemented with water pumped from Wolf Lake which is located across the highway to the north. Water is pumped to Pond 6, a mixing pond, and is distributed from there. Number 1 Spring is the largest of the three and has a flow of approximately 1400 gallons per minute. All ponds and raceways eventually drain into Wolf Lake. The hatchery plan is shown on page 10.

Description of ponds

Eight ponds were designated to be used in the fertilization experiment. They were chosen because of their comparable size, and divided into pairs according to type of bottom and past productivity. The Wolf Lake ponds in general are utilized mainly for culture of pond fishes. The study ponds were stocked April 18-20, 1946, with "eyed eggs" of the Common White Sucker, *Catostomus commersonii* (Lacepede). The following table shows the pond number, acreage, average depth, and stocking rates of the ponds included in the study.

Map of water area

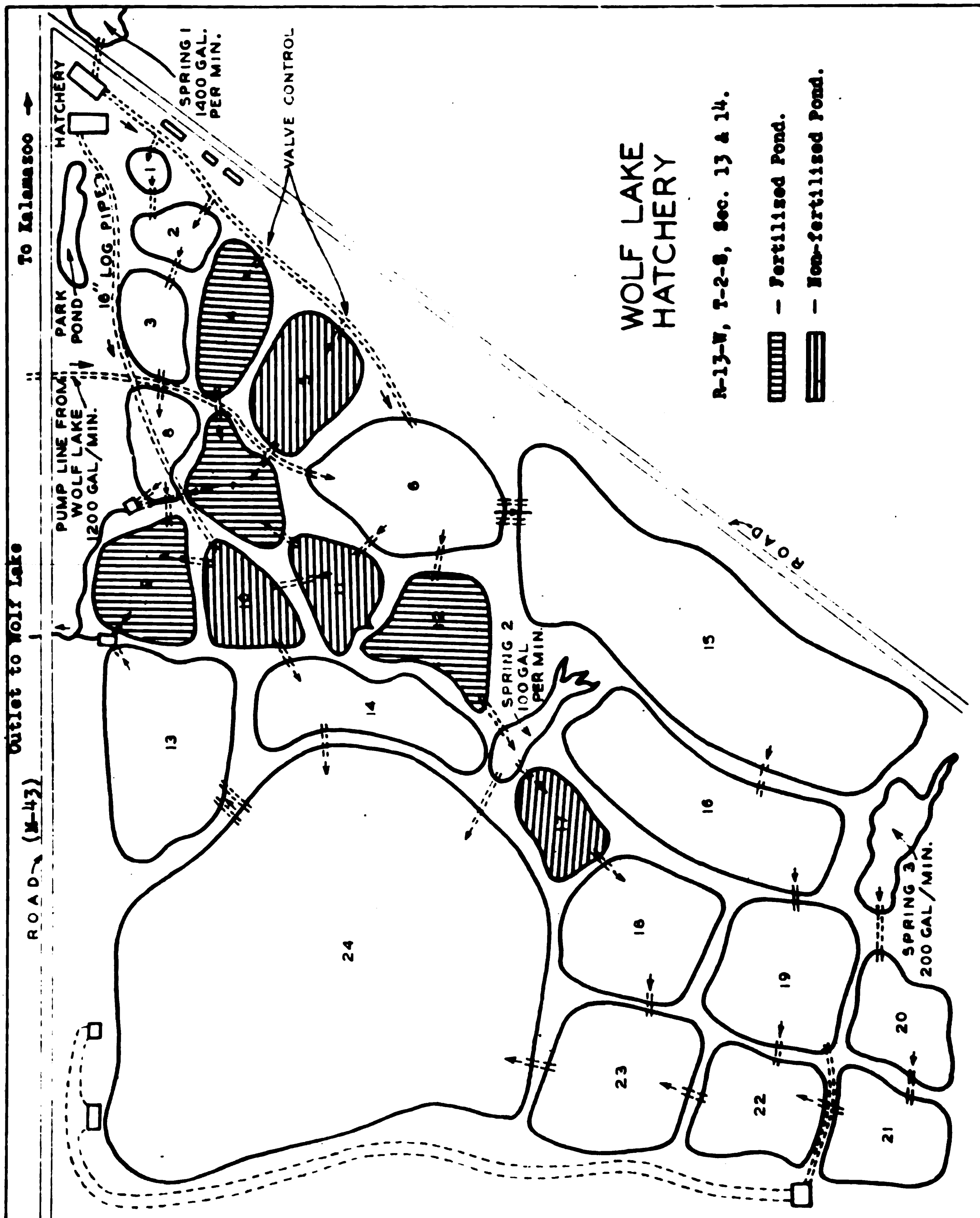


Table I

Size, depth, and stocking rates of study ponds.

Pond	Area(acres)	Ave. Depth(feet)	Stocking		
4	0.98	3.8	98,000	sucker	eggs
5	1.04	---	104,000	"	"
7	1.54	2.7	15,400	"	"
9	1.72	4.1	86,000	"	"
10	1.47	---	36,750	"	"
11	1.29	---	12,900	"	"
12	1.78	3.4	44,500	"	"
17	1.27	---	63,500	"	"

The average surface area for the above ponds was 1.38 acres, the largest having 1.78 acres and the smallest containing 0.98 acres. The average depth for the four fertilized ponds, namely 4, 7, 9, and 12 was 3.5 feet. Ponds 4 and 5 were ideally paired. They lie side by side, both have sandy bottom soil and nearly the same area and average depth. Ponds 7 and 11 were likewise paired because of their close proximity, size and depth. The bottom soils of the latter ponds did not agree as closely as those from Ponds 4 and 5. Pond 7 contained more organic matter on the bottom than did Pond 11. Ponds 12 and 10, and Ponds 9 and 17 were also paired partly because of their size and bottom soils relationship, and partly because of necessity. Considerable manipulation of the flow diagram was necessary so that pond levels could be maintained and still not contaminate control ponds with overflow or seepage from fertilized ponds.

One of the main problems in the selection of ponds for carrying out fertilizer experiments is seepage. The ideal situation is to possess a pond which has a minimum loss of water by this type of run-off. Seepage was a problem in Ponds 4, 5, 7, 10, and 11. The maintenance of normal levels in these ponds required additional water, which caused a dilution of the fertilizer. It has been suggested that Bentonite or some such material be added to control seepage particularily in Ponds 4, 5, 7, and 11, before further experimentation of this nature is carried on.

Higher aquatic plants were represented by comparatively few species. On July 9, 1946, a survey was made of the plants in each pond. Lists of species found and relative abundance of each was recorded on analysis record blanks as shown on page iii of the appendix. The results are listed in Table II. Identifications were made from Fassett (1940).

Table II

Abundance of higher aquatic plants in study ponds.

Pond 4

Name	Abundance
Chara sp.	Medium
Najas flexilis (Willd.)Ros.& Sch. (Bushy Pondweed)	Sparse
Potamogeton sp.	Sparse

Pond 5

Name	Abundance
Chara sp.	Medium
Najas flexilis (Willd.)Ros.& Sch. (Bushy Pondweed)	Medium
Potamogeton foliosus Raf. (Leafy Pondweed)	Sparse

Pond 7

Name	Abundance
Chara sp.	Abundant
Najas flexilis (Willd.)Ros.& Sch. (Bushy Pondweed)	Medium
Potamogeton sp.	Medium
Ceratophyllum demersum L. (Coontail)	Sparse

Table II (Cont.)

Pond 9

Name	Abundance
Najas flexilis (Willd.)Ros.& Sch. (Bushy Pondweed)	Abundant
Potamogeton foliosus Raf. (Leafy Pondweed)	Medium
Chara sp.	Sparse

Pond 10

Name	Abundance
Anacharis canadensis (Michx.) Planchon (Waterweed)	Medium
Chara sp.	Medium
Potamogeton sp.	Sparse

Pond 11

Name	Abundance
Potamogeton sp.	Abundant
Najas flexilis (Willd.)Ros.& Sch. (Bushy Pondweed)	Medium
Chara sp.	Medium

Pond 12

Name	Abundance
Potamogeton foliosus Raf. (Leafy Pondweed)	Abundant
Najas flexilis (Willd.)Ros.& Sch. (Bushy Pondweed)	Abundant
Chara sp.	Medium

Pond 17

Name	Abundance
Potamogeton foliosus Raf. (Leafy Pondweed)	Medium

Table II (Cont.)

Name	Abundance
Najas flexilis (Willd.)Ros.& Sch. (Bushy Pondweed)	Medium
Chara sp.	Medium

The ponds, in order of relative abundance of aquatic plant growth were; 12, 17, 9, 11, 10, 7, 5, and 4. During early August the plant growth reached a maximum after which a decline occurred. This was characterized by the plants "settling" to the bottom, and becoming brownish in color. Plants of the pondweed *Najas* formed floating mats at the surface which tended to drift toward the outlet. In fertilized Ponds 7, 9, and 12 this condition brought about a large amount of bacterial activity with subsequent decomposition. The presence of bacteria colonies was noted in centrifuged samples from these ponds.

Existence of higher aquatic plants in the fertilized ponds appears to depend upon the amount of light penetration. At the start of the experiment Ponds 4 and 5 were populated, as noted in Table II, with growths of *Chara* sp., which were medium in abundance. Upon draining Pond 4 was void of all plants while Pond 5 maintained its plant growth. It is assumed that the absence of plants in Pond 4 was caused directly by persistence of the phytoplankton bloom throughout the observation period. Blooms in the other fertilized ponds did not begin until later in the experiments; therefore, the results from these ponds cannot be compared with those from Pond 4.

METHODS AND MATERIALS

The data for this paper with the exception of that from greenhouse experiments were obtained from a series of daily observations and weekly collections made at the Wolf Lake State Fish Hatchery. The daily records were kept by Mr. C. T. Yoder, Fisheries Biologist for the Institute for Fisheries Research, who lived at the hatchery. Monday and Tuesday of each week were spent at the hatchery and with assistance from Mr. Yoder the fertilizer was applied and the necessary plankton collections were made. Water samples were taken for chemical analysis.

A period of three months preceeding the field work was devoted to greenhouse experiments.

Greenhouse experiments

Tests show that the amounts of dissolved solids and gases in natural waters vary in composition. Two alternatives must be considered when predicting a fertilizer for use in a certain body of water. One alternative is to treat water samples with different fertilizer ratios to determine the most nearly correct combination of elements. The amount of organic matter produced determines this ratio. The second alternative is to make chemical analyses on water samples to determine which element or combination of elements are deficient, or present in excessive quantities. This will give indications of which nutrients are limiting plant growth either by their presence or by their absence. A fertilizer ratio is the proportion of nitrogen as elemental nitrogen, phosphorus as phosphorus pentoxide, and potassium as potash in a fertilizer formula, example; a 4-8-4 fertilizer formula has a 1-2-1 ratio. With this in mind an experiment was started to determine a suitable fertilizer for Michigan waters, using the method of treating water with various fertilizers. Water samples from the Harrisville, Hastings, Drayton Plains, and Wolf Lake State Fish Hatcheries located in the Lower Peninsula were brought to the campus for testing. A series of 14 one-gallon crocks were filled with source water samples from the various collections. These were treated with 13 possible nutrient combinations of N-P-K and the results are shown in Chart 1. The common expression N-P-K will be used to indicate the nutrients found in commercial fertilizer formulas. The crocks were kept in the soils experimental greenhouse on the Michigan State College campus.

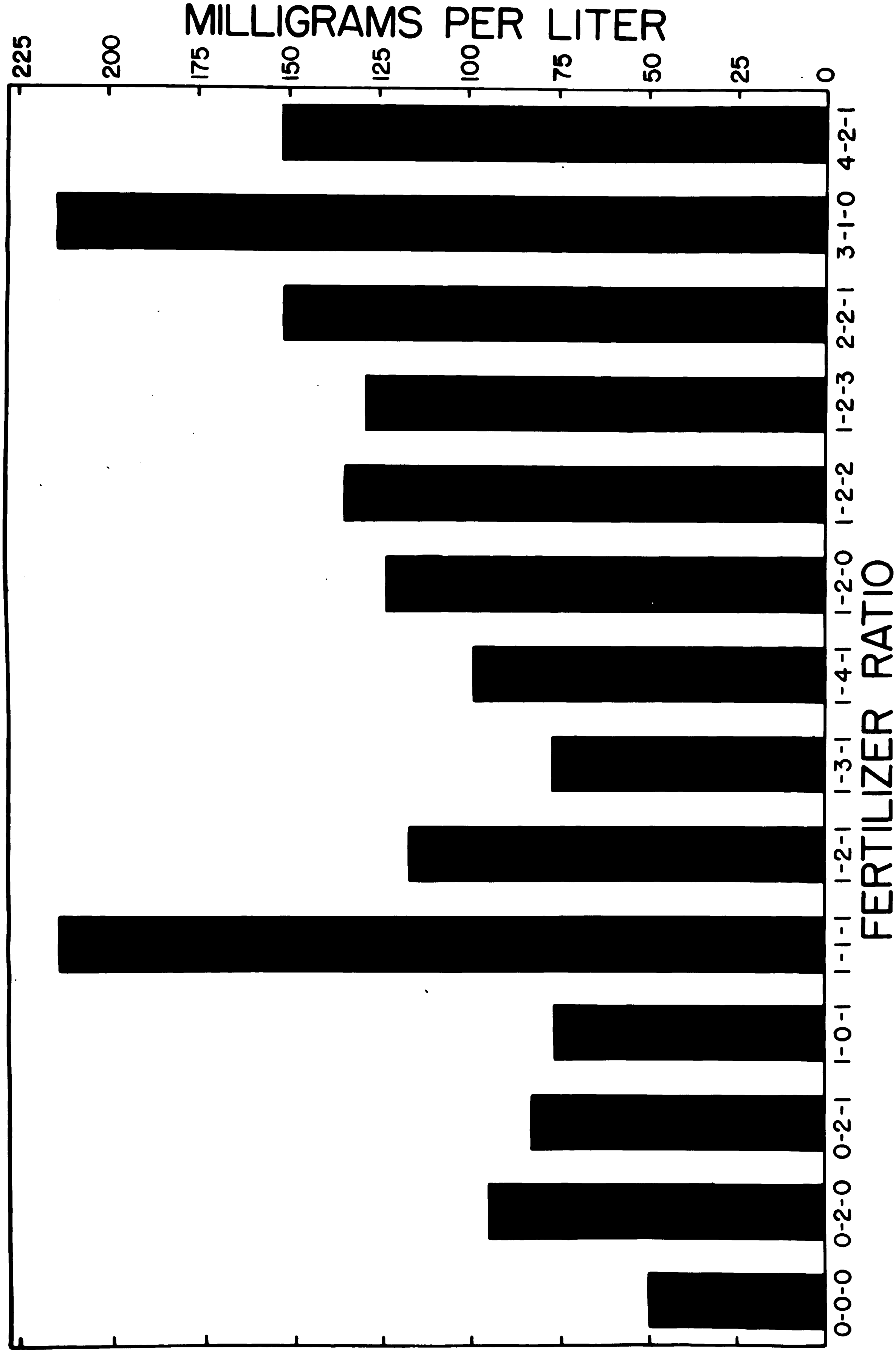
It is assumed that 150 pounds per acre is a suitable fertilizer application, this figure was proposed by Dr. R. L. Cook of the Soils Department of Michigan State College and was used in these tests. Increments of each nutrient are computed in form of grams of pure chemical per gallon of sample water. The following chemicals were used as sources of nitrogen, phosphorus, and potash; $NaNO_3$=16.47% nitrogen, $Ca(H_2PO_4)_2$=56.3% P_2O_5, and K_2SO_4=53.86% K_2O. 0.0375 grams $Ca(H_2PO_4)_2$=.021 grams P_2O_5 per gallon or equivalent to 150.07 pounds of 0-20-0 per acre per two feet of water. Therefore .128 grams of $NaNO_3$ equals .021 grams of nitrogen per gallon and .039 grams of K_2SO_4 equals .021 grams of K_2O per gallon. The required portions of the above were dissolved in a known amount of water and added directly to the experimental pots.

Chart 1 shows the various ratios of nitrogen, phosphorus, and potassium used, and the milligrams of organic matter per liter produced by the different fertilizer ratios when applied to the Wolf Lake Hatchery water supply. It is apparent that the high nitrogen-high phosphorus ratios, as 2-2-1, 3-1-0, and 4-2-1, consistently produced greater amounts of organic matter. Examination of organisms found in the 3-1-0 sample showed the following genera present; Oscillatoria sp., Scenedesmus sp., Chlorella sp., Dinoflagellate cysts, Pandorina sp., Amoeba sp., Pediastrum sp., Ankistrodesmus sp., Schizochlamys sp., and Nephrocytium sp. From these experiments came the fertilizer ratio best suited to all waters tested and to the Wolf Lake Hatchery source.

On the basis of the amount of organic matter produced the fertilizer formula, or percentage of available nutrients present, 10-6-4 was derived. The amount of this fertilizer required to produce the desired results is arbitrary without first knowing exactly what nutrients are already present in the water and in what quantities. To commence the experiment a 100 pound application of fertilizer per acre surface area was arbitrarily selected. Also it has been observed that a plankton bloom will last from two to four weeks. Therefore, 100 pounds of 10-6-4 fertilizer was to be applied per acre

Chart 1

Total particulate organic matter produced in fertilizer pot-experiments using different fertilizer ratios.


MILLIGRAMS PER LITER
225
200
175
150
125
100
75
50
25
0
FERTILIZER RATIO
4-2-1
3-1-0
2-2-1
1-2-3
1-2-2
1-2-0
1-4-1
1-3-1
1-2-1
1-1-1
1-0-1
0-2-1
0-2-0
0-0-0

every three weeks.

Fertilization of ponds.

The application of fertilizers was to include both organic and inorganic fertilizer materials. Organic fertilizer in the form of barnyard manure was applied on April 20, 1946, at the rate of one ton for each one and one-half acres. This has been done in many hatcheries in years past to bring about a zooplankton bloom to encourage the growth of the advanced fry and fingerling stages. The words bloom, pulse, or swarm will be used synonymously to describe a condition where an increase in numbers of an organism or organisms will bring about a lessening in the amount of light penetration or visibility. A reading of less than six feet with the Secchi disk denotes a bloom and is usually accompanied with a darkening of the water, and sometimes the presence of a film over the pond surface. The manure was placed in piles near the inlet and at the edge of the ponds. As expected a bloom was produced in each of the fertilized ponds following the application of manure. The plan called for the addition of inorganic fertilizer before the pulse subsided, to hold the first bloom, but it was not until June 18, 1946, that the commercial fertilizer was available and the first treatment made. In Pond 4 what appeared to be a phytoplankton pulse began during the third week in June. This delay may have resulted from the colder spring water. All other fertilized ponds had become clear.

The following program of inorganic fertilizer application was carried out, beginning June 18, 1946, and concluded on August 26, 1946. This part of the experiment was outlined to determine if a greater plankton bloom could be produced in a shorter length of time and maintained by the addition of smaller increments of fertilizer weekly, rather than one large amount every three weeks.

Table III

Fertilized and non-fertilized ponds showing frequency of fertilizer application.

Pond	Manure (pounds)	Inorganic Fertilizer (pounds)
4	1296	33 weekly
5	----	Control for Pond 4
7	2040	51 weekly
11	----	Control for Pond 7
12	2360	115 every 2 weeks
10	----	Control for Pond 12
9	2280	178 every 3 weeks
17	----	Control for Pond 9

The above table shows the pounds of manure and inorganic fertilizer used. The commercial fertilizer was broadcast over the pond surface from a rowboat. This required circling the edge of the pond and one or two trips down the center of the pond. An ordinary pint dipper was found to be most convenient to use for spreading. Throwing with the wind also aided in faster, more efficient coverage.

Plankton collections

The problem of obtaining a representative sample in any population is a difficult one. After many trials and eliminations a method was devised for collecting plankton samples from each pond, and also a method of concentrating the plankton forms. A tubular sampler was designed, similar to that used by Swingle and Smith of Alabama, but lack of materials held up its completion until the summer's collecting was nearly completed. In its place a one liter Erlenmeyer flask, attached to the end of a five foot handle was used. The flask was seated inside of a cut-away tin can of slightly larger diameter, which was placed on the end of the handle. By lowering the flask vertically, from the pond surface to the bottom at a constant rate, the container collected a sample of water from top to bottom. This was accomplished from a boat and the samples were taken while rowing from the pond inlet to the pond outlet and return. By adjusting the rate of descent of the bottle, to the depth of water, it appeared that the sampling was uniform. Samples were collected repeatedly into a clean, number two washtub. When the 10 gallon level was reached, the aggregate was mixed thoroughly by stirring and from this approximately 10 quarts were placed in a 12 quart galvanized pail, and two quarts in a mason jar.

At the laboratory in the hatchery headquarters building, duplicate three liter samples were concentrated by running through a Foerst Plankton Centrifuge. This technique is described in detail by Juday (1926). The residue from the centrifuge was placed in two ounce screw-top square glass jars, and preserved with 20 to 30 percent 6-3-1 preservative (6 parts of distilled water, 3 parts of 95% alcohol, and 1 part of commercial formalin).

A portion of the water from the two quart jar was used to determine pH, phenolphthalein, and methyl orange alkalinity. The remainder was saved in a quart milk bottle, and treated with one drop of Toluene to suppress further biological activity, sealed and placed in a cool storage. These samples are to be tested to determine the amounts of basic nutrients, as nitrates, phosphorus, potassium, calcium, iron, and magnesium found in the water from week to week. The results of this phase of the project will be described in a later paper.

Chemical and physical data

Records kept of the chemical and physical conditions, for purposes of this study, were made daily on temperature, wind, total hours of sunlight, and Secchi disk readings. All observations were made as nearly as possible to 10:00 A.M. Air temperatures and pond surface temperatures were taken with a pocket thermometer graduated in degrees Fahrenheit. Wind direction, velocity, and total hours of sunlight were estimated for each day. Secchi disk readings were recorded in ponds where blooms were present.

Weekly data consisted of chemical tests for pH, phenolphthalein and methyl orange alkalinity, and free carbon dioxide. These examinations were made for each pond prior to the application of fertilizer. No regular dissolved oxygen tests were made. It was thought that information derived from oxygen examinations was not essential as several observations indicated that dissolved oxygen did not reach a limiting point at any time except in the early morning periods in fertilized ponds with heavy blooms.

pH. The pH readings were made on a Macbeth constant line voltage pH meter. The pH showed a tendency to decrease in all ponds, and the trend was even greater in the fertilized ponds. Ammonium sulphate was used as a source of nitrogen in the fertilizer and may account for a portion of this decrease.

Phenolphthalein alkalinity was found by the common method using phenolphthalein as an indicator and titrating with N/50 H_2SO_4 as described in Amer. Public Health Assoc. (1936). This type of hardness decreased in all ponds from the early part of the experiment to zero in all ponds except Ponds 4 and 5 which maintained a small amount of phenolphthalein alkalinity throughout the study.

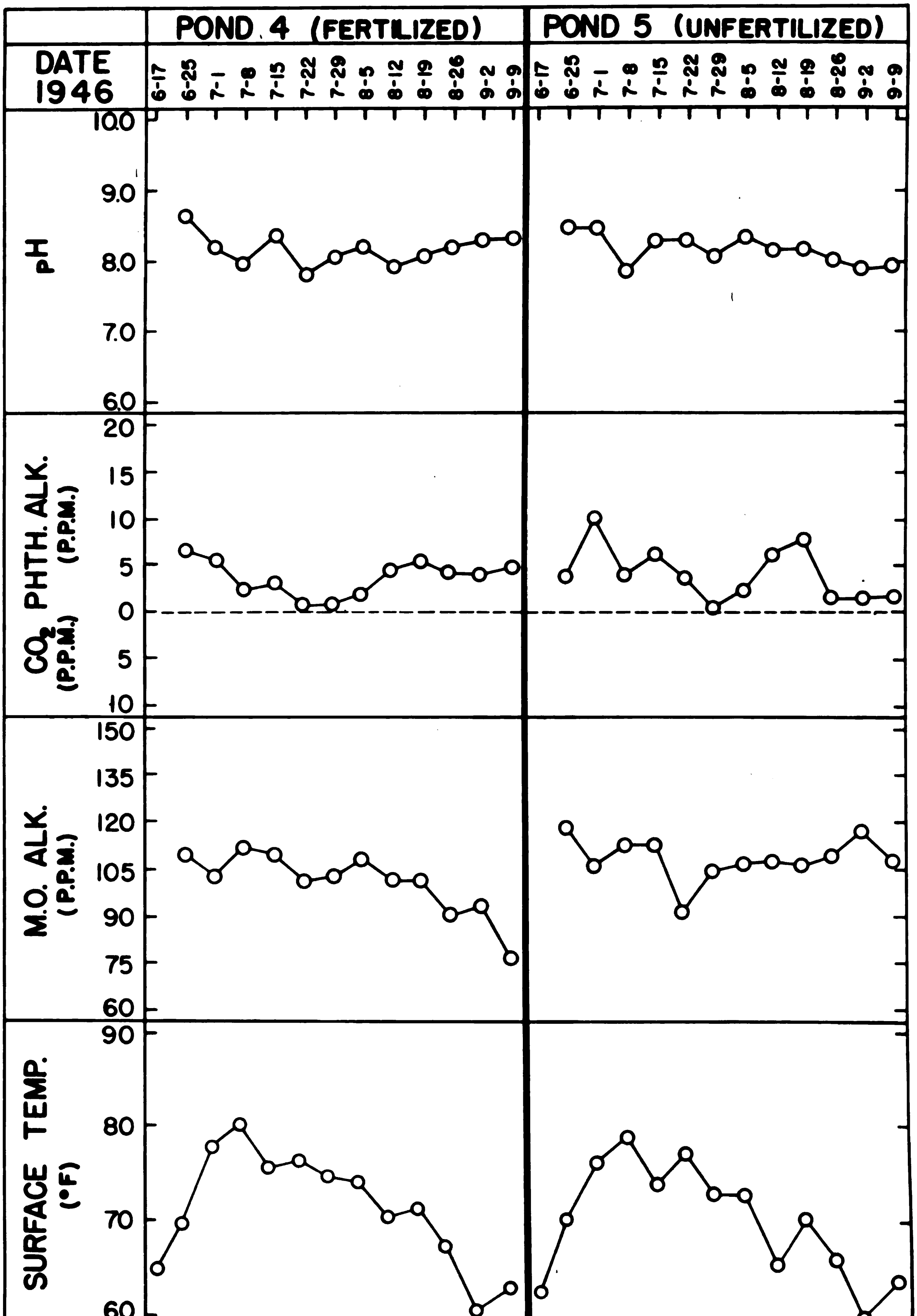
Dissolved carbon dioxide was determined by the standard method of phenolphthalein indicator and N/44 sodium hydroxide. This method is described in the Amer. Public Health Assoc. (1936). During the latter part of the experiment carbon dioxide was found in all ponds except 4 and 5. The respective charts show the presence of CO_2 where the phenolphthalein alkalinity reaches zero.

Methyl orange alkalinity was calculated by adding methyl orange indicator, to the sample used to compute the phenolphthalein alkalinity, and continue titration. Method used is described in Amer. Public Health Assoc. (1936). Addition of the results from both alkalinity readings equals parts per million of total hardness.

Temperature. Charts show average weekly surface temperatures for the study period. Surface temperatures fluctuate rapidly with regard to type of day. It is believed that temperatures taken two feet below the pond surface would give more reliable data.

Chart 2

Chemical and physical data from Ponds 4 and 5, consisting of pH, phenolphthalein (PHTH) alkalinity, carbon dioxide, methyl orange (M.O.) alkalinity, and pond surface temperatures.


POND 4 (FERTILIZED)
POND 5 (UNFERTILIZED)
DATE 1946
6-17 6-25 7-1 7-8 7-15 7-22 7-29 8-5 8-12 8-19 8-26 9-2 9-9
pH
10.0 9.0 8.0 7.0 6.0
CO2 (P.P.M.) PHTH. ALK. (P.P.M.)
20 15 10 5 0 5 10
M.O. ALK. (P.P.M.)
150 135 120 105 90 75 60
SURFACE TEMP. (°F)
90 80 70 60

<u>Comparison of paired ponds with regard to chemical and physical conditions</u>. Ponds 4 and 5, see Chart 2, showed a similar and constant relationship in all tests except for methyl orange alkalinity which decreased from 110 p.p.m., on June 25, 1946, to 75 p.p.m., on September 9, 1946, while the methyl orange reading for Pond 5 remained about the same.

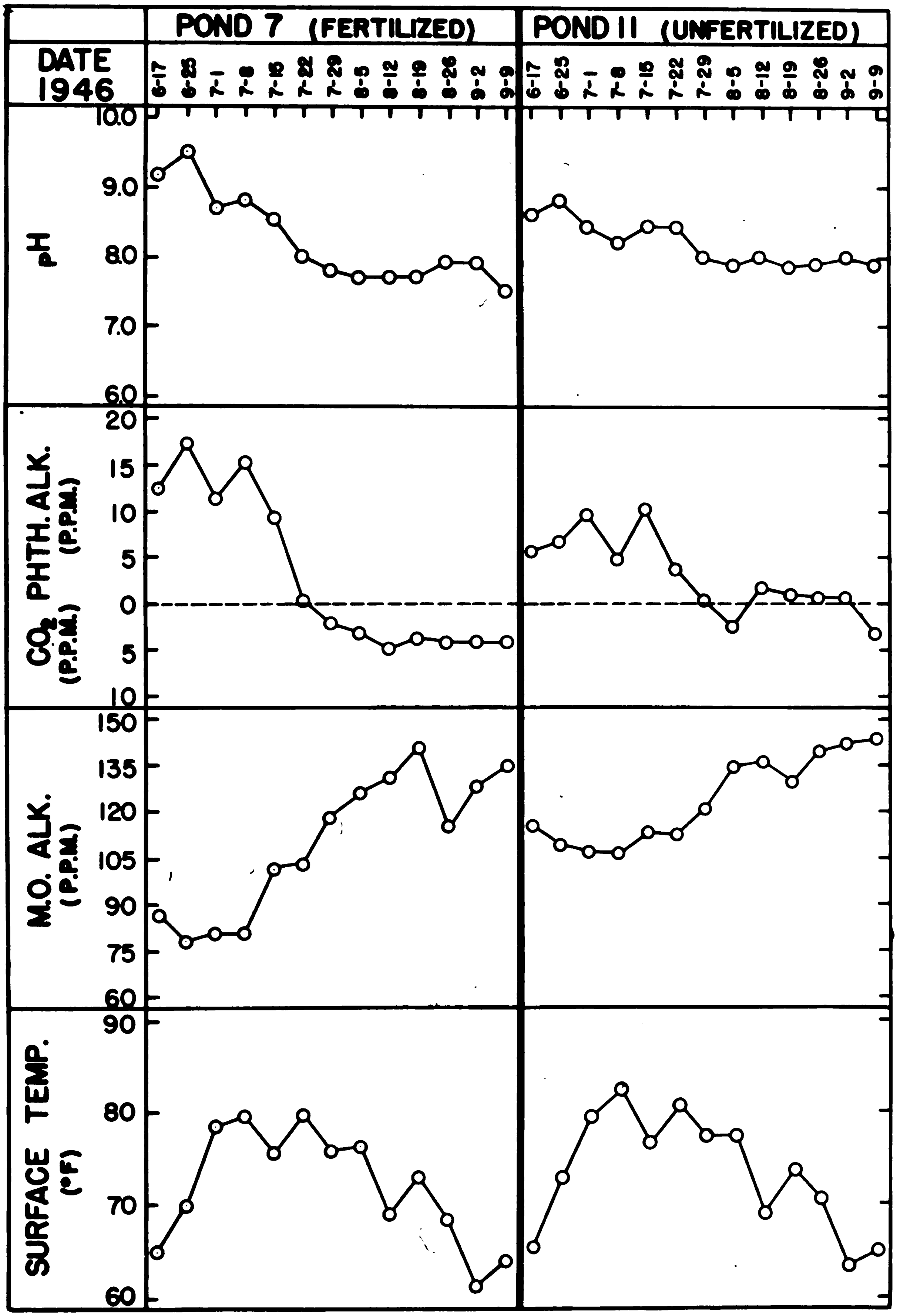
Ponds 7 and 11, see Chart 3, gave somewhat different trends. The pH dropped from 9.5 to 7.7 while in Pond 11 the pH decreased only slightly. On July 29, 1946, carbon dioxide was found in Pond 7. This condition continued throughout the rest of the study, even though a bloom was present and the methyl orange alkalinity was building up during this time. This would indicate a situation where only bicarbonate is present, for normal carbonate and hydroxide do not exist in any appreciable quantities in a solution more acid than about pH 8.2.

The presence of bicarbonate and free carbon dioxide must be attributed to the decomposition of bottom organic matter. This releases quantities of free carbon dioxide which tend to form bicarbonates and decrease the amounts of carbonates.

Ponds 9 and 17 show similar results to Ponds 7 and 11. The pH was higher in the fertilized Pond 9, reaching 9.4 on June 26, but gradually decreased during the summer to slightly below 8.0. The alkalinity in Pond 9 became somewhat erratic on July 15, 1946. A week of almost complete sunshine, averaging 13.6 hours per day, may have caused the peculiar

Chart 3

Chemical and physical data from Ponds 7 and 11, consisting of pH, phenolphthalein (PHTH) alkalinity, carbon dioxide, methyl orange (M.O.) alkalinity, and pond surface temperatures.

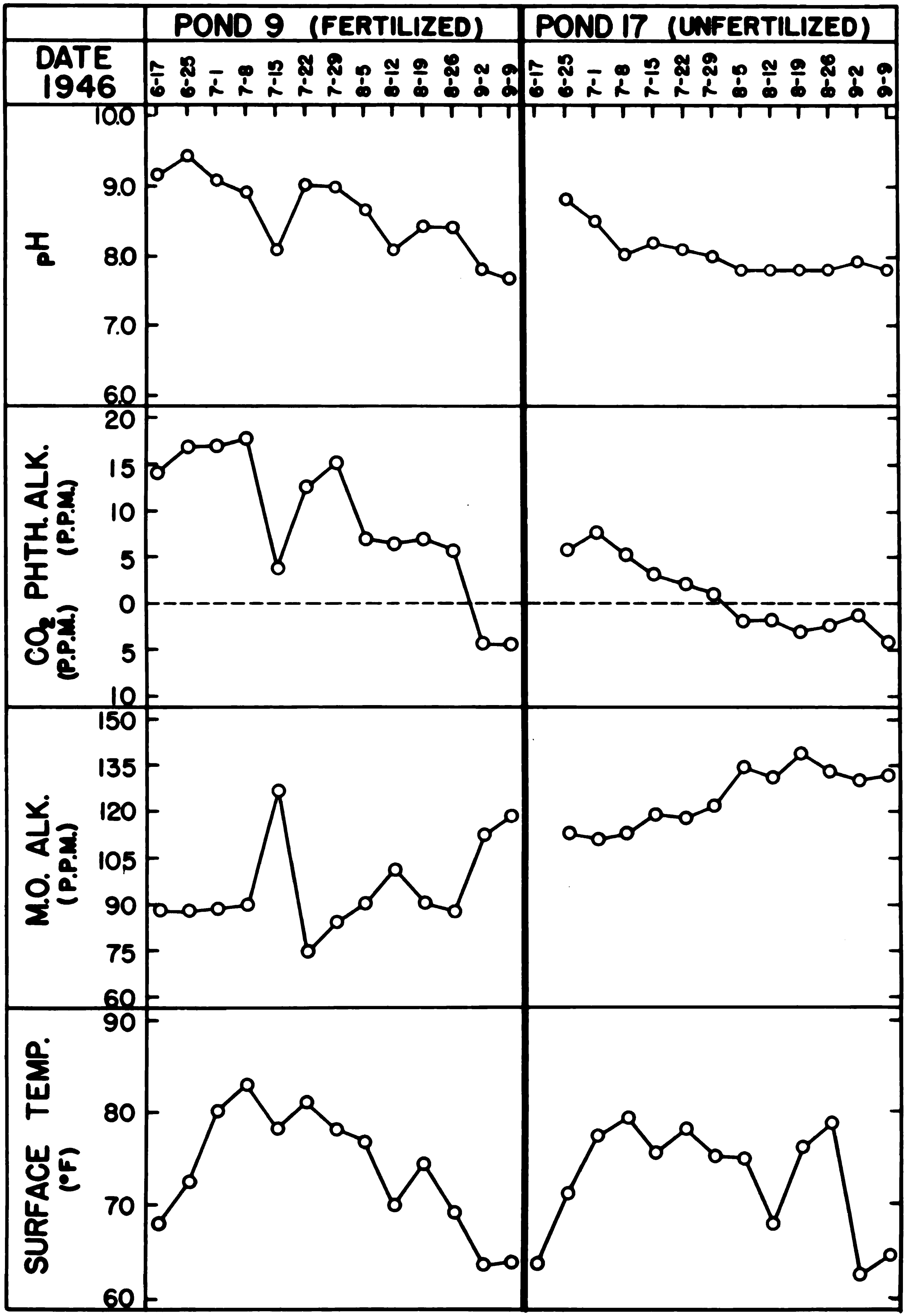

POND 7 (FERTILIZED)
POND 11 (UNFERTILIZED)
DATE 1946
6-17 6-25 7-1 7-8 7-15 7-22 7-29 8-5 8-12 8-19 8-26 9-2 9-9
pH
10.0 9.0 8.0 7.0 6.0
CO2 (P.P.M.) PHTH. ALK. (P.P.M.)
20 15 10 5 0 5 10
M.O. ALK. (P.P.M.)
150 135 120 105 90 75 60
SURFACE TEMP. (°F)
90 80 70 60

chemistry readings. The pH declined sharply as did the phenolphthalein alkalinity, while the methyl orange alkalinity rose correspondingly. Again decomposition of bottom organic matter may have contributed to this condition by releasing amounts of free carbon dioxide thus forming carbonic acid, and reducing the pH. The presence of carbonic acid caused the formation of bicarbonates from carbonates present in the water and bottom deposits. A bloom commenced on August 5 in Pond 9. This continued until September 1 when it declined suddenly. This can be seen in the readings for September 2 and 9. Amounts of carbon dioxide were present causing the bicarbonate form of hardness to be found in greatest quantities. In Pond 17, while there was no bloom at any time, higher aquatics were medium in abundance until July 30, when they were observed to have started "going down", a process where the whole plant seems to settle to the bottom similar to grain crops after a wind or hail storm. As indicated by Chart 4, on August 5, there was no phenolphthalein alkalinity, and 2.0 p.p.m. of carbon dioxide were present. Bicarbonates were present during the rest of the summer, the methyl orange alkalinity remained constant.

The two remaining Ponds, 12 and 10, manifest the same changes as the last two pairs. Observations in the unfertilized Pond 10 remained constant while in the fertilized Pond 12 the pH decreased from a pH of 9.8 on June 25 to slightly below 8.0 on August 5 and continued at nearly that reading. Bound carbonates and half-bound carbonates were

Chart 4

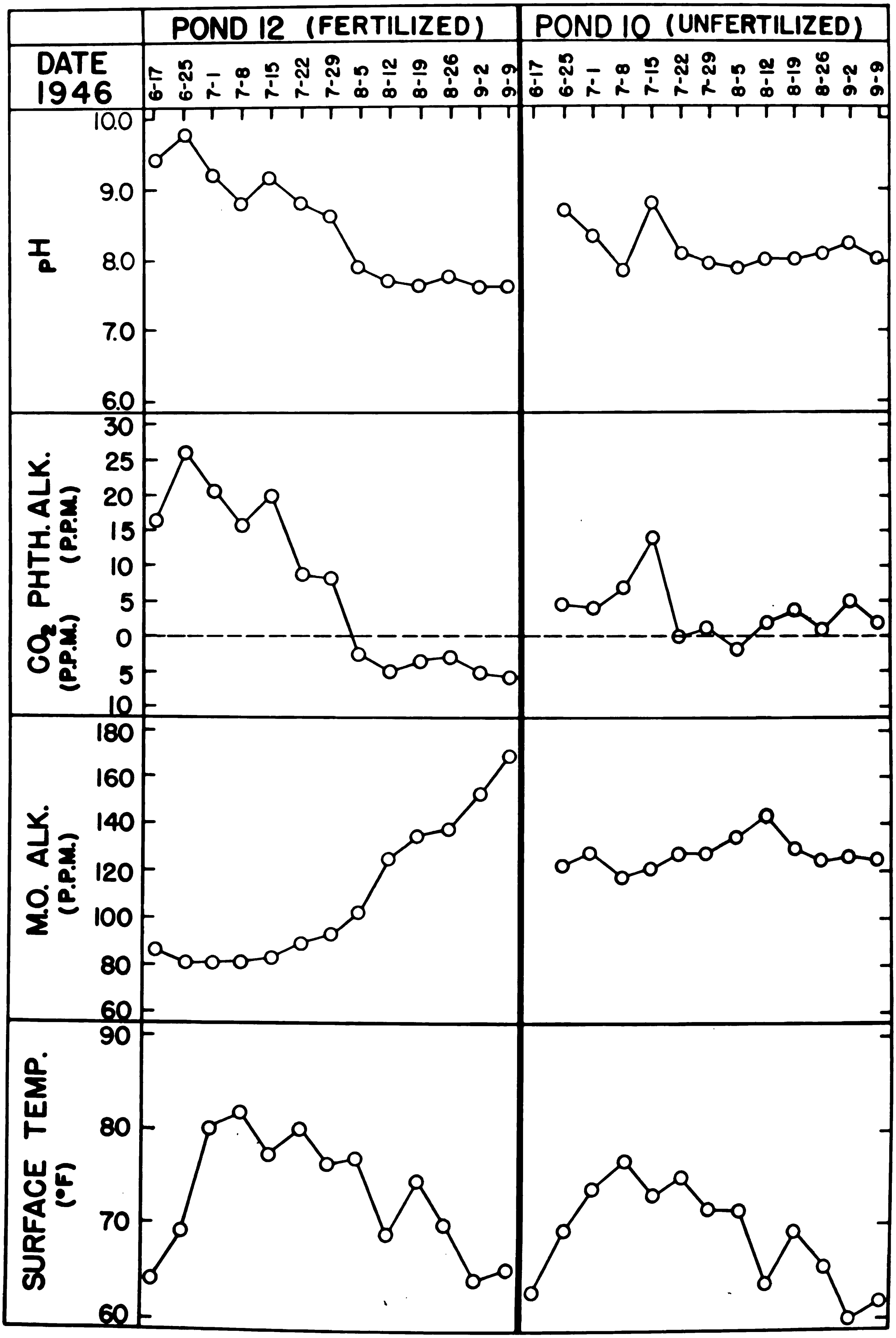
Chemical and physical data from Ponds 9 and 17, consisting of pH, phenolphthalein (PHTH) alkalinity, carbon dioxide, methyl orange (M.O.) alkalinity, and pond surface temperatures.


POND 9 (FERTILIZED)
POND 17 (UNFERTILIZED)
DATE 1946
6-17 6-25 7-1 7-8 7-15 7-22 7-29 8-5 8-12 8-19 8-26 9-2 9-9
pH
10.0 9.0 8.0 7.0 6.0
CO_2 (P.P.M.) PHTH. ALK. (P.P.M.)
20 15 10 5 0 5 10
M.O. ALK. (P.P.M.)
150 135 120 105 90 75 60
SURFACE TEMP. (°F)
90 80 70 60

found until the appearance of a light bloom on August 5. The growth of aquatic plants in Pond 12 was the most luxuriant of all, but started to go down in a manner similar to the plants described in Pond 17, during the last week in July. The water became dark-brownish in color. Again only half-bound carbonates were present at the conclusion of the experiment.

Chart 5

Chemical and physical data from Ponds 12 and 10, consisting of pH, phenolphthalein (PHTH) alkalinity, carbon dioxide, methyl orange (M.O.) alkalinity, and pond surface temperatures.


POND 12 (FERTILIZED)
POND 10 (UNFERTILIZED)
DATE 1946
6-17 6-25 7-1 7-8 7-15 7-22 7-29 8-5 8-12 8-19 8-26 9-2 9-9
pH
10.0 9.0 8.0 7.0 6.0
CO2 (P.P.M.) PHTH. ALK. (P.P.M.)
30 25 20 15 10 5 0 5 10
M.O. ALK. (P.P.M.)
180 160 140 120 100 80 60
SURFACE TEMP. (°F)
90 80 70 60

Tests for nutrients

Many factors have been taken into consideration in an attempt to explain the periodicity, production, and distribution of plankton. This may date back as far as 1830, when Liebig propounded the "Law of the Minimum", "which may be stated as follows: Each organism requires a certain number of food materials, and each of these materials must be present in a certain quantity. If one of these food substances is absent, the organism dies; if not absent but present in minimal quantity, the growth will be minimal, " Welch (1935). Other factors include temperature, sunshine, vertical circulation of the water, rainfall and flood, dissolved substances in the water, and nature of the geological formation. According to Chu (1942), all except the first two factors influence the growth of phytoplankton, mainly through the effect of the substances dissolved in the water.

Some authors went to great lengths in an endeavor to determine indices of plankton productivity. Pearsall (1930), and Chu (1942), made determinations of the concentrations of important minerals in natural waters, and studied their relationship to the fluctuation of plankton growth. Pearsall (1930), proposed that "It should however, be quite clear that in no case can we affirm that the mass of plankton (or of any other form of life) is proportional to the total concentration of substances dissolved in the nutrient medium. It is and must be some function of the limiting ion (or ions), i.e., the substance present in the smallest quantity proportionate to the amounts required by the plant." The general opinion was that certain optimum conditions built up through the summer months, and algae blooms would result. Later, certain limiting factors develop and bring about a "dying out" of the pulse. Typical of this type of observation is the work of Damann (1941), who made a two-year quantitative study of phytoplankton of Lake Michigan at Evanston, Illinois. Collections revealed two peaks in average monthly totals. The major peak occurring in June and the minor peak in November. Damann also found that hours of sunshine show a positive correlation with the total phytoplankton produced during the spring and summer.

Analyses of water samples from the source of water supply for the Wolf Lake Hatchery were not carried out until after completion of the experiment (December 15, 1946). Samples of water were collected from number 1 spring and from Pond 6, the mixing pond, which contains spring and lake water. Complete analysis was made by the Michigan Department of Health. Extracts from the report follow:

Table IV

Chemical analysis of Wolf Lake Hatchery source waters.*

Chemical Analysis	No.1 Spring(p.p.m.)	Pond 6 (p.p.m.)
N as NO_2	0.18	0.019
N as NO_3	none	none
Solids, total	186.00	184.00
SiO_2 (silica)	6.4	4.0
Fe_2O_3	0.12	0.1
Ca	44.00	44.00
Mg	15.2	15.6
Na and K	2.	trace
Cl	2.	2.
SO_4	28.8	23.9
HCO_3 (bicarbonate)	175.7	175.7
CO_3	none	none
Fluorine	.05	.05

* Data taken from analyses by the Michigan Department of Health, reports 495 and 496.

Inorganic fertilizer was applied in the dry form with filler. There has been some speculation as to the amount that goes directly to the pond bottom and that part which becomes available later. A table follows showing reserve nutrients in samples of bottom soil from the experimental ponds after draining. Analyses were performed by the Soils Department of Michigan State College, using the Spurway Tests.

Table V

Analysis of bottom soils upon completion of fertilization experiment.

Ponds	Nutrients in Pounds per Acre 6 Inches(furrow depth)						
	P	K	NO_3	Ca	Mg	Fe	Mn
* 4	20	30	40	6000	30-40	10	20
5	12	24	40	6000	30-40	16	16
* 7	15	22	40	5000	30-40	20	20
11	13	46	40	4000	30-40	20	10
* 9	19	80	40	6000	30-40	32	40
17	13	58	40	--	30-40	40	10
* 12	20	96	40	4000	30-40	20	20
10	12	34	40	5500	30-40	16	16

* Fertilized ponds

In order to interpret the above in p.p.m., the following conversions are taken from Spurway (1944). High, medium and low refer to that amount of nutrients required for growth of farm crops.

Table VI

Conversion of nutrient values in pounds per acre six inches to parts per million.

Calcium	1600	pounds	(high)	=	200	p.p.m.
Magnesium	16	"	(dangerous)	=	2	"
Potassium	40	"	(medium)	=	5	"
Phosphorus	20	"	(medium)	=	2.5	"
Nitrate	40	"	(low)	=	5	"
Manganese	32	"	(high)	=	4	"
Iron	32	"	(medium)	=	4	"

Table V indicates a deposition of phosphorus in the bottom soils of the fertilized ponds. How this took place would be worth-while knowing. A medium amount of potassium was present as it usually is throughout this area. Nitrates were low, whereas calcium and magnesium were extremely high. This is possibly due to the accumulation over the years. The ponds have been in operation for approximately 18 years. Iron content was medium in all ponds while the manganese was high and for some reason follows the same pattern as the phosphorus, being higher in all fertilized ponds.

Much work has been done in Wisconsin by Birge and Juday and co-workers in trying to correlate essential nutrients with plankton production. The problem is a very complex one and deserves more experimentation.

<u>Methods of plankton evaluation</u>

Several methods were used in an attempt to find a practical criterion of plankton production. Observations with the Secchi disk were made in the field. One other method, that of measuring the volume of settled plankton could be used in the field with a small amount of extra equipment. The three remaining methods are purely laboratory technicues. One requires the enumeration or counting of the individual cells per known volume of water. The second applies the use of a photoelectric colorimeter to measure the amount of light absorption. The third and last method is a quantitative determination of the total particulate organic matter. Each technique is described in the following paragraphs.

Secchi Disk

The Secchi disk is a piece of limnological apparatus composed of a disk 20 centimeters in diameter and is attached to a graduated line. The surface is painted black and white so that opposite quadrants are of the same color. A reading is taken by lowering the disk into the water until the disk just disappears from sight and then bringing it up slowly until it reappears. The average of these two readings are recorded as the Secchi disk reading. All readings were made at mid-forenoon to make the conditions as nearly standard as possible.

Welch (1935), relates that the Secchi disk was invented by A. Secchi in 1865, for use in studying the transparency of the waters of the Mediterranean Sea. This instrument was selected for this experiment as it provides a simple and a quick means of measuring the visibility of water light-penetration. It is also used widely in lake survey techniques.

Light Absorption

Percent of light transmitted or the percent of light absorption readings were made on a Will Corporation, Lumetron (photoelectric colorimeter), Model 400-A. A red filter, number 650, and absorption vials (22 millimeters in diameter) were standard equipment. The photoelectric cell is connected to a microammeter which is calibrated to read in percent of light transmitted. The red filter acts to absorb the rest of the colors of the visible spectrum and allows only the red light to pass through, therefore the reading in percent of light transmitted is the amount of light not absorbed by the sample. This value subtracted from 100 percent equals the quantity of red light absorption.

Before testing, samples were brought to a uniform volume of 50 milliliters, mixed thoroughly, and a portion placed in an absorption vial and the reading recorded. This method assumes that there is little or no turbidity caused by finely divided soil particles. This technique requires very little time but does require electricity to operate the colorimeter--this is important when considering field operations.

Volume of Organic Matter

The preserved plankton was allowed to settle in tapered, glass centrifuge tubes with a volume of 50 milliliters. These were allowed to stand overnight and were read the following morning as cubic centimeters of plankton. The larger particles and organisms settle out usually within an hour, while the smaller organisms, especially those fragmented after passing through the centrifuge, require a longer period of time. This method is used by some lake survey parties. Bottom organisms are commonly expressed in volume. It was for these reasons that this method was used for comparison.

Enumeration by Direct Count

A modified method of making counts was employed to determine numbers and kinds of organisms. The direct count is simple and requires a minimum of equipment. Modification of the counting system was developed at the New Jersey Oyster Investigation Laboratory by G. W. Martin and has since been used sucessfully at the Iowa Lakeside Laboratory, Martin (ms). The procedure for making counts is given below.

1. Measure diameter of low or high power field of the microscope. Compute area.
2. Compute area of 22 millimeter cover glass.
3. Compute number of fields low or high power magnification

there are in the area of a cover glass.

4. Concentrated plankton sample is brought up to a known volume. 3 liters concentrated to a volume of 50 cc.
5. A dropping pipette with rather small aperture is standardized so that the exact volume of a drop is known. Example 21 drops equal 1 cc.
6. Sample is completely stirred, and a drop of concentrate is allowed to fall on a clean slide and is immediately covered with a clean, round cover slip. Prefer 22 mm.
7. Place slide in mechanical stage of microscope and make a random count of organisms in 40 different fields. The total number divided by the number of fields equals the average number.
8. Multiply the average number of organisms per field by the number of fields in a 22 mm. cover slip. This equals the number of organisms per drop.
9. Multiplication of the number of organisms per drop by 21 equals the number of organisms per cc. This is divided by the concentration factor and multiplied by 1000 to obtain the number of organisms per liter of pond water.

All counts were made on a Bausch and Lomb, binocular microscope under high power, using a 10x ocular and a 97x objective. The organisms were all quite small, requiring high magnification. Also the concentration used was such that under low magnification, (10x ocular and 43x objective), the numbers of organisms were too abundant to count. This

was true for certain diatoms (Synedra) and green algae (Dictyosphaerium).

Calculation of number of organisms per liter is based on the area of a high power field. Calibration of the microscope used, revealed a high magnification field with a radius of 0.18 millimeters, and an area of 0.1017 square millimeters.

$$\frac{380.13 \text{ sq.mm. area of 22 mm. cover slip}}{0.1017 \text{ sq.mm. area of high power field}} = 3738 \text{ fields per cover slip}$$

therefore 3738 times the average number of organisms per field equaled number of organisms per cover slip.

Example: Total count of organisms per 40 fields equaled 4 or average number equaled 0.10. Therefore:

$3738 \times 0.10 = 373.8 =$ number per cover

$373.8 \times 21 = 7849.80 =$ number per cc.

$7849.8 \times 1000 =$ organisms per liter of concentrate.

$7{,}849{,}800 \div 60$ (concentration factor) $= 130{,}830$

or number of organisms per liter of original sample. Counts were tabulated on plankton data sheets shown on page ii of the appendix.

Tables were made so that actual numbers could be read off directly, using total number of organisms. All algae were identified to species whenever possible. Identifications were made from Smith (1920), (1935), Paschèr (1925), Wolle (1894), and Ward and Whipple (1918).

Gravimetric Determination of Organic Matter

The weight of dry organic matter was determined by the method described by Juday (1926). Several modifications were used, however, the technique was essentially the same. Duplicate plankton catches were transferred from the centrifuge bowl to 20-gram, Norton Alundum, weighed crucibles. The samples were then placed on a Lindberg laboratory hot plate and allowed to "simmer" to dryness. Crucibles were transferred to a desiccator for a period of 48 hours to bring to constant weight. A Sargent chain analytical balance was used to obtain the dry weight of the plankton. After weighing, crucibles were placed in an electric oven at 600° C for 30 minutes, removed and again brought to constant weight by method previously described. The difference between the final weighing, after ashing, and weight of the crucible plus the dry plankton is equal to the weight of dry organic matter, or total particulate organic matter. Weights are expressed in milligrams per liter, and for the sake of simplicity may be referred to as parts per million. The samples were not corrected for the loss sustained on ignition by the amount of pond water accompanying the catch. The samples had been transferred several times in making other determinations and the residue resulting from pond water was so minute it was believed to be unimportant. Duplicate samples were found to be very consistent in the weight of dry organic matter. The early summer readings from Ponds 4 and 7 showed average readings of 2.8

and 4.1 milligrams per liter, and at their peaks showed 9.0 and 18.3 milligrams of dry organic matter per liter. These figures are shown on Charts 6 and 7.

COMPARISON OF METHODS USED TO EVALUATE STANDING CROPS OF PLANKTON.

The weekly crops of plankton from the fertilized Ponds 4 and 7 were composed chiefly of green algae and diatoms. Charts 8 and 9 show groups of plankton found in the control and fertilized ponds. The blue-green algae and zooplankton remained low in numbers, and the Dinophyceae and Chrysophyceae were of little importance.

Comparison of the methods used in this experiment are illustrated in Charts 6 and 7. Each series of observations is shown graphically on the same chart by selecting an upper limit as a maximum. Weekly readings were calculated in terms of percent, using the maximum as 100 percent, and plotting points accordingly. It will be noted that there is a rather close comparison of all methods with a few noticeable deviations. Total particulate organic matter is the most generally accepted as the best criterion of biological productivity. Riley (1940), and Swingle and Smith (1939), have accepted this in their work. Each method will be treated separately and compared as such with the other methods.

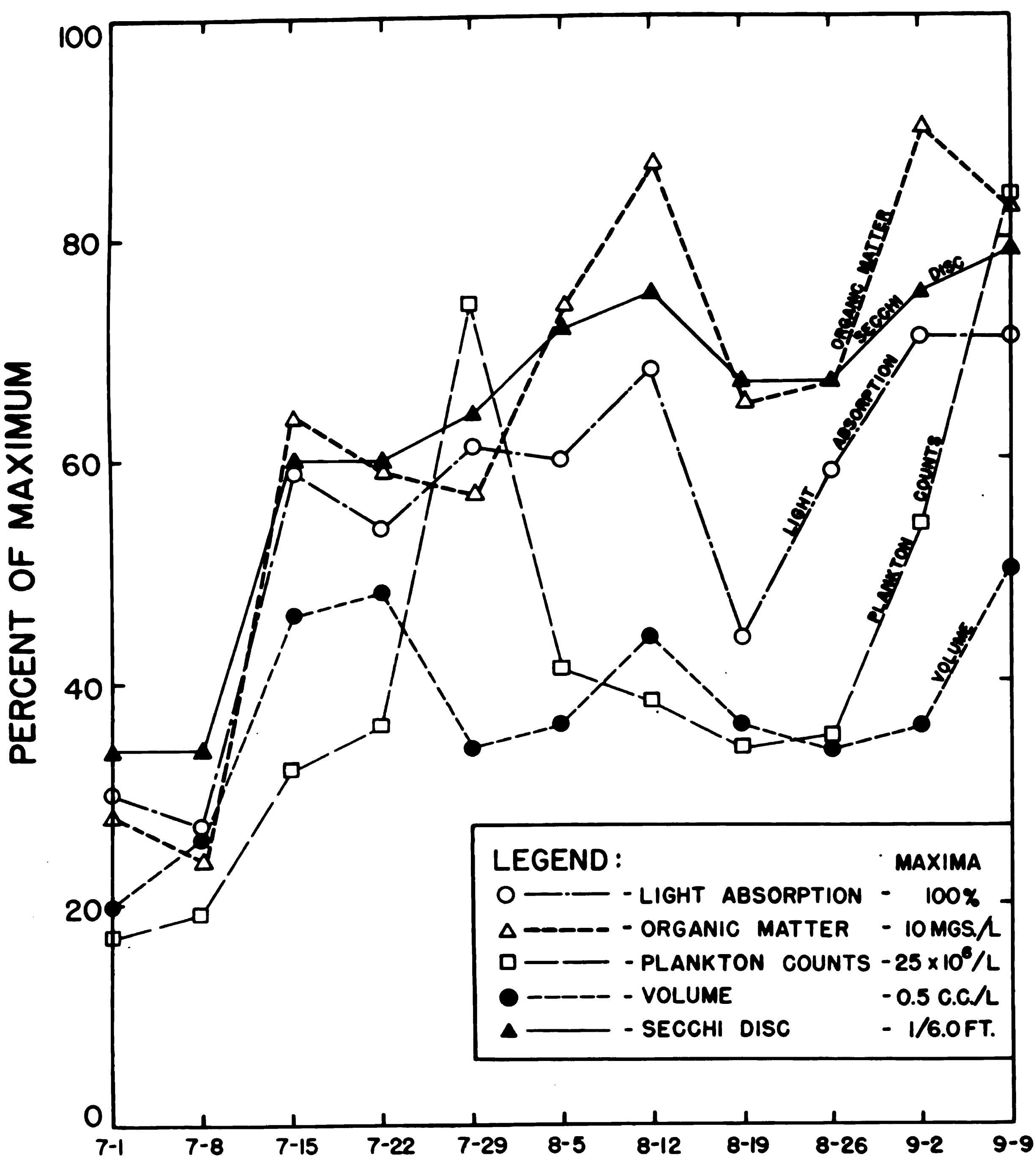
Secchi disk

Comparison of Secchi disk readings with those of organic matter and light absorption shows a very close relationship. This is shown on Charts 6 and 7. The use of this simple piece of equipment may serve as a good measuring stick to indicate an increase or a decrease of a bloom in a pond. It

Chart 6

Comparison of several methods of plankton evaluation (Pond 4)

(Lines show variations of plankton in terms of organic matter in milligrams per liter; plankton counts in numbers of organisms per liter; volumetric reading or organic matter in cubic-centimeters per liter; light absorption in percent; and Secchi disk reading in feet.)



PERCENT OF MAXIMUM
100
80
60
40
20
0
7-1
7-8
7-15
7-22
7-29
8-5
8-12
8-19
8-26
9-2
9-9
ORGANIC MATTER
SECCHI DISC
LIGHT ABSORPTION
PLANKTON COUNTS
VOLUME
LEGEND:
MAXIMA
- LIGHT ABSORPTION - 100%
- ORGANIC MATTER - 10 MGS./L
- PLANKTON COUNTS - 25 x 10⁶/L
- VOLUME - 0.5 C.C./L
- SECCHI DISC - 1/6.0 FT.

is conceivable that when a bloom declines below 18 inches or some other value, more fertilizer should be added.

Light absorption

Use of the chlorophyll content of plankton as an index of lake productivity has been used by several authors, among them Kosminski (1938), and Manning and Juday (1941). This method involves the use of an extraction process to separate the chlorophyll by acetone, or by other agents. It cannot be considered as a practical field method. Barrett (ms) obtained a high correlation by direct colorimeter readings correlated with the weight of dry organic matter. In view of this, colorimeter readings were made of all samples as described under methods of plankton evaluation. There are two factors which affect this type of reading, namely, variations in groups composing the plankton, and the presence of any turbidity due to finely divided soil particles. The samples were thought to be free of the latter type of contamination.

Results show a close comparison between light absorption and dry organic matter readings. This may be caused by the predominance of green algae. As can be seen on Chart 6, the light absorption in collections made on August 19 declined considerably as did the dry organic matter in the same samples. The answer to this is given on Chart 8, where a corresponding decrease in green algae is found.

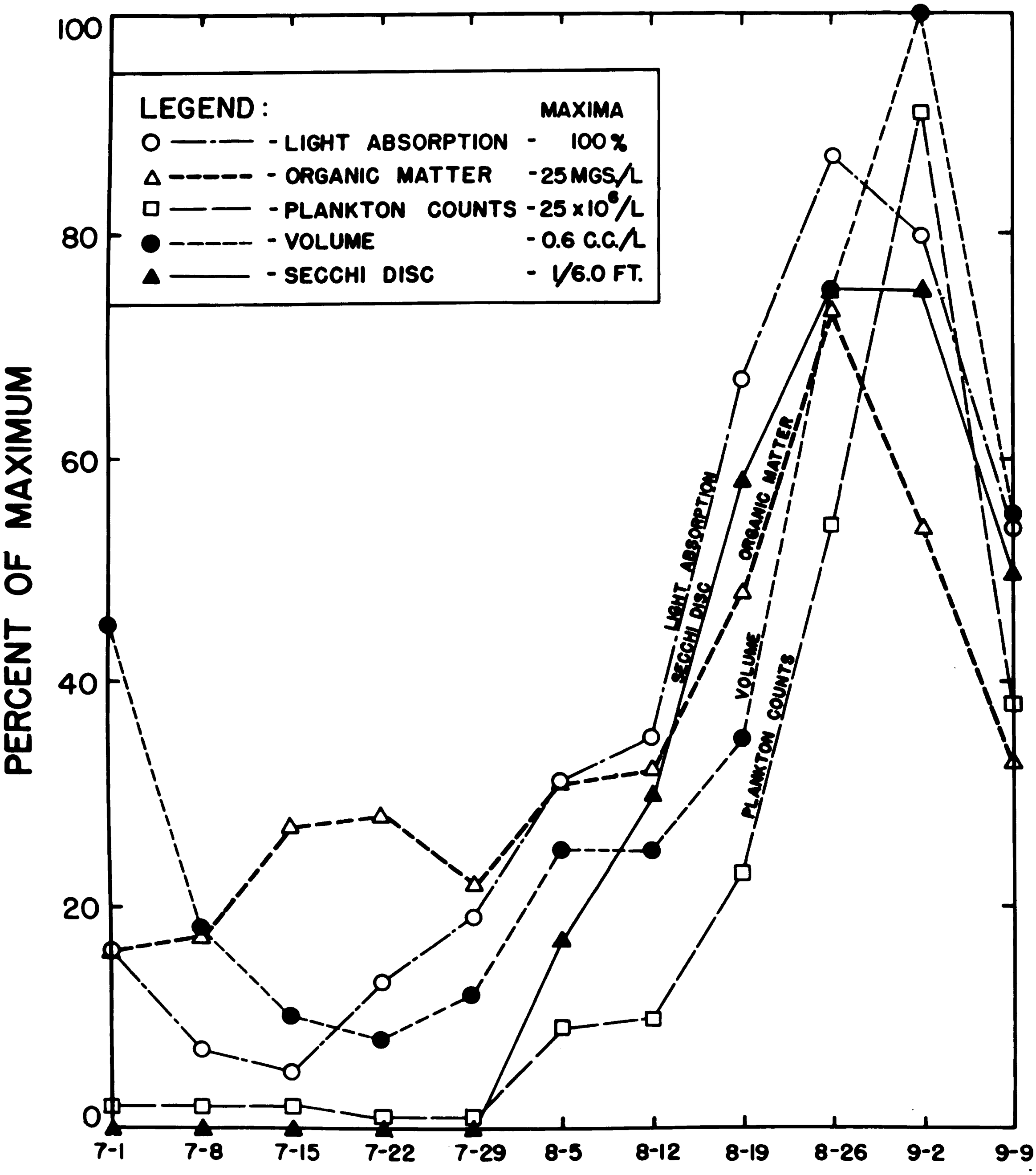
Volume of plankton

The volume of plankton was obtained by a settling

Chart 7

Comparison of several methods of plankton evaluation (Pond 7)

(Lines show variations of plankton in terms of organic matter in milligrams per liter; plankton counts in numbers of organisms per liter; volumetric reading or organic matter in cubic-centimeters per liter; light absorption in percent; and Secchi disk reading in feet.)


LEGEND:
MAXIMA
LIGHT ABSORPTION - 100%
ORGANIC MATTER -25 MGS/L
PLANKTON COUNTS -25 x 10^6/L
VOLUME - 0.6 C.C./L
SECCHI DISC - 1/6.0 FT.
PERCENT OF MAXIMUM
100
80
60
40
20
0
7-1
7-8
7-15
7-22
7-29
8-5
8-12
8-19
8-26
9-2
9-9
LIGHT ABSORPTION
SECCHI DISC
ORGANIC MATTER
VOLUME
PLANKTON COUNTS

process. If the total particulate organic matter as determined by sampling is used as the best measure of pond productivity, then the volume measurement is not a reliable one. In several cases the volume and weight comparison showed inverse relationships. On Chart 7 it is shown that the volume on July 1 showed 45 percent of the maximum while all other measurements were below 20 percent. On August 26, the volume and weights of plankton were approximately the same, and on September 2, the volume had risen to 100 percent while the actual dry organic matter weight had decreased substantially. When compared to the counts, the difference is accounted for by the large number of minute cell-fragments of green algae. These experiments show that volume as determined by the settling method is not a reliable criterion of a standing crop of plankton.

Plankton counts

Plankton counts were determined by a direct method of counting. This method is perhaps the simplest; however, it is time-consuming and requires a great amount of skill in identification and manipulation. Results obtained from this method compared quite closely with the more generally accepted method of determining dry organic matter per unit volume. This has somewhat the same shortcomings as the volume calculation. When a bloom of an extremely small colonial form as *Dictyosphaerium pulchellum* existed the count of organisms per standing crop became greater in comparison to the rise in dry organic matter. This is shown in Chart 7 where on September 2, the line representing counts exceeded that representing the weight measurements. In Chart 6 on July 29, the fluctuation of the small diatom _Synedra_ sp. caused a sharp increase in numbers, with a lesser corresponding increase in weight.

Total particulate organic matter

Dry organic matter, as determined by method described in preceeding section, is generally accepted today as the best criterion of plankton production. Riley (1940), defines phytoplankton production as "the quantity of phytoplankton produced during a given unit time, expressed as the weight of dry organic matter". Since all but a small percent of the plankton taken was phytoplankton, the results are presented as milligrams of dry organic weight and are used as a basis of comparison with the other methods.

CHECK-LIST AND NUMERICAL TABULATION OF PLANKTON ORGANISMS.

Results from weekly plankton counts are tabulated by species, by total numbers, and graphically by groups of organisms. A detailed weekly count by species is presented in Table VII which is also a check-list of all organisms found in the experimental ponds. Organisms in this table are listed in order using the classification of Smith (1933). Notes on the important organisms will follow in the next section of the paper.

A comparison of total numbers of organisms present in samples each week is shown in Table VIII. Total and average counts for the period between July 1 and September 9, inclusive are also recorded in Table VIII. This shows the fluctuation in numbers of organisms during the collecting period. These results are also presented graphically in Charts 8 and 9.

It will be noted that the green algae made up a large portion of the bloom in both Ponds 4 and 7. In Pond 4, which has a sand bottom, a diatom pulse was also found. When sufficient data has been collected to show which plankton organisms are encouraged under certain conditions and by certain fertilizers, predictions may then be made regarding the alterations of food cycles. The knowledge of the kinds and abundance of plankton is necessary when taking stomach samples to determine what the fish eat, when certain plankters are present in the qualitative samples.

Table VII

Check-list and numerical tabulation of plankton organisms

from Ponds 4, 5, 7, and 11.

(Numbers represent individual organisms and colonies of each species as they occur in nature. *Dactylococcopsis* and *Dictyosphaerium* counts represent numbers of colonies)

Table VII

Organisms	Pond	July 1, 1946	July 8, 1946	July 15, 1946	July 22, 1946	July 29, 1946	Aug. 5, 1946	Aug. 12, 1946	Aug. 19, 1946	Aug. 26, 1946	Sept. 2, 1946	Sept. 9, 1946
MYXOPHYCEAE												
Chroococcus turgidus (Kütz.) Näg.	5	16,250	....	....	....	....	....	....	....	....	....	....
	11	81,750	....	....	....	....	....	....	....	....	32,750	....
Aphanocapsa pulchra (Kütz.) Rab.	4	....	....	16,250	49,000	....	114,250	65,500	229,000	212,750	....	....
	7	....	....	32,750	32,500	16,250	81,750	343,500	441,500	16,250	65,500	....
	11	....	16,250	16,250	65,250	....	....	....	....	....	16,250	....
Merismopedia elegans A. Br.	7	....	16,250	....	....	....	....	....	....	....	....	....
Rhabdoderma lineare Schmidle and Lauterb.	4	....	....	....	....	....	16,250	16,250	....	....	131,000	....
	5	....	....	....	....	....	16,250	....	....	....	....	....
	7	....	....	....	....	....	....	....	....	16,250	....	32,750
Dactylococcopsis rhaphidioides Hansgirg.	4	32,500	32,500	81,750	81,750	163,500	212,500	81,750	65,500	65,500	32,500	49,000
	5	16,250	....	16,250	....	....	16,250	16,250	....	....	....	....
	7	....	....	....	....	....	114,250	16,250	32,500	32,500	32,500	32,500
	11	32,500	16,250	16,250	16,250	49,000	....	16,250	....	....	....	....
Aphanothece sp.	11	....	16,250	....	....	....	....	....	....	....	....	....
Coelosphaerium Naegelianum Unger.	11	....	....	....	....	....	....	....	....	....	....	16,250
Oscillatoria sp.	7	65,500	....	16,250	....	....	....	....	....	....	....	....
	11	....	16,250	....	....	....	....	....	....	....	....	....
Phormidium sp.	4	16,250	....	....	....	....	....	....	....	....	32,750	....
	5	16,250	....	....	....	....	....	....	....	....	....	....
Lyngbya sp.	7	....	49,000	....	....	....	....	....	....	....	....	....
Unidentified filament.	4	81,500	....	....	....	....	....	....	32,750	....	....	....
	7	....	131,000	114,500	32,500	16,250	16,250	....	....	....	....	....
	11	....	....	....	....	....	16,250	32,500	....	....	....	....
Unidentified cell.	4	81,750	65,500	....	....	....	....	....	....	65,250	130,500	32,750
	5	49,000	147,000	65,750	16,250	....	49,000	16,250	16,250	32,750	49,000	49,000
	7	65,750	81,500	163,500	....	65,500	....	16,250	392,500	....	....	....
	11	16,250	179,500	65,750	32,250	32,500	....	65,750	....	65,750	65,500	65,750
CHRYSOPHYCEAE												
Mallomonas sp.	4	16,250	....	....	....	16,250	....	....	....	16,250	....	....
Dinobryon sp.	11	....	....	....	....	....	....	....	....	....	16,250	49,000
BACILLARIEAE												
Cyclotella sp.	4	32,750	65,750	327,000	163,500	507,000	245,250	196,000	180,000	114,500	98,000	32,750
	5	....	16,250	32,750	16,250	....	....	....	....	49,000	49,000	....
	7	....	....	....	16,250	....	....	49,000	32,750	228,500	425,000	32,750
	11	....	....	....	....	32,750	....	....	....	....	....	....
Synedra sp.	4	2,583,750	3,892,250	3,009,000	3,990,250	12,429,000	4,644,500	5,380,250	4,726,250	3,974,000	6,018,250	6,656,250
	5	130,750	179,750	16,250	65,500	32,750	65,750	114,500	98,000	16,250	32,500	32,500
	7	....	....	....	....	49,000	81,750	180,000	245,250	343,250	752,250	686,750
	11	81,750	32,750	16,250	49,000	16,250	....	....	49,000	16,250	16,250	16,250
Achnanthes sp.	4	294,250	147,250	278,000	98,000	32,750	32,750	[illegible]	16,250	....	....	....
	5	228,750	65,500	16,250	65,500	16,250	....	....	....	65,500	16,250	....
	7	49,000	....	49,000	....	16,250	16,250	16,250	32,750	32,750	32,750	....
	11	....	....	....	....	32,750	16,250	....	....	....	16,250	....
Navicula sp.	4	245,250	98,250	212,500	147,250	81,750	49,000	65,500	....	....	32,750	....
	5	32,750	16,250	98,250	65,500	....	49,000	32,500	81,750	114,500	130,750	98,250
	7	32,500	32,500	....	....	....	16,250	49,000	....	....	....	....
	11	....	....	49,000	....	16,250	16,250	16,250	32,750	16,250	32,500	32,750
Gomphonema sp.	4	114,500	32,500	98,250	....	32,750	16,250	16,250	16,250	....	....	....
	5	81,750	49,000	49,000	....	....	49,000	16,250	49,000	16,250	81,750	98,000
	7	32,750	32,500	....	65,500	32,500	16,250	81,750	163,500	98,250	196,000	130,750
	11	16,250	....	16,250	....	....	16,250	....	....	....	....	....
Cymbella sp.	4	....	....	65,500	16,250	32,500	....	....	....	....	....	....
	5	65,250	32,500	....	32,750	....	....	....	....	....	32,500	32,500
	7	....	....	....	....	....	....	32,750	32,750	32,750	....	....
	11	....	....	....	....	....	....	....	....	....	....	....
Amphora sp.	4	....	16,250	16,250	16,250	....	16,250	....	....	....	....	....
	7	....	....	....	....	....	....	....	....	49,000	32,750	32,750
Epithemia sp.	4	16,250	....	....	....	....	....	....	....	....	....	....
	5	....	....	....	....	....	....	....	....	....	32,750	....
	7	....	....	16,250	....	....	....	....	....	....	....	....
	11	16,250	....	....	....	....	....	....	....	....	....	....
Nitzschia tabellaria Grun., Cleve	4	49,000	16,250	98,250	49,000	49,000	65,250	[illegible]	....	16,250	16,250	16,250
	5	....	....	....	....	....	....	....	....	....	....	....
	7	....	....	....	....	....	....	....	....	32,750	....	....
	11	....	....	....	....	....	....	....	....	....	....	16,250
Nitzschia sp.	4	278,000	16,250	....	32,750	....	....	....	....	....	....	....
	5	180,000	....	....	....	....	....	....	....	....	....	....
	11	....	16,250	....	....	....	....	....	....	....	....	....
Unidentified diatom.	4	81,750	32,500	....	16,250	....	16,250	65,750	16,250	....	....	....
	5	16,250	81,750	16,250	81,750	49,000	16,250	16,250	32,750	....	....	81,750
	7	....	....	....	....	16,250	....	49,000	....	....	....	....
	11	....	32,750	....	....	....	....	....	49,000	....	16,250	49,000
CHLOROPHYCEAE												
Chlamydomonas sp.	4	....	....	163,500	49,000	....	32,750	....	....	....	....	32,750
	5	....	....	49,000	16,250	16,250	....	....	....	....	....	....
	7	....	....	16,250	....	....	1,586,250	425,500	212,500	65,750	....	163,500
	11	....	....	98,000	245,250	....	....	....	....	....	....	....
Sphaerocystis sp.	4	....	....	....	....	....	....	....	16,250	[illegible]	32,750	....
	11	....	....	....	16,250	....	....	....	....	....	....	....
Oedogonium sp.	7	....	16,250	....	....	....	....	....	....	....	....	....
	11	....	....	....	....	....	16,250	16,250	....	....	....	....
Golenkinia sp.	4	....	98,000	....	....	....	....	....	....	....	....	....
Pediastrum Boryanum (Turp.) Menegh.	4	....	32,500	....	....	....	16,250	....	16,250	....	98,250	....
	5	....	....	....	16,250	....	....	....	16,250	....	....	....

Coelastrum cambricum Arch.	4	114,500	98,250	2,796,500	3,352,500	3,777,750	2,629,000	1,799,000	507,000	507,000	163,500	425,250
	5			16,250	16,250			49,000				16,250
	7						16,250	98,250				32,750
	11							16,250				
Dictyosphaerium pulchellum Wood.	4			65,500	65,500	65,500	65,500	65,500	32,500	1[illegible],474,750	5,037,000	11,055,500
	5							32,500				
	7				32,500	32,500	65,500	131,000	3,925,000	11,807,500	19,245,000	6,619,750
	11	49,000							65,500	212,750	98,000	98,000
Oöcystis crassa Wittr.	4				16,250							
	7		32,750	32,750					245,250			
	11				16,250							
Oöcystis elliptica Hemmgirg.	4						49,000					
	7							49,000				
Oöcystis solitaria Wittrock.	5				32,750				49,000			
Cocystis sp.	7							458,000				
	11	32,500										
Ankistrodesmus spiralis (Turn.) Lemm.	4				16,250	81,750	81,750	98,250	387,250	163,500	556,000	946,500
	7								49,000	49,000	523,500	539,500
Schroederia sp.	4						81,750	65,750	16,250	16,250	32,750	
	7							32,750	81,750	163,500	130,750	
Closteridium sp.	7							16,250				
Kirchneriella obesa (W.West) Schmidle.	4								16,250			
	7								49,000			
	11					16,250						
Kirchneriella sp.	4										32,750	49,000
	7										32,750	
Quadrigula Chodati (Tanner-Fullman) G. M. Smith.	4											196,250
Quadrigula lacustris (Chod.) G.M. Smith.	4							49,000	16,250			
	11							65,750				
Tetraedron caudatum (Corda) Hansg.	4											32,750
Tetraedron minimum (A. Br.) Hansg.	4			32,500	16,250							
	7				16,250				32,750			
	11		16,250	16,250				16,250			16,250	
Tetraedron sp.	4						32,750	376,000	392,500	392,500	490,750	646,750
	5				16,250							16,250
	11									16,250		
Scenedesmus obliquus (Turpin) Kützing.	4	16,250		16,250	32,750	32,750	49,000	81,750	65,250	49,000	196,500	98,500
	5	16,250	16,250								16,250	
Scenedesmus arcuatus Lemmermann.	4					65,750			81,750		131,000	
	5										16,250	16,250
Scenedesmus quadricauda (Turpin) de Brebisson.	4	65,750	65,750	294,500	179,750	474,250	376,000	376,000	392,500	392,000	490,750	686,750
	5	16,250	16,250		16,250		16,250					16,250
	7		16,250					16,250	49,000	65,500	130,750	65,750
Scenedesmus sp.	4							49,000		65,750		
Crucigenia irregularis Wille.	4				16,250		32,750	32,750	16,250			
	7						65,500	343,500	1,799,000	261,500		163,500
	11				16,250		16,250					
Actinastrum gracillimum G.M. Smith.	4					65,750						
	7								376,000	130,750	834,000	539,750
Micractinium pusillum Fresenius.	4			180,000	425,250	392,500	834,000	752,250	654,000		65,500	
	7											98,000
Closterium acutum (Lyng.) DeBréb.	7										65,500	
Closterium sp.	4										32,750	
	7				16,250							
Cosmarium circulare Reinsch.	4			32,750		49,000	130,750	114,500	196,000	114,500	130,750	294,250
	5	16,250										
	7											32,750
	11											32,750
Cosmarium depressum (Nag.) Lund.	4					16,250	130,750	147,250	654,000	1,079,500		261,750
	5		16,250									
	7						16,250	16,250			81,750	32,750
Cosmarium sp.	7											32,750
Unidentified cell.	11					32,750		16,250			32,500	
DINOPHYCEAE												
Peridinium sp.	4		16,250	32,500		32,750						
	5	16,250										
	11		16,250									
Ceratium hirundinella (O.F.M.) Schrank.	7		49,000	16,250			49,000	16,250				
EUGLENOPHYCEAE												
Phacus sp.	7						16,250					
Trachelomonas sp.	5	16,250					49,000	114,250	130,750	130,750	65,500	
	7		16,250					16,250				
	11		16,250	16,250								
PROTOZOA												
Unidentified protozoa.	4	179,750	81,750	245,250	49,000	32,500	16,250			49,000	98,250	16,250
	5	81,750	32,500			16,250	16,250					
	7	49,000							16,250	16,250	32,750	
	11	16,250		16,250		16,250	49,000	16,250	16,[illegible]			
ROTATORIA												
Anuraea sp.	4	16,250										
	5	16,250										
	7		16,250	16,250								
CLADOCERA												
Daphnia sp.	7	32,500					16,250	16,250	32,750		32,750	16,250
	11	32,500				16,250			32,500	49,000		
COPEPODA												
Cyclops (Nauplius).	7				16,250							
Diaptomus sp.	7					16,250						

Table VIII

Comparison of weekly plankton counts from fertilized and non-fertilized ponds.

Date of Collection 1946	Plankton count in Numbers of Organisms per l.			
	Pond 4	Pond 5	Pond 7	Pond 11
1 July	4,317,000	1,014,000	376,000	376,000
8 July	4,775,000	654,000	458,000	376,000
15 July	8,062,000	392,500	441,500	327,000
22 July	8,880,000	441,500	212,500	474,500
29 July	18,430,500	131,000	310,500	245,500
5 August	10,155,500	310,500	2,191,500	147,000
12 August	9,485,000	327,000	2,534,500	294,500
19 August	8,536,500	294,500	8,373,000	245,500
26 August	8,749,500	327,000	13,573,500	376,000
2 September	13,606,500	474,500	22,748,500	359,500
9 September	20,884,000	507,000	9,485,500	425,000
Total number	115,881,500	4,873,500	60,705,000	3,646,500
Average number	10,534,682	443,046	5,518,637	331,500

Chart 8

Groups of plankton organisms found in Ponds 4 and 5.

ABUNDANCE OF PLANKTON
(NUMBER OF ORGANISMS IN MILLIONS / LITER)

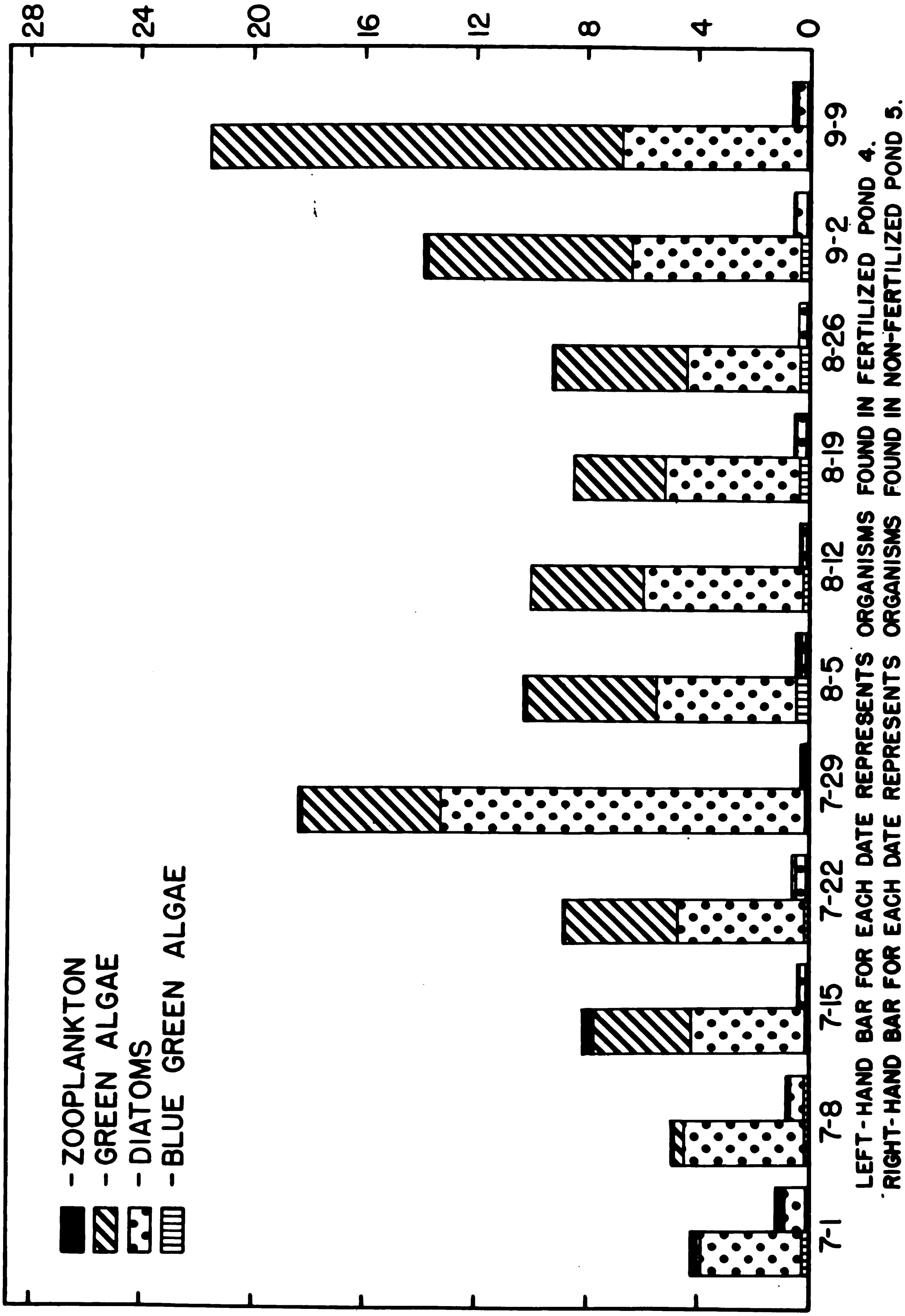
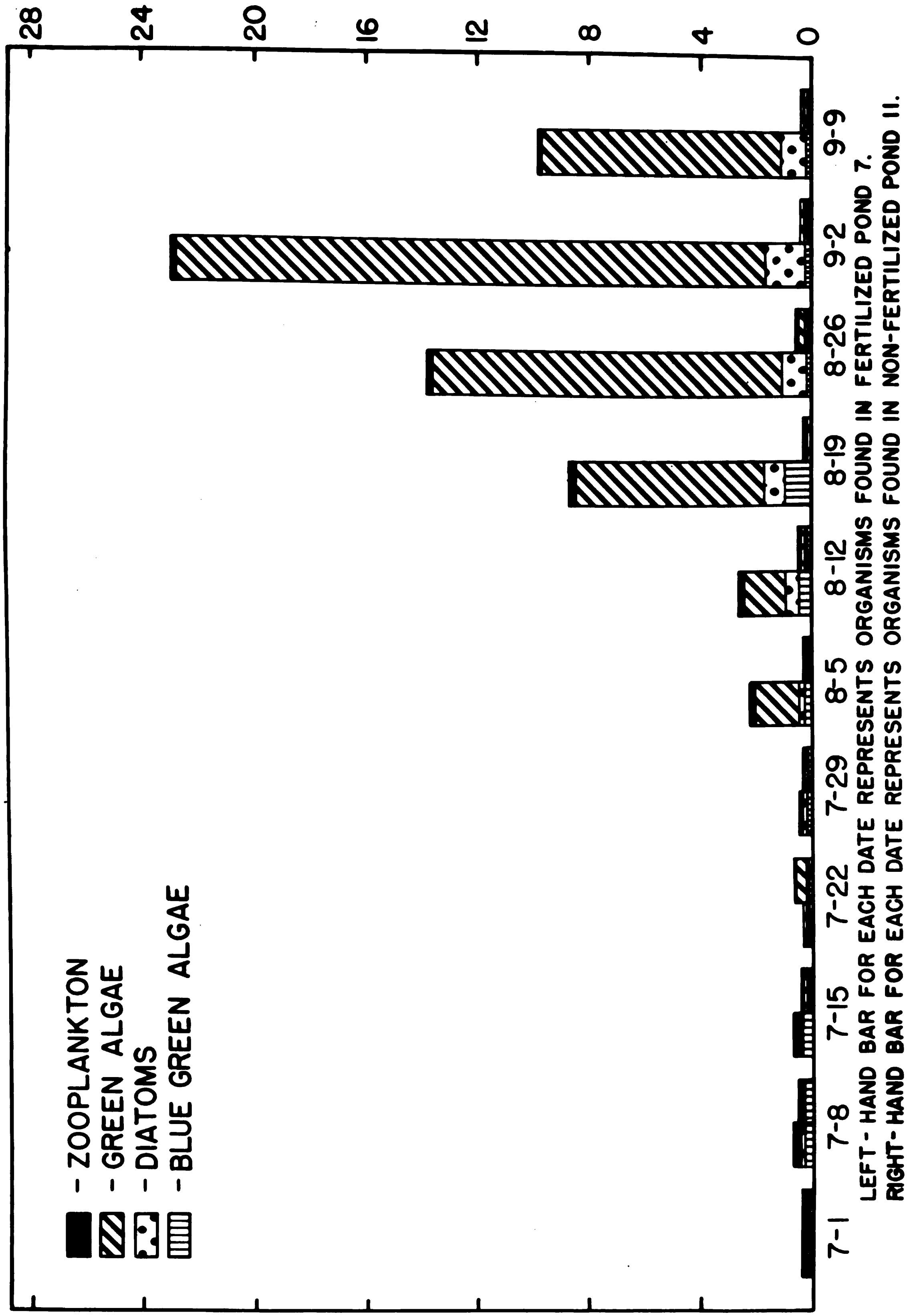


Chart 9

Groups of plankton organisms found in Ponds 7 and 11.

ABUNDANCE OF PLANKTON

(NUMBER OF ORGANISMS IN MILLIONS / LITER)



NOTES ON CONSTITUENT ORGANISMS

Knowledge of the kinds of plankton organisms produced in fertilized and non-fertilized ponds is very important for several reasons. It is essential that we know what specific groups are encouraged by the fertilizer formula. Stomach analysis of fish in the pond would not be complete without knowing which plankters are present and also size of different plankters plays an important role in interpreting evaluation data.

Laboratory analysis was completed on one-half of the ponds. Time was not available to make counts from all ponds. The pairs 4 and 5, and 7 and 11, were chosen from the eight for the following reasons. Ponds 4 and 5 were sand bottom ponds and the fertilized pond had a bloom throughout the entire collecting period. The bottom soils of Ponds 7 and 11 contained more organic matter and were sampled for bottom organisms as well. All fertilized ponds supported a bloom. The more important organisms found in Ponds 4, 5, 7, and 11 are summarized in the following paragraphs.

Myxophyceae (Blue-green Algae)

This group of algae was represented by ten genera. Two of these were medium in abundance while the rest were scarce. The blue-greens did not at any time become the most abundant form.

Aphanocapsa: _Aphanocapsa pulchra_ Kütz. Rab. colonies were found in all ponds except Pond 5. The numbers of colonies increased noticeably in the fertilized ponds as compared to the non-fertilized ponds.

Dactylococcopsis: Dactylococcopsis rhaphidioides Hansgirg. appeared in all ponds in medium numbers throughout the collecting season. Colonies of this species, when separated by a Foerst Centrifuge, were broken into fragments usually of one or two cells each. Counts show a tendency for this species to have been encouraged by fertilization in Pond 4.

Chrysophyceae (Brown Algae)

Only two members of this group of algae were found namely, Mallomonas sp., and Dinobryon sp. Their occurrence was sparse.

Bacillarieae (Diatoms)

Ponds 4 and 5, the sand bottom ponds, supported medium to abundant numbers of this group. Collections from Ponds 7 and 11 showed fewer numbers with the exception that during the "bloom-period" in Pond 7 there was a corresponding increase in practically all of the commonly occurring diatoms.

Cyclotella: Different species of this genus appeared in all collections taken from Pond 4, while in other ponds it appeared infrequently.

Synedra: Individuals of this genus were abundant in Pond 4 and common in the other three ponds. In Pond 4 the combined species count reached a maximum of 12,429,000 organisms per liter on July 29, 1946. Fluctuation of numbers indicate that this diatom was greatly affected in the fertilized ponds compared with its response in the non-fertilized ponds.

Achnanthes: This diatom occurred in sparse numbers in all ponds. Counts show little or no response to fertilization.

Gomphonema: Several different species were present and counts show that diatoms of this genus occurred in all ponds. There was no definite pattern in fluctuation of numbers with regard to fertilization.

Nitzschia: Nitzschia tabellaria Grun. Cleve. appeared in medium abundance in practically all collections from Pond 4. This condition remained roughly the same throughout the summer.

Chlorophyceae (Green Algae)

The green algae were represented by at least 34 species. Most of these occurred only sparingly, while five species reached abundance exceeding one million per liter. Some of the more important forms are listed below.

Chlamydomonas: This algae occurred in sparse numbers in all ponds except Pond 7 where on August 5, 1946, it reached a maximum count of 1,586,250 organisms per liter.

Coelastrum: Coelastrum cambricum Arch. appeared commonly in all collections from Pond 4 and sparingly in the rest of the ponds. A count of colonies reached a maximum of 3,777,750 organisms per liter on July 29, 1946, in Pond 4.

Dictyosphaerium: Dictyosphaerium pulchellum Wood. became very abundant in the two fertilized ponds. The greatest abundance recorded in Pond 4 was on September 9, 1946, when the count reached 11,055,500 organisms per liter. In Pond 7 this species reached a maximum of 19,265,000 organisms per liter on September 2, 1946. Like the genus *Dactylococcopsis* this algae, when concentrated through the Foerst Centrifuge, broke into fragments containing four or more cells.

Ankistrodesmus: *Ankistrodesmus spiralis* Turner. occurred in only the fertilized ponds and built-up in numbers during the summer to nearly one million on September 9, 1946. The cresent-shaped cells were usually separated by the centrifuging process, breaking up the clusters that are found in nature.

Scenedesmus: This genus was represented by three common species, namely, *S. quadricauda*, *S. obliquus*, and *S. arcuatus*. Of these *S. quadricauda* was the most common. All three species occurred primarily in Pond 4.

Crucigenia: *Crucigenia irregularis* Wille. was found to be sparse in occurrence in Ponds 4 and 11, and medium in occurrence in Pond 7, reaching a maximum of 1,799,000 organisms per liter on August 19, 1946.

Miractinium: *Miractinium puscillum* Fresenius. occurred only in fertilized ponds, and reached medium abundance in Pond 4.

Cosmarium: *Cosmarium circulare* Reinsch., and *C. depressum* (Nageli) Lundell. were the two species common in Pond 4 collections. These species occurred sparingly in all other ponds. *C. depressum* was found in greatest abundance on August 26, 1946, when the count reached 1,079,500 organisms per liter.

Dinophyceae (Dinoflagellates)

This group was represented by *Peridinium* sp., and *Ceratium hirundinella* (O.F.M.) Schrank. They were found for the most part, in the fertilized ponds.

Euglenophyceae (Euglenoids)

Members of this group were *Phacus* sp., and *Trachelomonas* sp., the latter occurring in sparse numbers in Pond 7.

Protozoa

Protozoa were found in the collections of all ponds. Practically all were badly treated by the centrifuge separation. Identification was not attempted.

Rotatoria

This group was represented by a single organism *Anuraea* sp.

Crustacea

Cladocera and Copepods were found in very sparse numbers. It is believed that possibly more were present than actually appeared in the counts. This may be attributed to a selective method of collecting.

DISCUSSION AND CONCLUSION

Much experimentation has been undertaken in the past two decades to determine the effect of different organic and inorganic fertilizers upon water areas. Assuming that the addition of essential nutrients does encourage the growth of micro-organisms, both plant and animal, it still leaves a big question unanswered, namely; what place does plankton occupy in the production of more pounds of fish per acre? The phytoplankton (plant) and zooplankton (animal) are thought by many biologists to be essential in these important basic food chains.

Authors give various reasons for the fluctuation of plankton, and the comparative values of plant and animal plankton in the primary pond-food cycles. Pearsall (1922), discussed factors influencing the distribution of free floating vegetation. He based his ideas on four hundred complete analyses from surface waters of English lakes. Akehurst (1931), also made observations for four years on pond life, with special reference to the possible causations of swarming of phytoplankton. He states that "factors as certain toxins play an important part in the swarming of phytoplankton". A "toxin" being defined as "an excretion product or products of undefined chemical constitution, which may also serve as an accessory food and may inhibit or stimulate growth". The author further divides the phytoplankton into two main groups according to food reserves, roughly starch and oil. Toxic effects do not occur between an "oil" and a "starch". Swarming (increase and decrease) of genera of the two groups, is due to available quantity of accessory food. The toxin of the oil group becomes an accessory food of the starch group and conversely.

During this same period, Griffiths (1923), made observations on the ecology of phytoplankton in fresh water and made tentative assignments of factors which control the composition of the water solution and consequently determine the occurrence and distribution of the free-floating algae. These are summarized as follows:

1. The initial composition of the water is important and is characterized by whether the water contains a high or low alkali (K-Na / Ca-Mg) salts ratio. Water high in K and Na encourages the growth of Desmids.
2. The presence of sediments, whose decomposition products provide nutriment for the phytoplankton organisms.
3. The occurrence of a seasonal vertical circulation of water is accompanied by submerged sediments and water mixing with the upper layers in the spring and autumn. Diatoms are associated with this factor.
4. Occurrence of a whole-volume horizontal circulation caused by wind, stirs the shallow lying sediments. Protococcales are associated with this condition.
5. Presence of submerged aquatics modify the water quality by their photosynthetic activities.

Ponds respond differently to the addition of fertilizing substances. The use of a complete fertilizer, one that contains amounts of N-P-K, in the Wolf Lake Hatchery Ponds encouraged water blooms of phytoplankton in all fertilized ponds. While at the Drayton Plains State Fish Hatchery near Pontiac, Michigan, ponds fertilized with the same fertilizer did not produce a phytoplankton bloom but encouraged a zooplankton bloom in one pond and a growth of filamentous algae in other fertilized ponds. This same phenomena has been observed in other parts of the country. Surber (1943), was not able to produce a phytoplankton bloom in hard water ponds, at the U. S. Fishery Experimental Station, Leetown, West Virginia, by the addition of inorganic fertilizer. He associates "water blooms" of filamentous algae as Spirogyra and Oscillatoria with plant decay. It is noted in the text of this study that a similar condition was present in several of the Wolf Lake Ponds. Surber believes that "....plant decay apparently releases nutrients into the water which stimulate the developement of certain kinds of algae....." In Alabama, Smith and Swingle (1939) "....found that phytoplankton constituted the bulk of the organic matter in ponds fertilized with inorganic fertilizers....." This is typical of results obtained by other authors.

When considering the comparative values of different plankton constituents that compose the sestonic organic matter, three groups of organisms are important, phytoplankton, zooplankton, and bacteria. (Sestonic refers to

all of the organisms living and non-living which float or swim in the water.) Meehean (1935), states that "samples taken in ponds, especially with reference to Oklahoma and Louisiana, have revealed that phytoplankton is of minor importance as to volume and numbers", he further concludes "In fertilized ponds the organic matter from zooplankton and phytoplankton sources is insignificant as compared with that from bacteria", (Smith and Swingle (1939)). Smith and Swingle (1939), on the other hand, found that "phytoplankton constituted the bulk of the organic matter in ponds fertilized with inorganic fertilizers".

The findings of the latter authors represent more nearly the conditions found in this study. The weights expressed in this paper are composed primarily of green algae. The maximum amount of dry organic matter produced in Pond 7 was 18.3 mgs./ liter. This figure is only a little over one-half of the 31.0 p.p.m. of organic matter produced in a fertilized pond by Smith and Swingle (1939). Their figure represented the average plankton production in Pond 15 which was treated with inorganic fertilizer. This may be attributed to the fact that their experiments run for nearly two months longer, and because of the warmer conditions. Birge and Juday (1934), found a mean average of 43 mgs. / liter of organic matter in the water of Wisconsin lakes.

Blooms were produced in all fertilized ponds while no blooms appeared in the non-fertilized ponds. Since the

ponds were paired to be as nearly similar as possible the resultant blooms must manifest, at least to some degree, the response of plankton to fertilizer. In the ponds selected for detailed study plankton counts showed a definite increase of the green algae in the fertilized ponds and to a lesser extent the diatoms. However, in Pond 9 the writer examined enough samples to indicate that the bloom present was due to a blue-green algae Anabaena Lemmermanni P. Richter. The chemical conditions in this pond must have been suitable for the increase of this species. Factors which contribute to the response of different groups are not too well known. It was endeavored in this paper to explain the kinds and amounts, of plankton produced in certain ponds with accompanied chemical and physical data. Fluctuations of the more important constituent organisms are discussed in a separate section.

SUMMARY

1. Plankton blooms were produced in all fertilized ponds at the Wolf Lake State Fish Hatchery but there were none in the paired control ponds.
2. The plankton of the two fertilized ponds studied in detail consisted primarily of green algae.
3. Results show that the Secchi disk and light absorption readings compare closely with the total dry organic matter expressed as milligrams per liter.
4. Volumes and counts of organisms depend greatly upon the size of the organism. A fluctuation in numbers of a small species as the green algae Dictyosphaerium may not show a corresponding change in the weight measurement.
5. Greenhouse experiments brought out a need for practical field tests to determine the presence of an excess or a deficiency of essential elements already in the water before application of the fertilizer.
6. It is believed that the addition of fertilizer nutrients to a body of water should be carried out on a p.p.m. basis.
7. Presence of a lasting plankton bloom produced early in the season will discourage the growth of higher aquatics.
8. The chemistry of the water in the fertilized sand bottom ponds differed from that of the fertilized ponds containing organic bottom soils. Carbonates and bicarbonates were present throughout the experiment with a corresponding decrease in total hardness in the sand bottom ponds. Carbonates and

bicarbonates were present in the organic bottom ponds during the early part of the study. Later only bicarbonates were present. This was accompanied by an increase in the total hardness.

9. Qualitative examination of samples is important in a study of this nature. The kinds of organisms produced under certain conditions and those species utilized by the fish should be known in order to encourage the more desirable forms.

10. Ponds fertilized weekly, every two weeks, and every three weeks produced blooms which all began during the same week. This indicates that in these particular ponds more frequent applications of fertilizer did not encourage an earlier bloom.

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APPENDIX

PLANKTON DATA SHEET

Name of Lake________ County________ Twp.________ Date________ #________

Sample or Station Number________ Depth________ Size of Sample________ Type of Sample________

Concentration________ Computation Factor________ Date of Computation________

pH________ Alkalinity________ Initial________

ORGANISMS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total	Ave.	No. Per Liter	

LAKE AND STREAM SURVEY   I.F.R.   AQUATIC VEGETATION ANALYSIS RECORD

COUNTY: COLLECTOR:

LAKE: T.   R.   SEC.   DATE:

COLL NO.	SCIENTIFIC NAME	STATION & ABUND., AREA IN ACRES					REL ABU	BOT TYPE	DEPTH RANGE	REMARKS

NOTE. Under remarks enter fl.- flowering stage; fr.- fruiting stage; B- winter buds; C- covered with marl.
ABUNDANCE: S- sparse; M- medium; D- dense.