A QUANTITATIVE STUDY OF PLANKTON IN A NEW POND AT AN ALTITUDE OF 3,300 FEET

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An Abstract of A Thesis
Presented to
the Faculty of
Appalachian State Teachers College

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts in Education

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August 1953

ABSTRACT

Because of the progressive increase in the number of ponds, in their enlarging economic importance, and in the possibilities of using these in conjunction with biology classes, a study was made of a .4 acre pond at an elevation of 3,300 feet.

Cold water ponds are known to be less productive than warm water ponds, but little work has been done on cold water farm ponds at high altitudes.

The quantity of plankton is an index to the productivity of a pond, because it is the basis of the food supply of the fish.

Several methods for quantitative study of plankton were tried, and from the standpoint of simplicity of operation and the least expensive equipment, the Sedgwick-Rafter sand filter is recommended for concentration, and for the final determination the Sedgwick-Rafter Counting Cell and the ash-less filter paper methods are recommended.

The limited production of plankton in the Poplar Grove Pond was perhaps due to the low temperature. However, the chemical conditions did not limit productivity; except the deficiency of inorganic salts, since the Poplar Grove Pond was not properly fertilized.

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ACKNOWLEDGMENTS

Credit must be given for the encouragement, guidance, and enthusiastic interest shown by Mr. F. Ray Derrick, of the Biology Department of Appalachian State Teachers College. Advice, particularly in arrangement and exposition of this thesis, came from Dr. A. Van Pelt, also, of the Biology Department. Without the assistance and suggestions for illustrations and graphs, by Dr. John R. Shaffer, this thesis would have been more difficult to read and understand. Mr. B. W. Stalling generously allowed the study and sampling of his ponds, and furnished inorganic fertilizer at intervals. Credit and thanks should go to my wife, Joyce, for her encouragement and assistance.

CHAPTER I

I. INTRODUCTION

Although the civilization of man has centered along rivers for irrigation and navigation, the backbone of the culture has been largely along agricultural lines for over 3,000 years. Man has exploited the land and has had to intensify his farming efforts and methods to meet the needs of increasing populations.

Aquaculture, a relatively new science, is rapidly gaining recognition. Since water covers most of the earth's surface, it contains vast resources largely available for future use.

However, to gain the most good from these resources, man must learn the methods of intensive efforts and methods of conservation and balance.

Water, as well as land, varies in its productive capacity, depending upon environmental factors. Less work has been done on these factors in cold waters than in warm waters, and less in inland waters than in ocean waters.

This thesis is a preliminary quantitative investigation of plankton, the free-floating unicellular and multicellular animals and plants, in Stalling's pond near Poplar Grove, North Carolina, at an altitude of about 3,300 feet. The tests were conducted between January 26, 1953 and August 5, 1953.

Methods of quantitative study of plankton will be briefly described and compared. Combinations of methods will be tested for accuracy, for convenience of transportation of equipment, and for ease of manipulation.

Qualitative study will be briefly treated since this is not a taxonomic study. Methods of preservation for identification and for classroom study will be briefly outlined for teacher usage.

The equipment used for quantitative study was selected with the public school teacher in mind. The requirements of low cost and simple operation were of primary importance.

The recent increase in the number of new ponds built in North Carolina gives the teachers of this state many more opportunities to use these ponds in connection with field trips for biology. For example, there are 261 ponds over one-fourth acre in size and 325 ponds total in Gaston County, according to Hannon Seitz, Soil Conservationist of Gaston County. The natural curiosity of the students will lead them toward the water with its varied and spectacular life forms. The concepts of the complexity of the food cycle, and of the dynamics of population can be most conveniently taught by the use of aquatic life.

Apparatus and suggestions for culturing plankton from freshwater ponds for classroom and aquarium study will be briefly described. Optimum conditions will be considered and will be suggested for teacher guidance. Several classroom experiments with plankton will be described.

Practical aspects of pond culture, interpreting the results of the plankton quantitative analysis, will be suggested with changes made on this particular pond by the addition of inorganic fertilizers. Changes in nitrate content, carbon dioxide, oxygen, and in acidity will be considered.

II. DEFINITION OF THE TERM POND

The terms lake and pond are in very common use, but the distinctions between them are not only arbitrary, but also very diverse. For limnological purposes a definition of the term pond may be proposed, but none seems to be wholly ideal for the differentiation.

Forel (1892) defined a lake as a body of standing water occupying a basin and lacking continuity with the sea. He also defined a pond as a lake of slight depth. Muttkowski (1918) formulated a set of criteria which would make a lake include only those bodies of standing water which are of considerable expanse and which are deep enough to stratify thermally, thus eliminating almost all ponds. Carpenter (1928) holds that the true difference between a lake and a pond is one of depth and not area. Thienemann (1926) and

Lenz (1928) apparently accept Forel's definition. Welch (1935) chooses to employ the term <u>pond</u> for that class of very small, very shallow bodies of standing water in which quiet water and extensive occupancy by higher aquatic plants are common characteristics. This thesis shall use the term <u>pond</u> to mean a standing body of water less than an acre in area, and less than ten feet in depth.

The pond under consideration in this thesis is a pond resulting from a dam which obstructs water from at least two small streams and several springs. It is considered a new pond, being less than three years old.

CHAPTER II

GEOLOGY AND TOPOGRAPHY OF THE REGION

The pond considered in this thesis is located near the center of Watauga County, North Carolina, in the Southern Appalachian Plateau, which averages about 3,000 feet above sea level.

Yoder (1938) reports the latitude of Boone, the county seat of Watauga County and approximately near the center of this county, is 36° 15' N., and the longitude is 81° 40' W.

The rocks of the region are largely gneisses and schists containing a high mica content. The geologic age of these rocks is Pre-Cambrian, among the oldest in the North American continent. The soil developed on these rocks is acidic in nature. The water issuing from springs and in streams has a particularly low mineral content. Davis and Cobb (1928) consider the whole area to be covered with Ash Loam. The dam itself contains a large portion of clay to insure against leakage.

The natural vegetation of the region is highly varied with forests listed as mixed hardwoods. The higher elevations have spruce and fir, while the lower elevations have mostly hardwoods. Hemlock and white pine are abundant in many places.

The pond is located at Poplar Grove near Hodges Gap, which is three miles west of Boone, North Carolina. The elevation is 3,376 feet above sea level.

I. THE BOTTOM DEPOSITS

The bottom deposits include silt and some rocks. Occasionally stumps or sunken tree roots are present. There is a very small amount of organic detrites. No thorough study was made of the bottom deposits.

II. THE MORPHOMETRY OF THE POND

The pond has an approximate "L" shape, with one leg being 166 feet and the other measuring 136 feet. The maximum effective length or the maximum effective width, is the greatest distance along which wind and wave action occur without any kind of land interruption. The mean width is the area of the pond divided by the maximum length.

The mean depth-maximum depth relation, the mean depth divided by the maximum depth is .474. Expressed as a decimal value, this serves as an index figure to indicate the character of the approach to basin shape to conical forms.

The maximum depth surface relation, the maximum depth divided by the square root of the surface, is .0748. Expressed as a decimal value, this is an indication of the relation of depth to horizonal extent.

The longest axis of the pond runs N-S, and the other axis runs NE-SW.

The approximate surface area of the pond is 16,000 square feet or .368 acres. The approximate length of the shore line is 630 feet.

For this pond the shore development (the ratio of the actual length of shore line to the length of the circumference of a circle, the area of which is equal to that of the pond) is 1.4.

The Morphometry of the Pond

Dimension	Maximum	Maximum Effective	Mean
Length	302.0 Ft.	166 Ft.	
Width	84.0 Ft.	78-84 Ft.	53.3 Ft.
Depth	9.5 Ft.		4.5 Ft.

Other information about the pond may be ascertained from the outline map (Figure 1) and the topographical map (Figure 2.) The scale for both maps is one inch equals thirty feet.

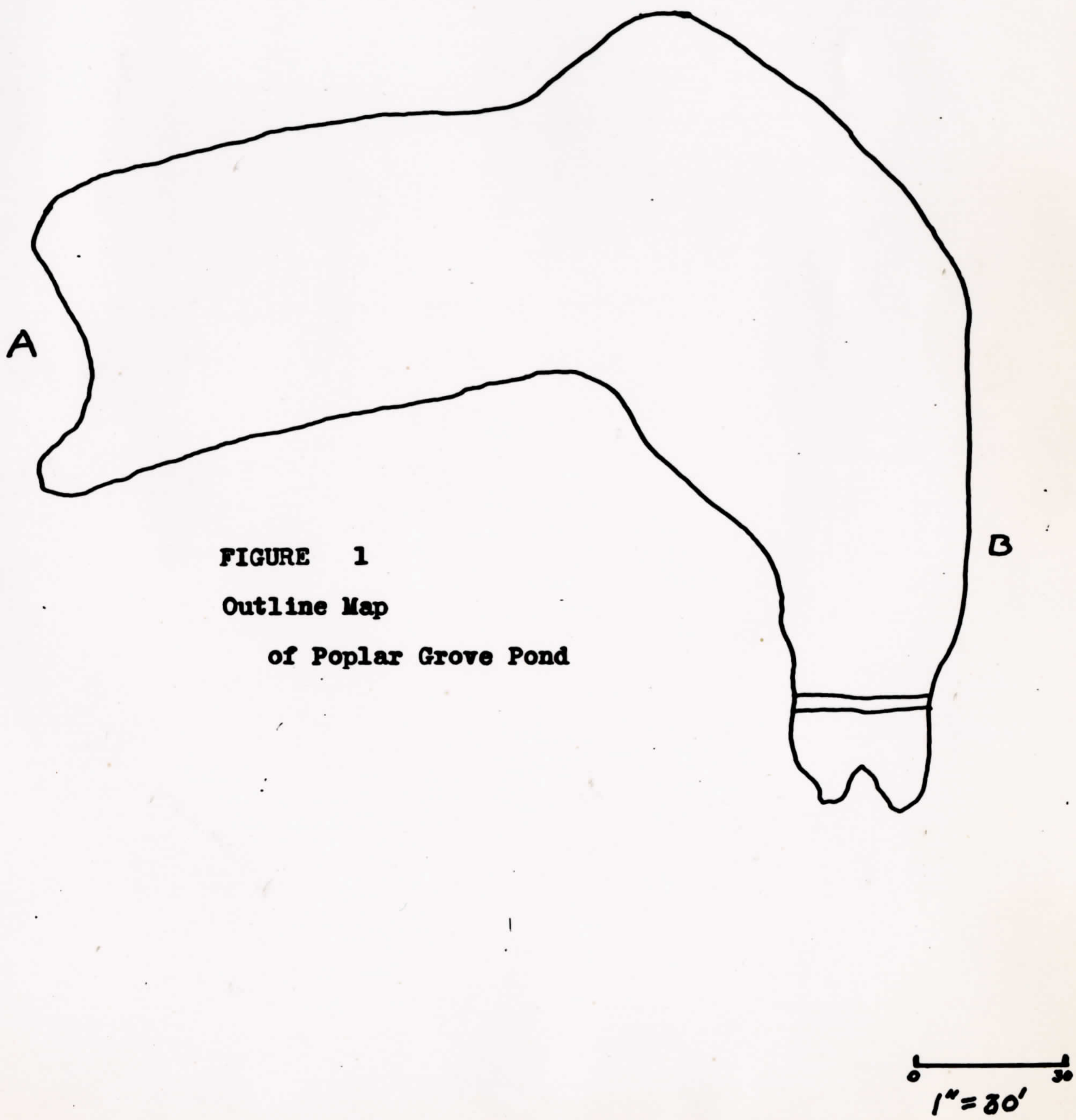


FIGURE 1

Outline Map

of Poplar Grove Pond

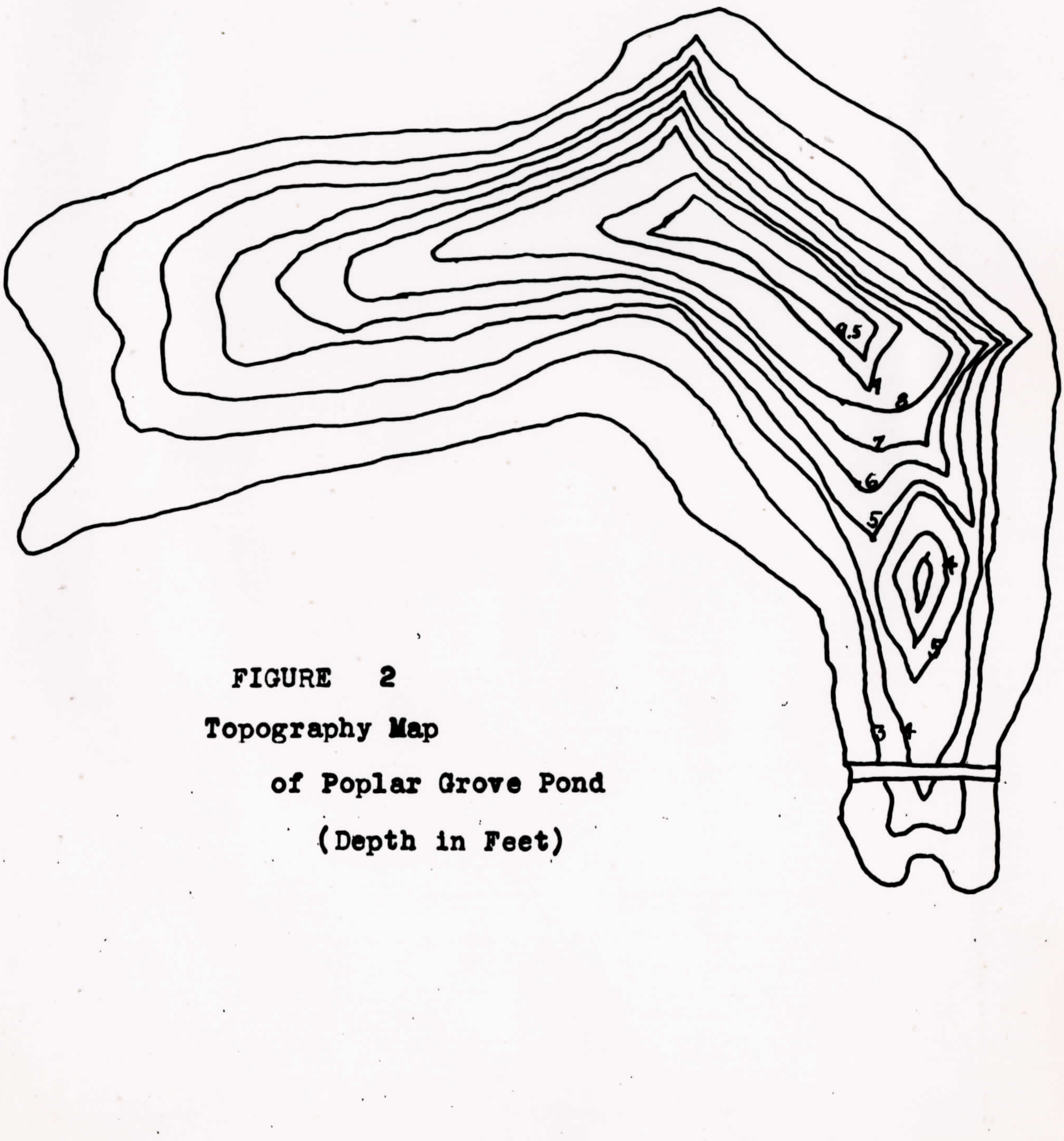


FIGURE 2
Topography Map
of Poplar Grove Pond
(Depth in Feet)

CHAPTER III

THE PHYSICAL AND CHEMICAL FACTORS OF THE POND

The plankton growth in any pond is influenced by both physical and chemical factors. The physical factors, such as temperature and light, and the chemical factors, such as dissolved oxygen content, free carbon dioxide, and alkalinity have not been extensively studied for this altitude in cold water ponds. A study of these factors may shed some light on the problems of pond culture under higher altitude conditions.

I. PHYSICAL FACTORS OF THE POND

Climatic Data of Watauga County. Yoder (1938) reports that the climate of Watauga County is classed as modified continental type. The winters are mild to severe with summers cool and pleasant. The lowest temperature recorded since weather data has been recorded is -12° F., rarely however, does the temperature fall below -1° or -2° F. The total snowfall averages eight inches per year, although some years may have three or four times this amount, while others have little or none. For the coldest month, January, the average temperature is 34° F.

The summer temperature averages 10° cooler than nearby Piedmont counties to the East. The hottest temperature recorded is 96° F. The July average temperature is 69° F.

Rainfall for the year is well distributed. Total precipitation for the year is about 57 inches. There is no wind data available nor cloud formation recorded.

Subsurface Temperatures of the Pond. The temperatures most influential upon plankton are those of the water, rather than those temperatures which fluctuate in the air because of sunlight and weather.

To measure the subsurface temperature requires an instrument which will retain the coldest temperature reading, while being raised through the warmer layers of the shallow water. An instrument of this type is the Taylor Maximum-Minimum Thermometer which is shown in Figure 5. The construction of this thermometer is such that the movement of the column of mercury will change the position of two pieces of iron wire. (These remain in the highest and lowest positions reached, respectively, and a small horseshoe magnet is used to move the iron wire for resetting.)

In this study a Taylor Maximum-Minimum Thermometer was lowered by a cord into the pond near a bridge, and allowed to rest near the bottom. The depth at this point is about four feet. The maximum temperature reading taken is the temperature reached in air. The minimum temperature reading measured is the temperature reached in water.

Figure 3.

VIEW OF POPLAR GROVE POND

Figure 4

CLOSE-UP VIEW OF POND

Figure 5

A TAYLOR MAXIMUM-MINIMUM THERMOMETER

TABLE I

RESULTS OF PHYSICAL AND CHEMICAL TESTS
POPLAR GROVE POND, 1953

Date	Time	Weather	Air Temp.	Water Temp.	Free Carbon Dioxide	Methyl Orange Alkalinity	Oxygen	pH
Jan. 26	3:30 p.m.	Cold-ice	42°F.	31°F.	2.5		6.6	
Jan. 31	2:30 p.m.	Cloudy	65°F.	44°F.	3.0	0	10.0	
Feb. 10	4:00 p.m.	Clear	44°F.	40°F.				
Feb. 21	3:30 p.m.	After rain	52°F.	49°F.	5.0	0	8.0	6.0
Mar. 4	4:00 p.m.	Clear	60°F.	57°F.	9.0	0	7.2	6.7
Mar. 13	3:30 p.m.	Cloudy	61°F.	50°F.	10.0		11.0	6.6
Apr. 2	4:00 p.m.	Clear	54°F.	50°F.				
Apr. 30	3:55 p.m.	Cloudy	59°F.	56°F.				
May 7	3:35 p.m.	Cloudy	68°F.	62°F.		0	13.2	7.3
June 1	3:20 p.m.	Clear	76°F.	75°F.	10.0		9.4	
June 9	10:15 a.m.	After rain	83°F.	73°F.	12.0	.1	7.0	
June 27	10:45 a.m.	After rain	80°F.	75°F.	3.0	0	10.0	6.9
June 29	5:00 p.m.	Cloudy	72°F.	71°F.	2.0		9.9	7.1
July 8	4:55 p.m.	Cloudy	81°F.	75°F.	2.5	0	10.4	7.0
July 10	4:00 p.m.	Clear	78°F.	76°F.	2.5	0	10.4	6.9
July 17	4:20 p.m.	Clear			7.0	0	7.0	6.9
July 27	5:30 p.m.	Clear	80°F.	68°F.	2.7		10.0	6.9
July 29	7:10 p.m.	Clear	76°F.	72°F.	3.0	0	7.6	6.8
Aug. 5	5:45 p.m.	Clear	76°F.	76°F.	7.0	0	11.7	7.1

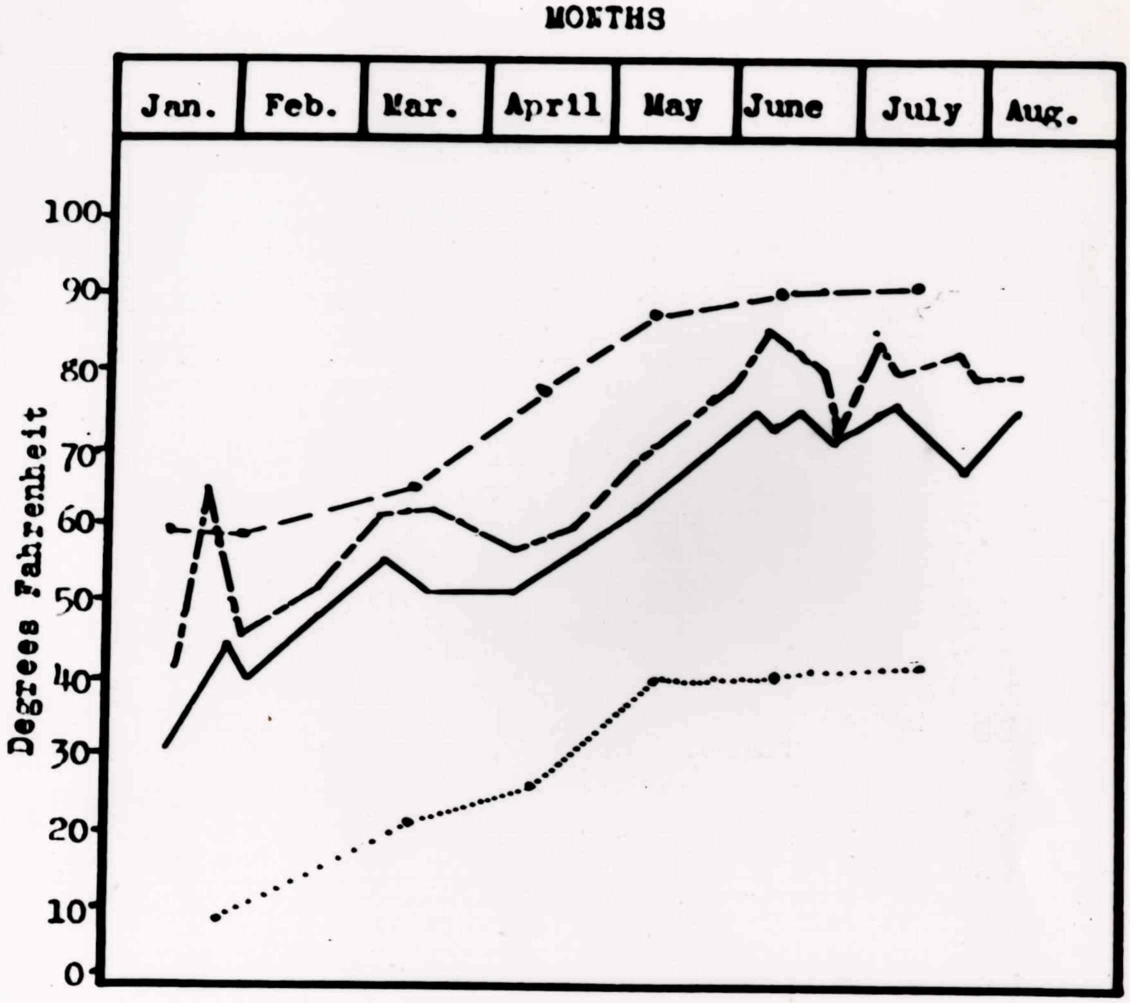


Figure 6 TEMPERATURES AT POPLAR GROVE POND

Extreme Maximum Air Temperature — — —

Extreme Minimum Air Temperature ....

Observed Air Temperature - - - - -

Observed Water Temperature ———

Color. Chemical methods of comparing colors were not used, but the color of the water in the pond and in sample flasks was noted. The color ranged from clear to light brown. The brown color was caused by silt and/or diatom formations. Very seldom was a definite green color imparted to the water.

Water Movement. Movement of the water in the pond is confined to the inflow from the streams and springs, to the outflow through a drainage pipe, and to the wave action and currents provoked by the wind.

Light Penetration. The method used for the measurement of light penetration in the pond was the limit-of-visibility test. In this test, the Secchi disk was used, as in Figure 7. This disk is a circular metal plate, twenty centimeters in diameter. The upper surface of which is divided into four equal parts and so painted that two quadrants opposite each other are white and the other two are black. The disk is attached to a measured cord and lowered flat into the water until it disappears from view. The depth at which it reappears is noted and is averaged with the depth at which it disappeared. This average is considered to be the limit of visibility.

Figure 7

Testing Water Visibility

TABLE II

REPRESENTATIVE SECCHI DISK READINGS
POPLAR GROVE POND, 1953

Date of Sample	Limit of Visibility
May 7	30 inches
June 1	
June 9	20 inches
June 27	24 inches
June 29	
July 8	36 inches
July 10	
July 17	42 inches
July 27	33 inches
July 29	38 inches

II. CHEMICAL FACTORS OF THE POND

Dissolved Oxygen. The water of a pond must have dissolved oxygen to support plankton forms, as well as, the higher fishes. Oxygen is necessary for decomposition of organic matter, as well as, for respiration of life forms. Some oxygen may come from the photosynthesis process in green algae and from the action of the wind and waves in mixing air in water. Therefore, the amount of oxygen in the water can be a definite biotic limitation.

The method used in the chemical determination of the oxygen content of water in parts per million, was that outlined by Theroux, Elridge, and Mallmann (1943) and called the Winkler Method. Since the water did not contain a high ratio of iron or organic matter, the Rideal-Stewart modification was not used.

In Figure 8, the equipment is shown in use. A wooden box with spacers provides a convenient method of transportation by automobile and of storage, but is not designed for hand carrying.

Figure 8

TESTING DISSOLVED OXYGEN

The sample of water to be tested for oxygen was obtained in a 125 cc. or 250 cc. flask with as little agitation as possible. Immediately after collection the one cc. of the manganous sulfate solution was added by pipette, dipping the end just below the surface of the water. Then, one cc. of the alkaline potassium iodide solution was added. The flask was then stoppered, excluding air bubbles, inverted several times, and set aside so that the precipitate dissolved almost completely when one cc. of concentrated sulfuric acid was added. The solution was allowed to stand at least five minutes before one hundred cc. of the solution was withdrawn into an Erlenmeyer flask, over a piece of white paper. Titration was begun by adding a solution of .025 N. sodium thiosulphate from a burette or graduated pipette until a faint lemon-yellow coloration was obtained. Two drops of starch indicator were added at this point, and titration was continued until the green, blue-green, and blue coloration just disappeared. The number of cc. of thiosulphate solution used was recorded and multiplied by two to give the parts per million of dissolved oxygen in the original sample.

The manganous sulphate solution is made by dissolving about 480 grams of manganous sulphate crystals ($MnSO_4 \cdot 4H_2O$) or 400 grams of $MnSO_4 \cdot 2H_2O$ in sufficient distilled water to make one liter.

The alkaline potassium iodide solution is made by dissolving about 500 grams of sodium hydroxide, 20 grams of sodium azide, and 150 grams of potassium iodide in sufficient distilled water to make one liter. (The sodium azide may be omitted if it is certain that nitrite interference will not be encountered in the dissolved oxygen test. The standard sodium thiosulphate solution should be 0.025 Normal. This can be made up directly by dissolving 6.205 grams of sodium thiosulphate in a liter of distilled water. It is important that crystals which have become white, owing to the loss of water during crystallization, should not be used. The suggestion is made that five cc. of chloroform may be added, as a preservative.

The starch indicator solution can be produced by making a thin paste of about two grams of starch in cold water, and by pouring this into 200 cc. of boiling water. The solution should be well stirred and lumps taken out. A few drops of chloroform may be added as a preservative.

The results of the oxygen tests at Poplar Grove are given in Figure 9, in parts per million. No sample gives less than 6.6 parts per million. This reading was made through a thin ice covering which blocked the action of the wind.

Free Carbon Dioxide. Although carbon dioxide is usually considered as a waste product of oxidation, it is an important material in the process of photosynthesis in green algae of the pond. In some ponds an abundance of carbon dioxide comes from the decomposition of organic matter to the detriment of pond life. However, too little carbon dioxide can limit productivity and cause less green plankton growth.

The method for chemical analysis of carbon dioxide is described by Welch (1948) as to procedure, and by Theroux, Eldridge and Mallmann (1943) as to chemical consideration.

This test should be made at the time the sample is taken, because of the ease with which the carbon dioxide

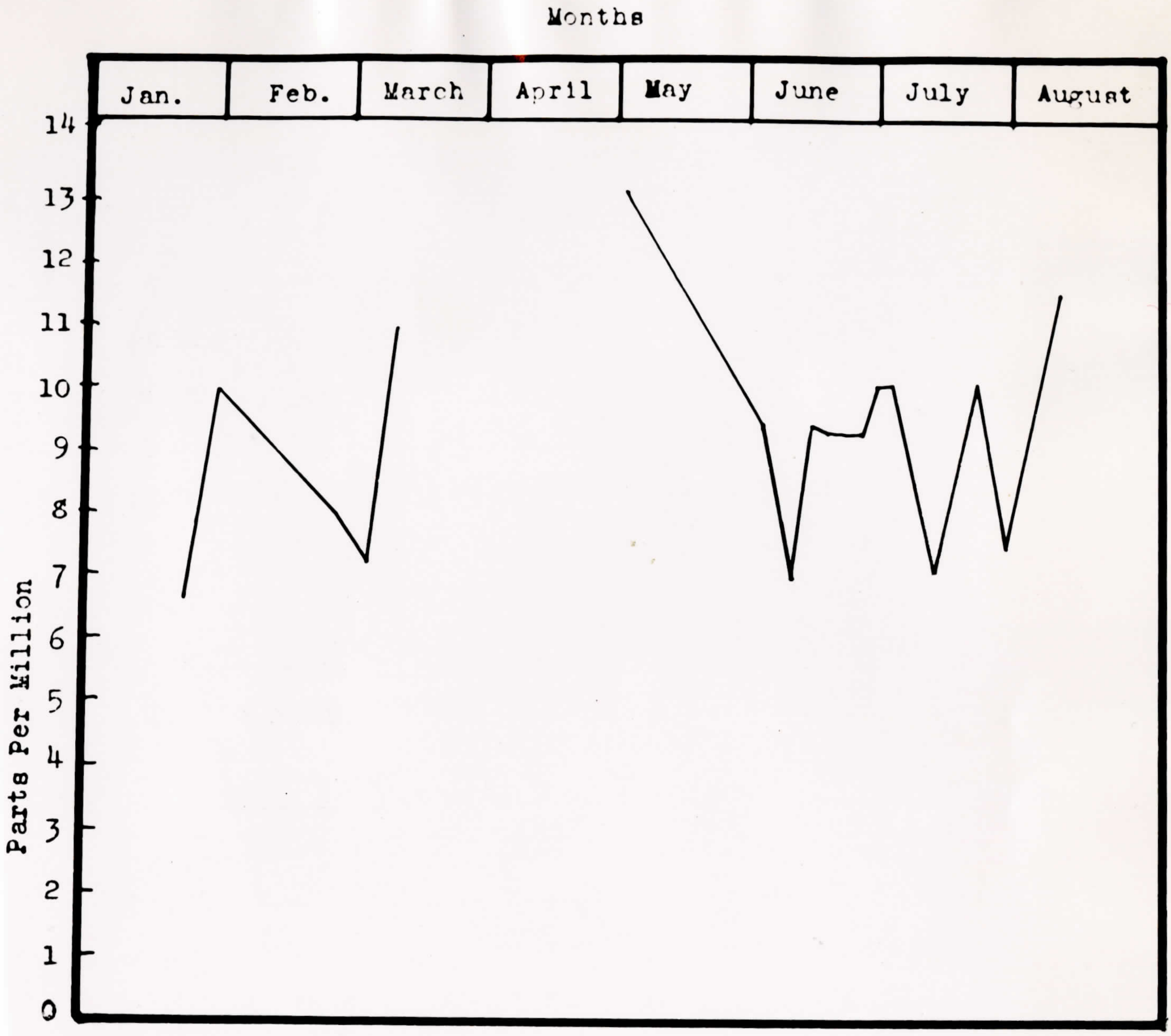


Figure 9 DISSOLVED OXYGEN IN WATER

OF POPLAR GROVE POND

escapes. If this is not possible, the sample bottle should be completely filled and stoppered and the sample kept at a temperature lower than that at the time it was collected. Ten drops of phenolpthalein indicator are added to a 100 cc. Nessler tube. The tube is filled to the 100 cc. mark with the water to be tested with as little agitation as possible. N/44 sodium hydroxide solution is used to titrate until a just perceptible pink coloration occurs which will remain for a minute or so.

The number of cc. of N/44 sodium hydroxide solution used to obtain a pink color will, when multiplied by ten, give the number of parts per million of carbon dioxide in the sample. Figure 10 is a graph of the fluctuations in the carbon dioxide content of the water at Poplar Grove Pond.

The phenolpthalein indicator may be made by dissolving about five grams of phenolpthalein in one liter of 50% alcohol. This solution is neutralized with very dilute sodium hydroxide. One liter of 50% alcohol may be made by diluting 526 cc. of 95% alcohol to one liter with boiled distilled water.

Standard N/44 sodium hydroxide solution may be prepared by using .91 grams of sodium hydroxide and making to one liter with distilled water. Normality of solution may be checked by titration against a standard acid. Dissolving the surface coating of sodium hydroxide which will stick, and pouring off the fluid is said by Theroux to remove any carbonate coating.

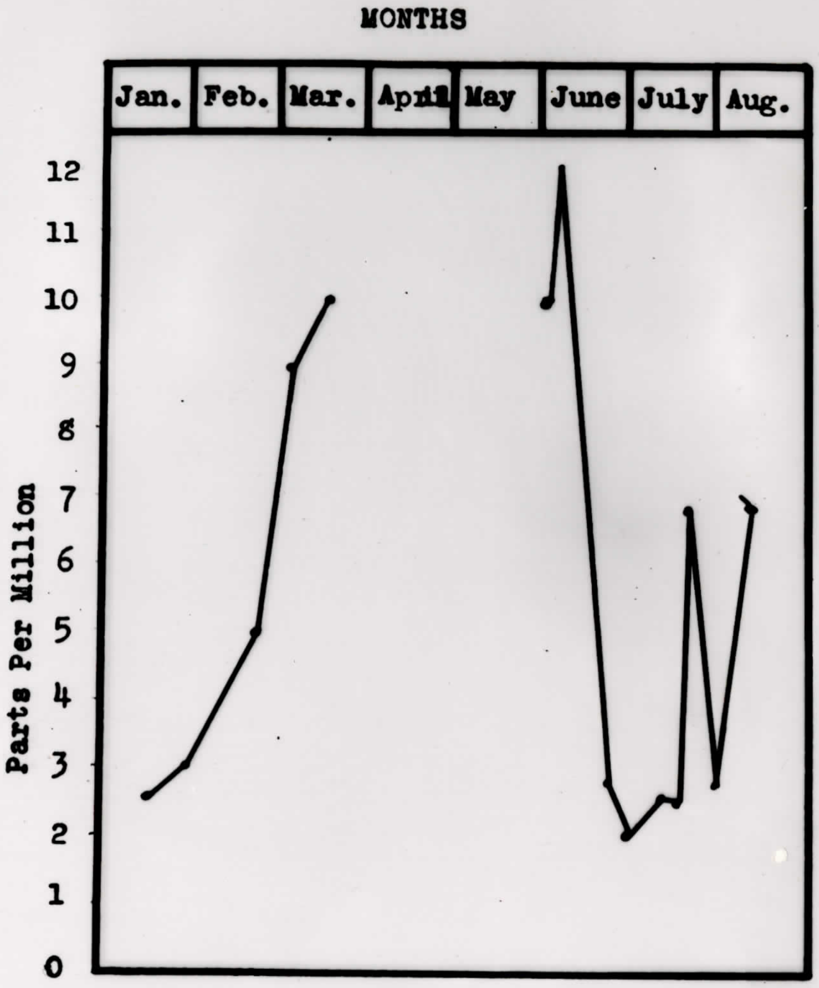


Figure 10 FREE CARBON DIOXIDE

Alkalinity. There are two types of alkalinity conditions in water. One is the estimation of total alkalinity using methyl-orange indicator. The other is an estimation of the carbonate alkalinity (CO_3-ion) using phenolpthalein indicator.

Welch (1948) describes the procedure for determining methyl-orange alkalinity. 100 cc. of the water sample are put into a 250 cc. Erlenmeyer flask over a white background. Five drops of methyl-orange indicator are added, and if the solution becomes yellow; hydroxides, normal carbonates, or bicarbonates are present. N/50 H_2SO_4 (0.02N) solution is used to titrate against the white background until a faint pink coloration appears. This end-point can be seen best if a blank sample is used for comparison. The number of cc. of N/50 H_2SO_4 solution is multiplied by ten to give in parts per million of calcium carbonate, the methyl-orange alkalinity.

Methyl-orange indicator may be made by dissolving about .5 grams of methyl-orange in one liter of distilled water.

The standard 0.02N sulphuric acid may be made by dilution of stock acid. Twenty divided by the normality of the stock surphuric acid will give the number of cc. of stock surphuric acid necessary, upon addition of distilled water, to produce one liter of 0.02N sulphuric acid.

The procedure for determining phenolpthalein alkalinity is to measure 100 cc. of the water sample into a

Nessler tube. Upon addition of five drops of phenolpthalein indicator the solution will turn pink, if hydroxides or carbonates are present. Titration with N/50 sulphuric acid is continued until the pink color just disappears. The number of cc. of N/50 sulphuric acid required is multiplied by ten to give the phenolpthalein alkalinity.

The water of Poplar Grove Pond is usually acidic rather than basic in reaction.

Hydrogen Ion Concentration. There are two methods of determining the hydrogen ion concentration of water. One, is the electrometric method which requires specialized and expensive electrical equipment. The other, is the colorimetric method which uses a sensitive indicator solution and a graded, colored standard for comparison.

Although colored glass standards, or a pH test paper can be used, a Taylor solution comparator, pictured in Figure 11, was available for use on the waters of this pond. This device, colorimetric in function, uses a block with openings for solution tubes and for comparison with standard tubes mounted on slides in order of pH. (Welch defines the pH scale as the "logarithm of the reciprocal of the normality of free hydrogen ions.") The indicator used was bromthymol blue which has a range from 6.0 to 7.6 in pH. The color changes from yellow to blue. The directions furnished by the Taylor Company were followed.

The fluctuations in pH at Poplar Grove Pond are shown by a graph in Figure 12. The acid soils and rocks have considerable influence on the pond plankton.

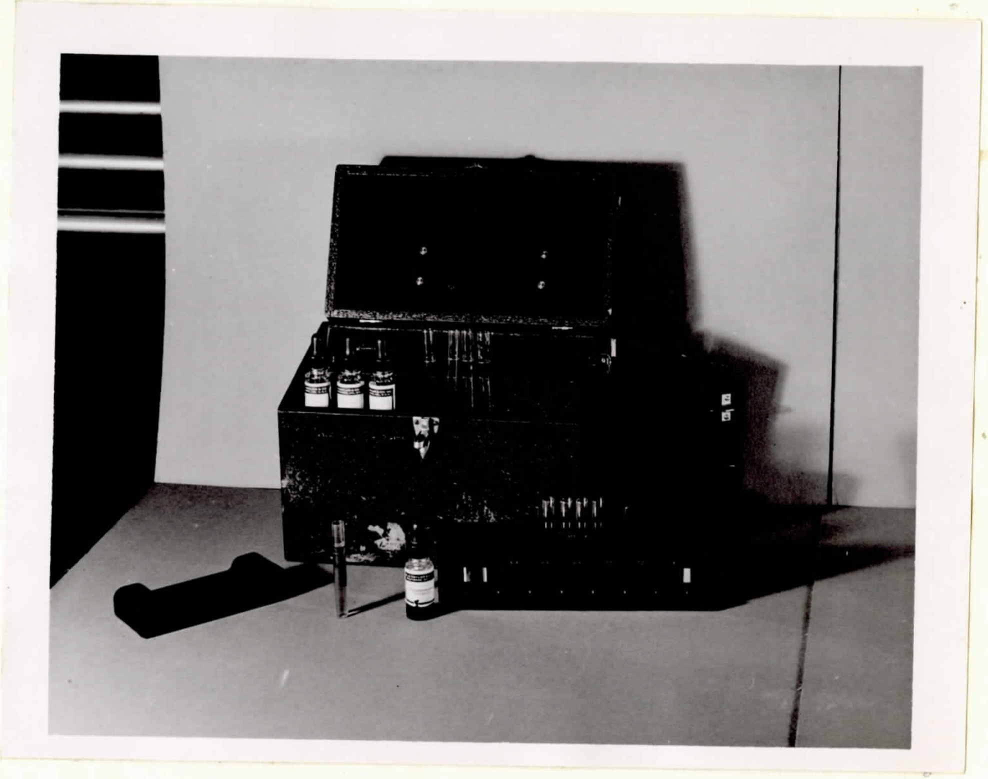


Figure 11 TAYLOR pH COMPARATOR

Nitrates. The method described by Griffin (1921) was employed to detect quantitatively the amount of nitrogen, as nitrates, present.

The phenolsulphonic acid method (for waters low in chlorine) uses prepared standards for colorimetric comparison. However, it was found that the method is complicated and that the standards deteriorated within several weeks. Phenol (carbolic acid) is a strong acid, as is concentrated sulphuric acid, and concentrated ammonium hydroxide is a strong base. All of these dangerous chemicals are used in the above method. Further details may be gained from technical, analytical chemistry books, if the teacher desires.

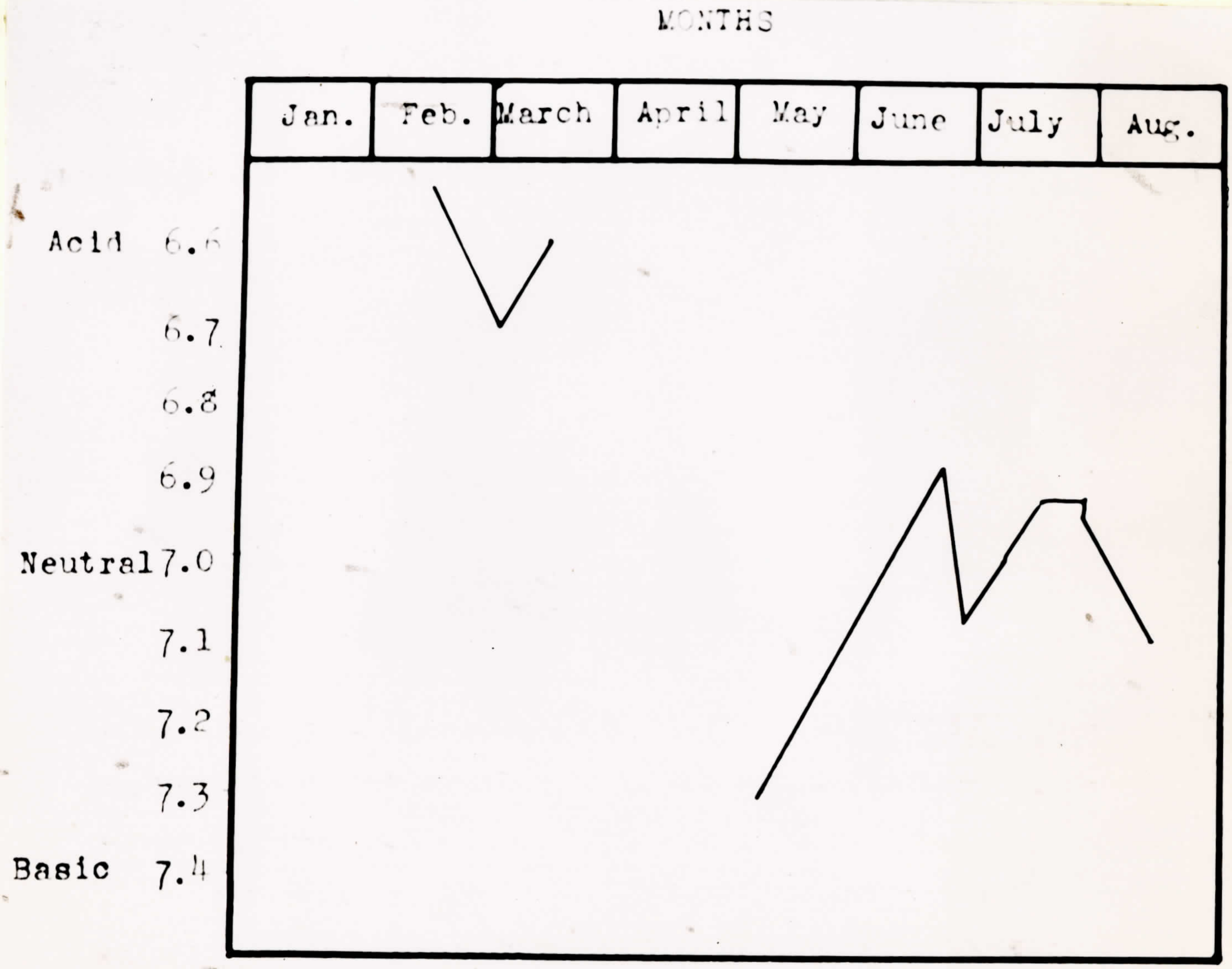


Figure 12 ACIDITY OR pH OF POPLAR GROVE POND WATER

CHAPTER IV

METHODS OF PLANKTON ANALYSIS

According to Welch (1935) the term plankton was first proposed by the oceanographer, Victor Hensen, in 1887 to designate that heterogeneous assemblage of minute organisms and finally divided, nonliving materials then known to occur in the waters of the sea and to float about at the will of the waves and other water movements. Commonly, however, the term plankton is restricted to the organisms only. A single organism in the plankton is known as a plankter.

Plankton of various fresh waters differ widely in quality. According to Welch (1935) these can include all the classes of Algae, except the Phodophyceae; Bacteria; Fungi; all Protozoa, except the Sporozoa; Coelentera; Trochelminthes, such as Rotifera and Molluscoidea, represented by free-swimming larvae and statoblasts of Bryozoa (Polyzoa); Crustacea, especially Cladocera, Copepoda, and Ostracoda; and limnetic insect larvae, such as Corethra. Other organisms at some drifting stage can be found in the plankton.

I. QUANTITATIVE ANALYSIS

Sampling. All equipment for sampling purposes is designed to insure, as nearly as possible, the securing of a representative sample or cross-section of the plankton population of the pond.

Although a bottle trap, made of a weighted bottle with a suitable stopper release apparatus, is often used, the more motile forms of plankton may react negatively to the vacuum and to the dark or opaque object.

A glass tube, pictured in Figure 13, which was approximately one-half inch in diameter and about four feet in length, was used in a vertical position to obtain representative samples from a station. A rubber stopper was suspended from a cord running through the tube. Before lowering the glass tube, vertically into the water, the stopper was pulled down, allowing the water to enter the tube. Upon reaching the full depth, the cord was pulled raising the stopper and closing the tube. The water sample was poured into the sample bottle from the top. If necessary, repeated samples were taken to fill larger containers. Samples were not taken separately at different depths, but a vertical sample of all depths was taken at one time.

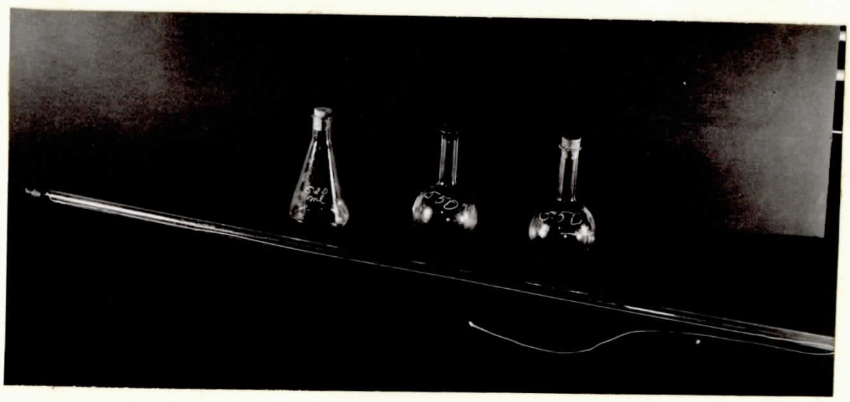


Figure 13. SAMPLER TUBE AND COLLECTION FLASKS

Concentration. In order to facilitate analysis various methods were used in order to concentrate the plankton of the water sample. The main problem of concentration was the loss of some of the very small organisms in the process.

A method requiring much time is the settling method. A Squibb separatory funnel, Figure 14, can be used, or a series of glass tubes, Figure 15, may be placed in a vertical position so that gravity aids in concentration. An assumption must be made that the majority of the organisms will fall into a lower fraction of the total height after they are killed with a solution of dilute formaldehyde. This assumption may be correct enough for an estimate in classroom practice, but for concise data there may be a large error because of the plankton which adhere to the sides of the glass tubes.

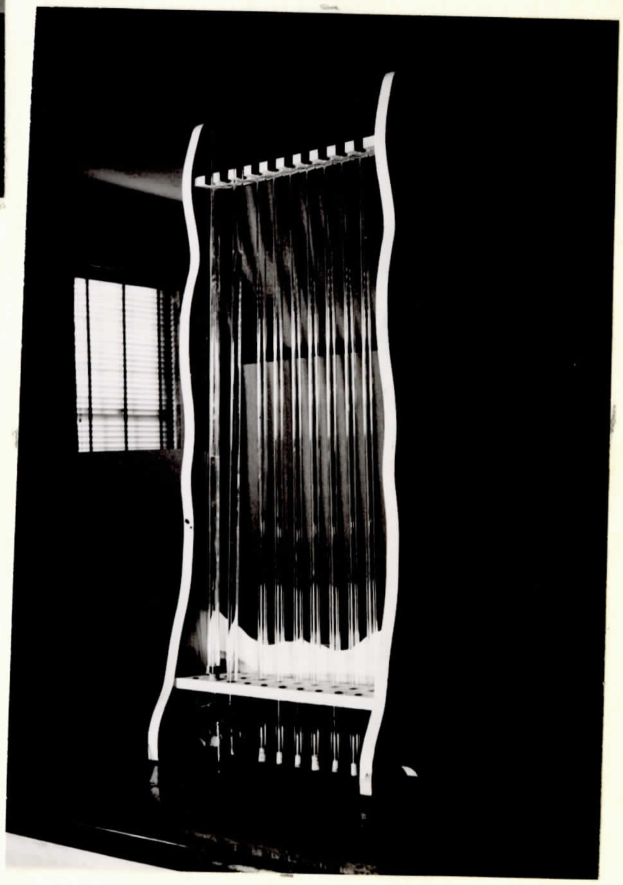
Another method employed by Welch (1948) uses the Foerst electric centrifuge, which has a motor with a maximum speed of 20,000 r.p.m. to turn a bowl with slanting sides. The lighter water flows out over the small diameter of the bowl, while the organisms are kept in the wider part by their weight under centrifugal force. The sample is allowed to flow or drip slowly into the bowl from a container. A laboratory-made centrifuge, using a hand-tool motor of 20,000 r.p.m., was not satisfactory because of the strain of sustained action. The time to run a 500 cc. sample was over thirty minutes, resulting in heating and final burning of the bearing. Special lathe work was required to turn the bowl from steel.

Figure 14.

SQUIBB SEPARATORY FUNNEL

Figure 15.

GLASS TUBE STAND



The method found to be most satisfactory during this investigation was the Sedgwick-Rafter sand filter method. The apparatus is shown in Figure 16. This process, described in detail by Welch (1948), utilizes a cylindrical glass funnel, a one-hole rubber stopper, and a small glass U-tube, some circles of special silk bolting cloth, and special sand, such as Berkshire or Ottawa sand. The cylindrical glass funnel, of 500 cc. capacity, is about 23 cm. long in the upper part which has a diameter of 5 cm., and the lower part diminishing until the diameter is 12 mm. for a length of about 7.5 cm. The terminal part of the funnel is about 6.5 cm. long and 12 mm. in diameter. This terminal end contains the U-tube in the stopper, a circle of the bolting cloth, and about 12 mm. of Berkshire sand.

Before operation the funnel is set up, the U-tube is placed in the stopper, and the bolting cloth is placed on top of the stopper and affixed in the funnel. Distilled water is added to fill the U-tube, and sand is poured into the funnel. The sides of the funnel are flushed with a stream of distilled water to wash down the sand. The original sample of plankton-bearing water is thoroughly mixed and volume recorded. This is poured gently into the funnel so as not to disturb the sand. When the water level reaches the height of the U-tube, this tube is removed, and the remaining fluid is allowed to seep through. About 5 to 10 cc. of distilled water is then used to flush out the sand after the stopper is gently removed. A small beaker is placed beneath the funnel for the purpose of obtaining the sand and for washing the plankton free from the sand particles with about 5 more cc. of distilled water. This water then is decanted into a small graduate cylinder, and the amount is recorded.

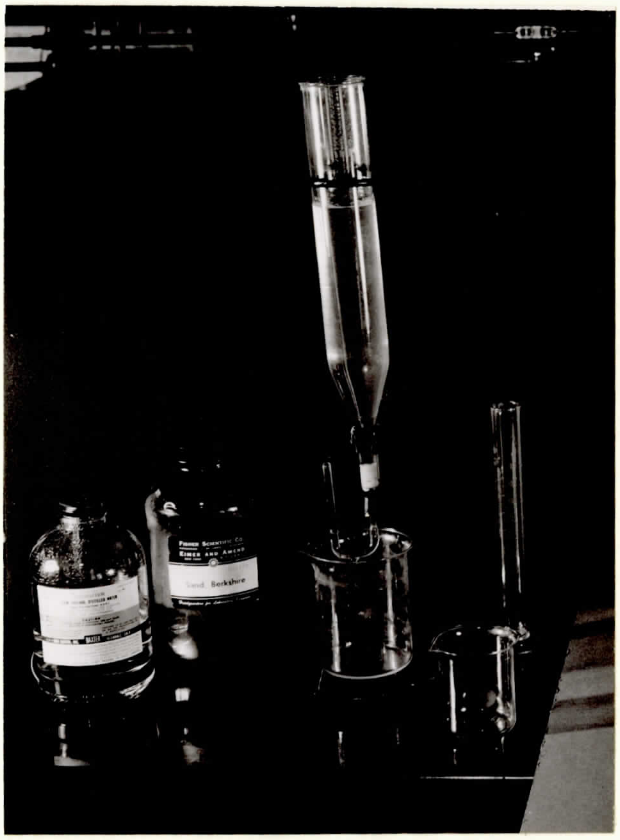


Figure 16. SEDGWICK-RAFTER SAND FILTER

<u>Separation</u>: Samples of water containing plankton concentrates may be filtered through hard surface filter paper fitted in a glass funnel, or some other appropriate supporting device, as shown in Figure 17. Because of the small size of

some plankters, the paper must have very fine mesh. Number 50 Whatman paper was used, and if the killing with formaldehyde was not done properly, some few small organisms, such as Euglena came through in test solutions.

Final Determination. The Number 50 Whatman paper is a special ashless chemical-filter variety which lends itself readily to the use of gravimetric analysis. The apparatus used, an electric furnace, or an extra hot Fisher burner, as in Figure 18, must oxidize all carbon of the paper and all organic matter, and leave mineral parts of the plankton and silt. This method of incineration of plankton is mentioned by Swingle (1950). The loss of weight of the dried sample when it is incinerated is considered to represent the organic matter present. The amount of plankton, as organic matter, is then calculated in parts per million. Dineen (1950) called this method, Seston and ash-free dry weights. The term Seston designates the whole heterogeneous mixture of living and non-living bodies which float or swim in water. According to Dineen, the dry weights were converted by Juday (1942) to approximate wet weights by multiplying by ten. This method of using special filter paper seemed to be the simpliest and most convenient separation method, as well as, the best quantitative method. With any method tried, the evaluation of Welch (1948) is that "No satisfactory method of separating the living plankton from other suspended materials has been devised."

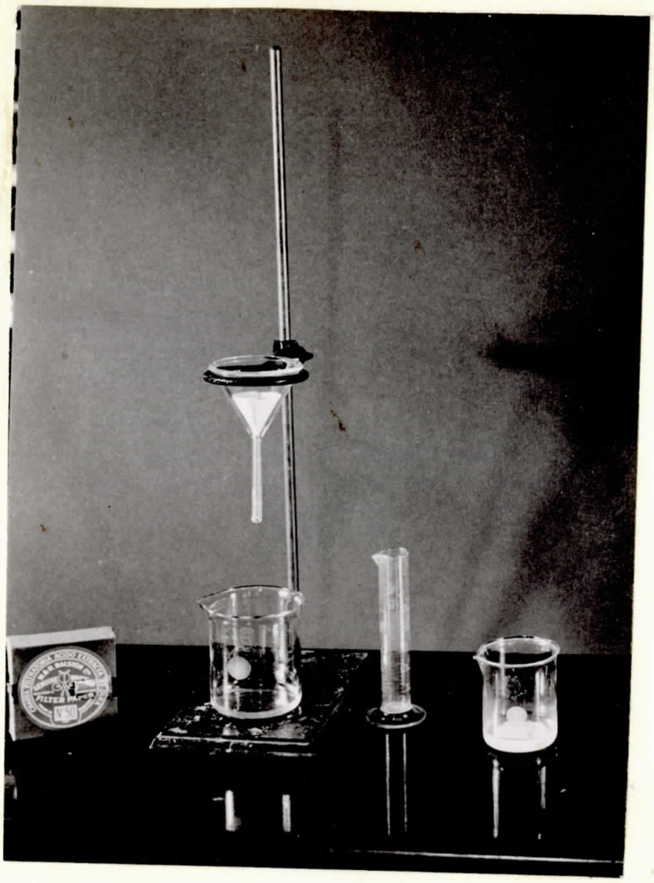


Figure 17.

FILTER PAPER STAND

Figure 18

IGNITION APPARATUS

A graph showing Seston or ignition weights is given in Figure 19. Silt is responsible for making some of the results higher than others. Another graph showing organic loss by months, at Poplar Grove Pond is shown in Figure 20. The results of the Gravimetric quantitative tests are given in Table III. Poplar Grove Pond does not have enough plankton for full growth of fish.

Another method, using a microscope and specialized equipment, as shown in Figure 21, is the Sedgwick-Rafter Counting Cell Method. This method uses a glass cell of microscope slide size and so constructed that it will hold exactly one cc. when covered by a special thin cover glass. An expensive Whipple ocular micrometer is used in conjunction with the cell. Calibration of the eyepiece requires a stage micrometer with rulings of dependable standard. The amount of plankton present is calculated in the number per liter by use of the counting cell. This method was used to check the action and efficiency of the gravimetric method in this thesis.

The ashless chemical-filter paper method and the Sedgwick-Rafter Counting Cell Method were found to be the most satisfactory from the standpoints of simplicity and accuracy.

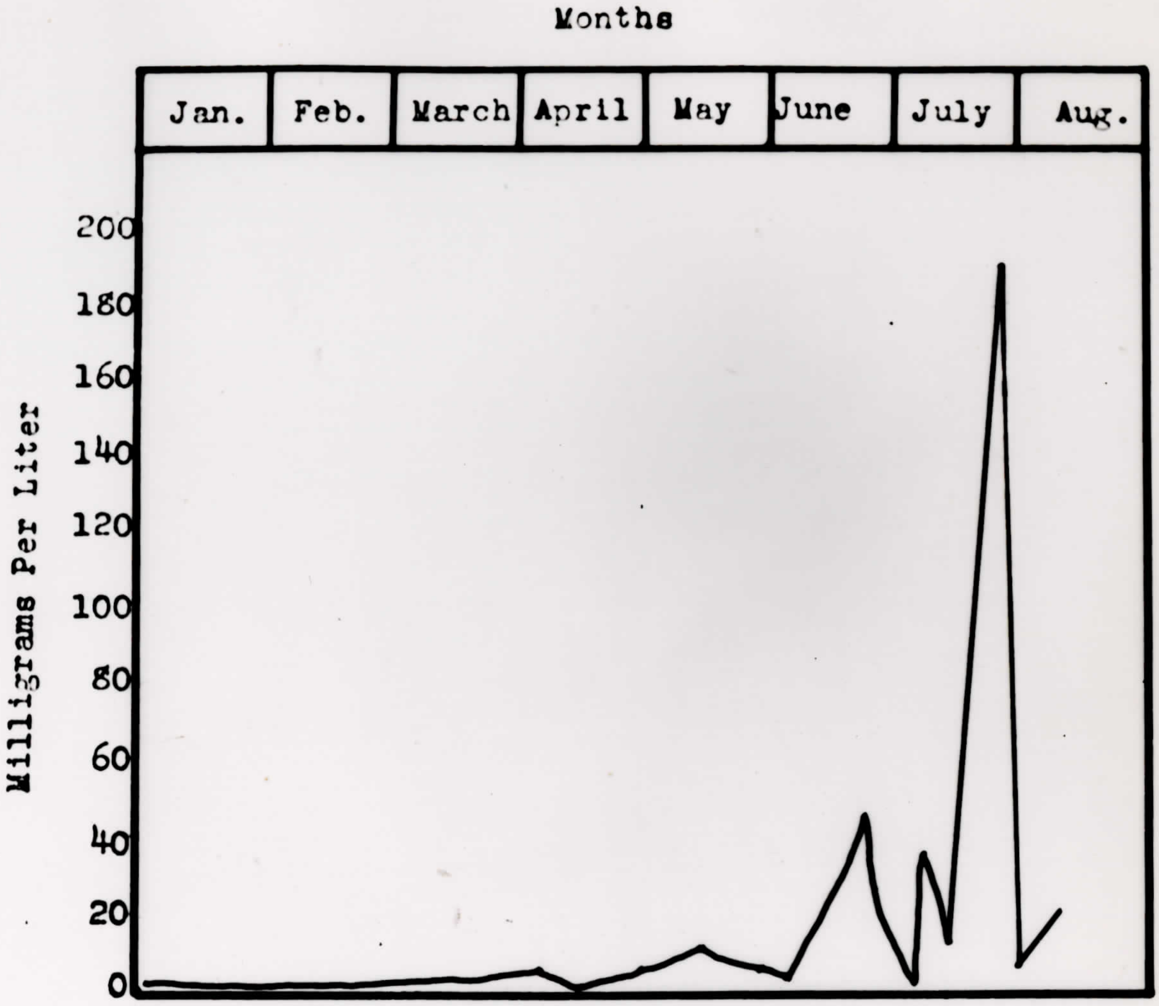


Figure 19 SESTON WEIGHTS OR TOTAL IGNITED WEIGHTS
POPLAR GROVE POND, 1953

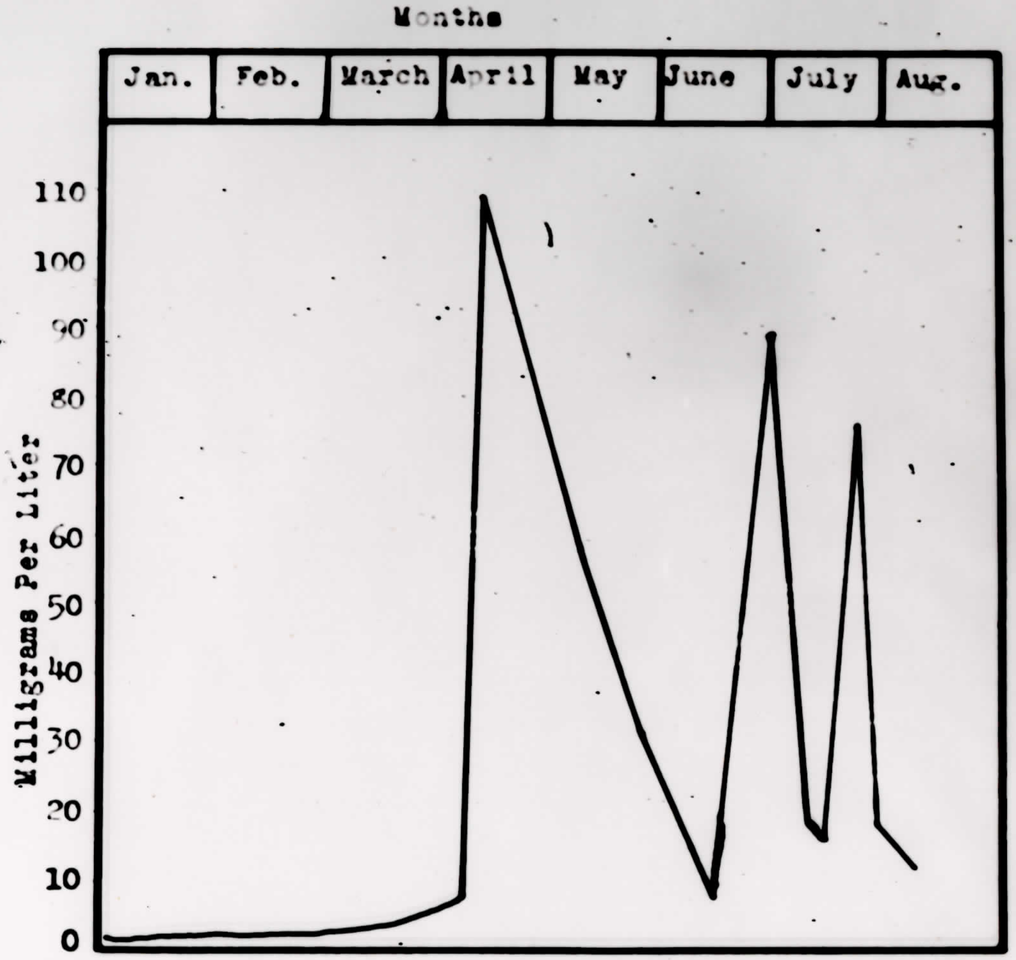


Figure 20 ORGANIC LOSS

DRY WEIGHT MINUS IGNITED WEIGHT

POPLAR GROVE POND

1953

TABLE III

LABORATORY DATA FROM GRAVIMETRIC QUANTITATIVE TESTS AT POPLAR GROVE POND, 1953

Date of Sample	Crucible Weight in Grams	Paper Weight in Grams	Crucible and Paper Weight in Grams	Crucible, Paper and Dried Sample Weight in Grams	Crucible and Ignited Sample Weight in Grams	Ignited Sample Weight in Grams
Apr. 2	6.8683	.3673	7.2356	7.0000	6.8700	.0016
Apr. 9	6.8709	.3650	7.2359	7.2390	6.8718	.0008
Apr. 10	6.7265	.3733	7.0998	7.0000	6.7272	.0006
Apr. 30	6.8700	.3660	7.2360	7.2490	6.8704	.0003
May 7	6.7262	.3660	7.0922	7.1078	6.7295	.0032
June 1	6.7250	.3800	7.1050	7.1150	6.7271	.0020
June 9	6.8710	.3690	7.2400	7.2466	6.8737	.0026
June 27	6.8680	.3710	7.2390	7.3102	6.8930	.0249**
June 29	6.7253	.3725	7.0978		6.7353	.0099*
July 8	6.8700				6.8720	.0019
July 10	6.7253	.3774	7.1027	7.1303	6.7433	.0179**
July 17	6.8670	.3770	7.2440	7.2587	6.8741	.0070
July 27	6.7250	.3725	7.0975	7.1610	6.7700	.0449**
July 29	6.8683	.3731	7.2414	7.2647	6.8750	.0066*
Aug. 5	6.7249	.3752	7.1001	7.1139	6.7335	.0085

*Silt present in the water.
**Silt greatly present in the water.

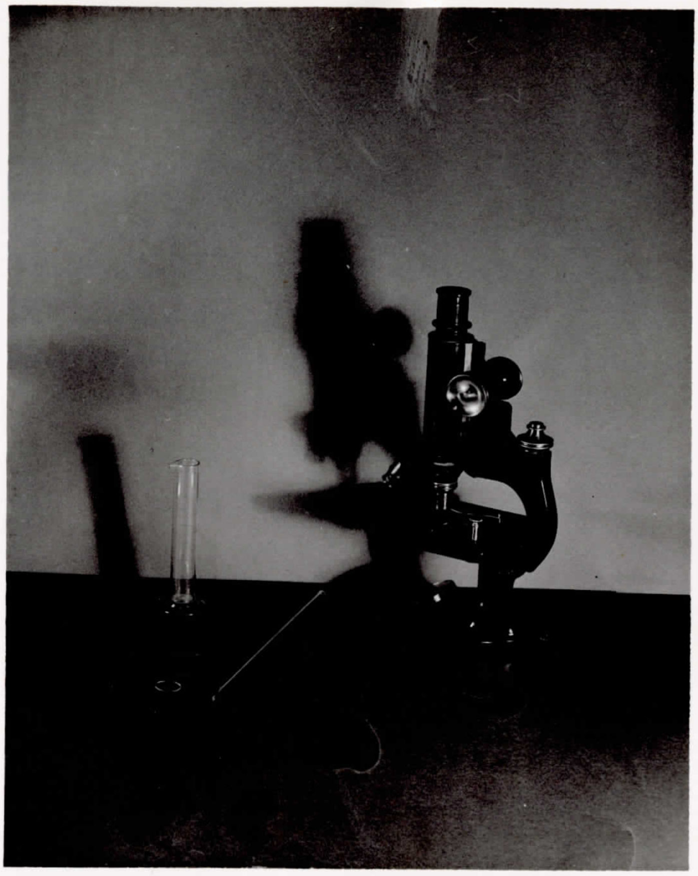


Figure 21

SEDGWICK-RAFTER COUNTING CELL, WHIPPLE OCULAR, AND MICROSCOPE WITH A DRAW TUBE

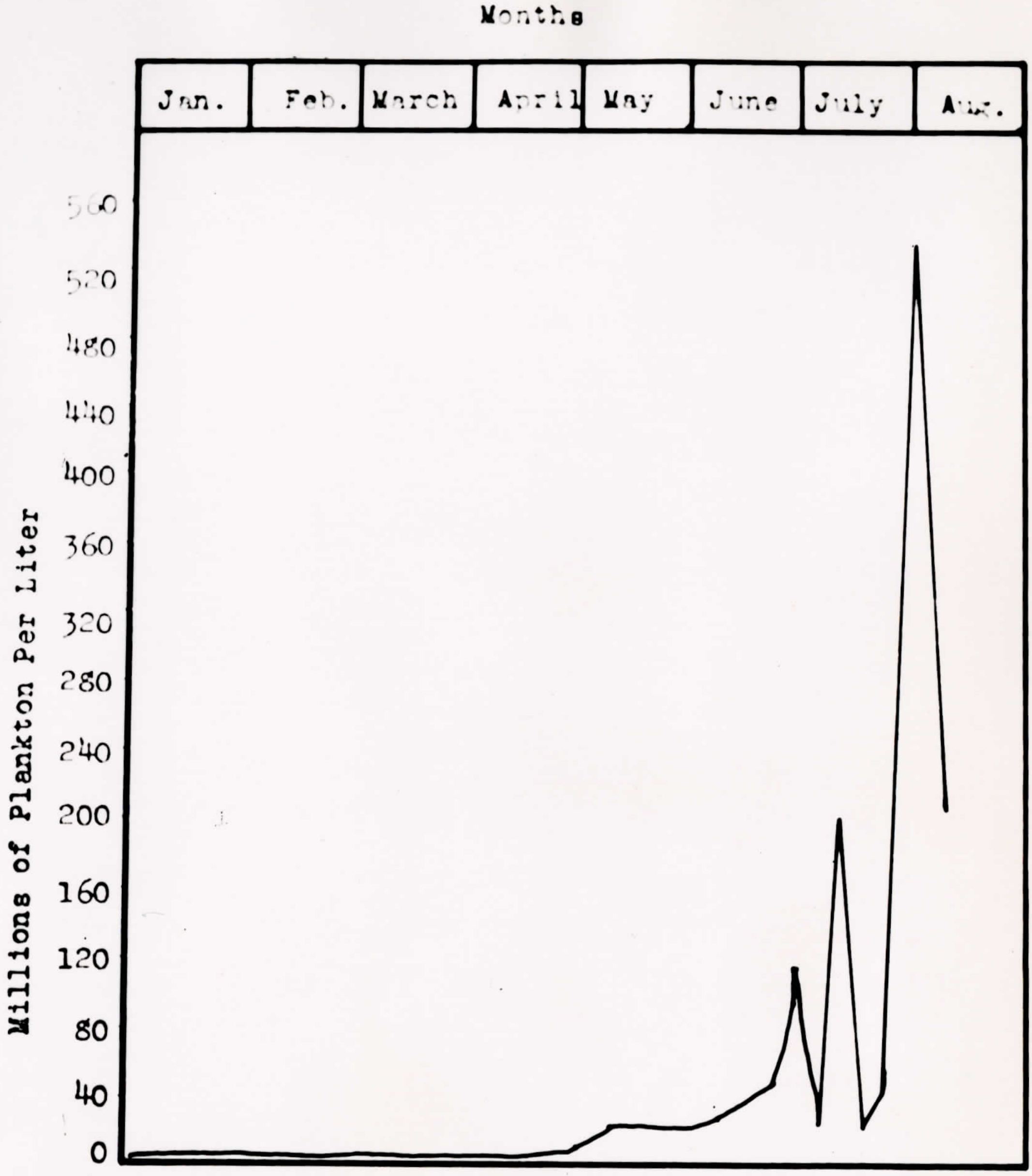


Figure 22 SEDGWICK-RAFTER CELL COUNT

(All Kinds And Sizes)

II. QUANTITATIVE ANALYSIS

Plankton Net Method. The objective of all qualitative analysis is to obtain recognizable, uninjured specimens. Plankton nets, according to Welch (1948), have errors because of the adherance of plankton in the mesh of the nets, which retards collection, and because of the spreading of mesh which is caused by water action and allows some of the plankters to escape. Also, the nets, of silk construction, require special attention for preservation.

Another method of similar principle uses a metal funnel with only the terminal end being made of silk mesh. This has similar disadvantages besides the added one of rusting.

Glass-Slide Rack Method. Slide racks, as pictured in Figure 23, are made of two long and two short pieces of wood, properly finished to avoid warping in water, and some large mesh screen wire. The long sides are lined with parallel notches to take the ordinary three by one inch microscope slides. The wire is hinged so that it will open for insertion of slides and will close to hold them. The mesh must be large enough to facilitate the free entrance of the water when the rack is submerged. At the end of submergence, the rack should be lifted slowly into a submerged pail and brought to the laboratory in its natural water. Single slides can be taken out and placed with water in a Petri dish. Microscopic examination is required.

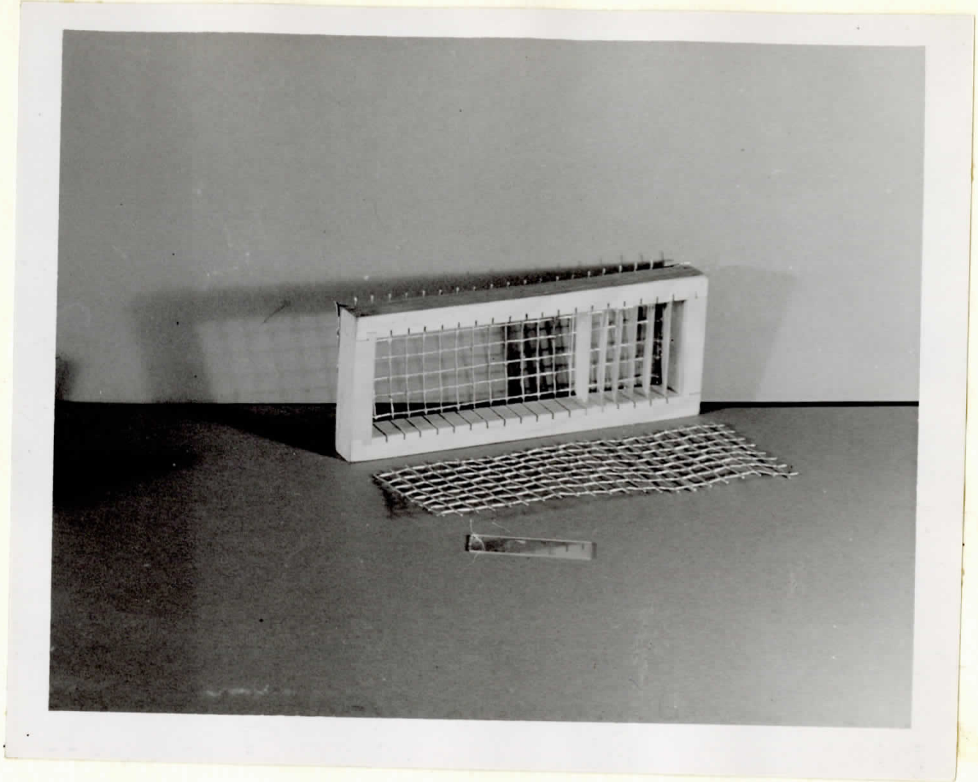


Figure 23

GLASS-SLIDE RACK

Centrifuge Method. If a centrifuge can be employed, it will separate plankton from water and make them available for placing on a microscope slide by the use of a dropper, pipette, or rubber spatula. The speed of revolutions; however, in some cases, is so great that the specimens are crushed beyond identification.

Tube Sampling and Sand Filter Method. For this thesis the glass tube sampler and the sand filter, described before, were used to obtain samples and concentrates. The samples used in the Sedgwick-Rafter Counting Cell were transferred to slides, dried and made into permanent slides for future examination, as shown in Figure 24. Balsam was used to seal the cover glasses firmly to the slide and to the specimen. Some of the permanent slides were made of ignited samples (or the ash after ignition.)

The frequency of the occurrence of plankton forms is shown in Figure 25.

Figure 24. PERMANENT SLIDES

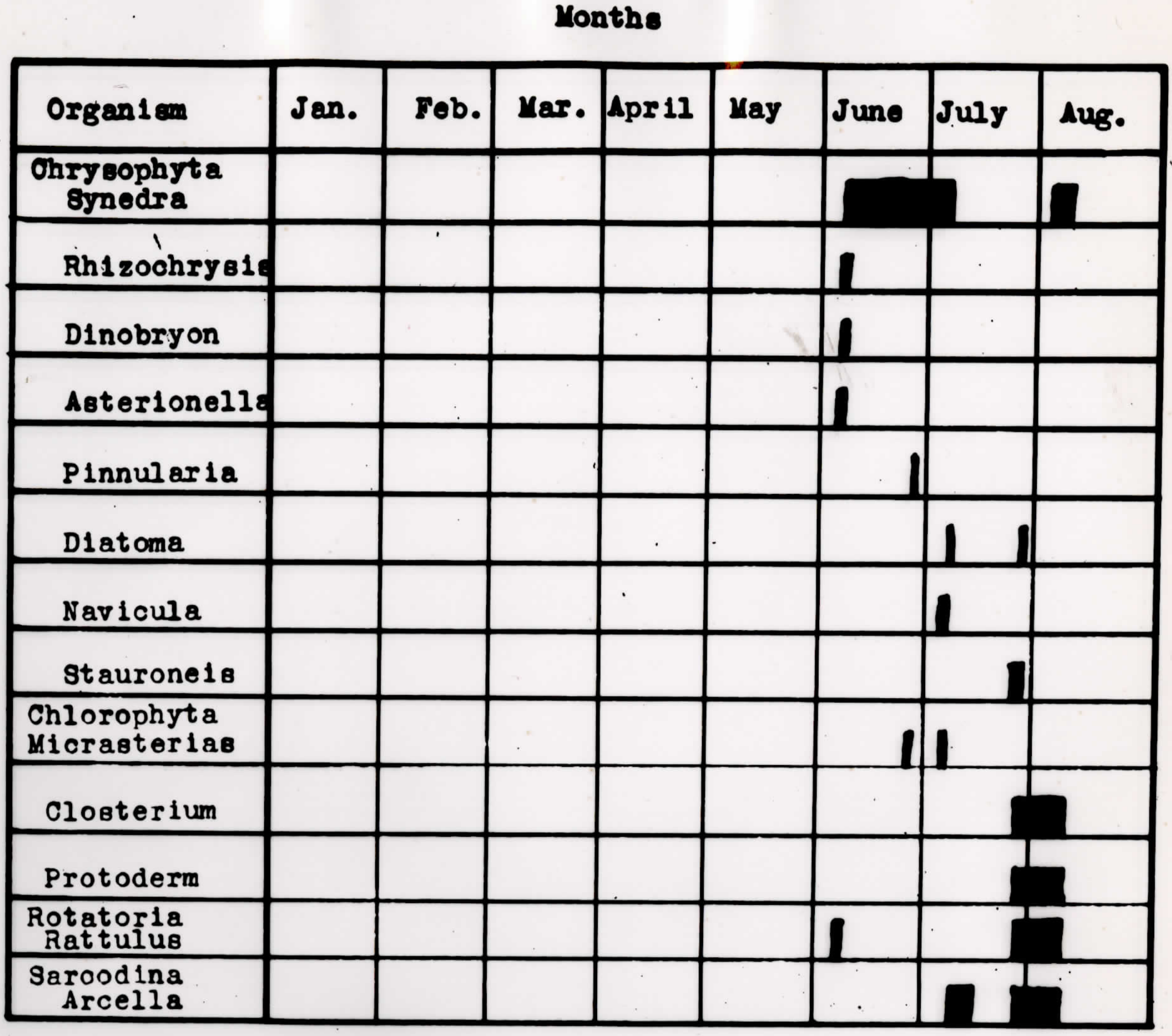


Figure 25 DATE OF OCCURRENCE OF PLANKTON ORGANISMS AT POPLAR GROVE POND, 1953.

TABLE IV

RESULTS OF QUANTITATIVE TESTS AT POPLAR GROVE POND, 1953

Date of Sample	Microscopic Count 1 L. Vol.	Seston Mg/L	Organic Loss Mg/L
Apr. 2	2,700,000	6.4	
Apr. 9	700,000	1.7	6.5
Apr. 10	4,200,000		
Apr. 30	10,000,000	2.6	108.0
May 7	20,000,000	12.0	58.5
June 1	34,600,000	7.8	30.9
June 9	36,300,000	5.0	7.7
June 27	60,000,000	47.6	88.3
June 29	126,000,000	19.1	
July 8	23,700,000	3.9	
July 10	200,700,000	34.3	18.5
July 17	24,000,000	14.1	15.5
July 27	50,000,000	183.0	76.0
July 29	545,000,000	6.9	17.4
Aug. 5	216,000,000	16.0	10.0

CHAPTER V

RECOMMENDATIONS AND SUMMARY

Practical Aspects With Recommendations. Many factors influence the productivity of a fish pond. These factors can not be isolated but react in a complex inter-related fashion. Therefore any recommendations must be assumed to be average or minimum suggestions.

Smith (1938) found that the organic matter in properly managed warm water ponds should be between 15 to 30 parts per million in the warmer months. The organic matter in Poplar Grove Pond is very low, averaging about 4.4 parts per million.

Recommended amounts of inorganic fertilizer were applied. This resulted in stunted fish. For example, the two year old bluegills removed from the pond weighed only about 3 ounces, while the two year old black bass weighed about 8 ounces.

Decaying scum or decomposing organic matter will use a large part of the supply of oxygen in the water. There should be an oxygen content of more than three parts per million. This is minimum, including the low period when light does not provide photosynthesis in green algae, and during the warm seasons when the oxygen absorption ability of the water is low. The Poplar Grove Pond has an ample supply of oxygen.

The acidity or pH of the pond water should be higher than 6.4 to yield zooplankton, which feed the fish. The low pH of 6.6 is still not dangerous to balance at Poplar Grove Pond.

The carbon dioxide, used to make oxygen and for food manufacture in algae, is quite high at certain seasons of the year, reaching above 12 parts per million in June.

The plankton of the pond are mostly desmids, diatoms, and a few rotifers, with very few algae. Desmids, diatoms, and rotifers produce little food and oxygen for other, larger forms. The reasons for the lack of algal forms may be (1) the water is often silted and muddy, (2) proper elements of inorganic chemicals are absent or lacking in sufficient quantity, i.e. nitrogen and phosphates, (3) proper "seeding" of the algal forms may not have been accomplished, perhaps because the wind action for seeding is deflected by the surrounding high hills, or because the pond is young.

More research must be done to ascertain effects of proper "seeding" of algal forms, with addition of the proper kind and amount of inorganic fertilizer.

Swingle (1950) suggests the addition of the following amounts of fertilizers to an acre of water at each application:

"100 pounds of 6-8-4 (N-P-K)
10 pounds of nitrate of soda

These may be applied separately or mixed before applying.

Where the 6-8-4 fertilizer is not available or where large amounts of fertilizer are required, the following mixture is recommended per acre of water:

40 pounds sulfate of ammonia
60 pounds superphosphate (16%)
5 pounds muriate of potash
15 pounds finely ground limestone

These materials may be mixed before applying, and used immediately or stored several months before using."

The fertilizers applied at Poplar Grove Pond were 100 pounds of 0-12-12; 10 pounds nitrate, 50 pounds potash and 50 pounds of phosphate about May 13 were applied; and 15 pounds 33% ammonium nitrate on June 2; and finally on June 27, about 25 more pounds of ammonium nitrate were applied. However, effective algal growth was not achieved.

Swingle suggests that applications of fertilizer should begin in the spring during warm weather after flood waters have receded. Whenever the water loses the brown or green color from algal growths so that in 12 inches of water the bottom can be seen, another application should be made. The last application should be made in September or October, after the pond has received between eight and fourteen applications per year. According to these recommendations Poplar Grove Pond was not properly fertilized.

The fertilizer for small ponds can be applied broadcast from the bank, while that for some ponds may be placed in a loose sack and towed in the water behind a boat. The wave action of the propeller may be used to spread the fertilizer for large ponds when the fertilizer is dumped from sacks in the boat, into the water.

The water in the Poplar Grove Pond is muddy and silted. Swingle recommends that the above mentioned fertilizer is not to be used until the muddy water is cleared to a depth of twelve inches. Two or three applications of barnyard manure at the rate of one ton per acre per application at 3-week intervals will normally clear the water so that the recommended fertilizer may be used.

The use of a mixture of 75 pounds of cottonseed meal and 25 pounds of superphosphate per acre of water at 2- to 3-week intervals will also clear muddy water and greatly increase fish production. After the mud settles the commercial fertilizer mixtures may be used as prescribed.

A closely fished pond will have bigger fish because the supply of food for each fish is increased. Pond owners need to bear this in mind, perhaps to invite a class of school children and teacher to fish it.

The problem of controlling pond weeds which shade algae, hide little fish, and destroy food sources for fish, is best treated by fertilization while the water is cold. The fertilization causes the leaves of the weeds to be coated with pondscum and algal growths. These growths cut the light supply to the leaves and cause their eventual decay. However, fertilizer must not be applied while the leaves are decaying rapidly, for such a rapid decay will use the available oxygen in the water and will kill the fish.

Summary. The following numbered points are given in brief for summary purposes:

1. This thesis is a quantitative study of the plankton in Stalling's New Pond near Poplar Grove, Watauga County, North Carolina, at an elevation of 3,300 feet, with some educational aspects of this study.
2. The rocks of the region are largely gneisses and schists, resulting in acid soils and water low in mineral content.

3. The pond has an area of about .368 acres, a maximum length of 302 feet, a maximum depth of 9.5 feet, and has its longest axis running N-S.
4. The air temperature of the region during time of testing the pond was at the maximum in July with 88° F. and at the minimum of 8° F. in February.
5. The water temperature of the region at the time of testing reached a minimum of 31° F. in January, and a maximum of 76° F. in July.
6. Methods for testing temperature and light penetration are discussed under a general catagory of the physical factors of the pond.
7. The methods for evaluating quantitatively the dissolved oxygen, free carbon dioxide, alkalinity, carbonates, and nitrates are discussed as chemical factors of pond culture.
8. Equipment and methods of sampling, concentration, separation, and final determination of plankton content of pond water are discussed under quantitative methods.
9. A long glass tube was used to sample the whole depth at once. A cord was used to pull the cork tight on the bottom of the tube. Thus light and shadow would not be changed to frighten the plankton.
10. The Sedgwick-Rafter sand filter is described and its use explained as the method recommended for concentration.

11. For separation No. 50 Whatman filter paper was used in a glass funnel. The paper was of the ashless variety for ignition purposes.

12. The Sedgwick-Rafter counting cell with Whipple ocular was used for microscopic count of plankton. The other final determination method was the drying and ignition of the Whatman filter paper sample.

13. For qualitative determinations the concentrated samples were examined under the microscope in conjunction with and after the counting cell was used.

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LITERATURE CITED

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APPENDIX

APPENDIX A.

DEMONSTRATIONS AND CULTURES

The name plankton includes both plants and animals which drift or are primarily influenced by the action of the water or waves in the pond.

In the plant catagory are Diatoms (Bacillariaceae), Desmids (Desmidiaceae), Blue Green Algae (Cyanophyceae), Green Algae (Chlorophyceae--all algae except Rhodophyceae), and miscellaneous plant fragments such as Bacteria, Fungi, and so forth.

The animal catagory includes the Protozoans, except Sporozoa, particularly the Mastigophora; the Rotifers (Trochelminthes), and (Gastrotricha); Crustaceans; Entomostraca (Copepoda, such as Cyclops and Diatomus), (Cladocera), and (Ostracoda); Eggs of aquatic Diptera and Ephemerida; Statoblasts of Bryozoa or Polyzoa (Molluscoidea); Coelenterata (Hydra, a facultative plankter); and Corethra (a Diptera larvae.)

Many of the genera are easily kept in the laboratory or classroom. Ordinary glass battery jars holding about a gallon make good aquaria. Two gallon jars are as good or better for some species, as in Figure 26. For some, ground glass tops for dishes or sealed fruit jar tops may be needed.

Cultures may be started at any time of the year. In winter, mud, sticks or stones, or water samples may be placed in the jars for inoculation.

Sometimes after a healthy culture has developed, the algae will disappear. Seasons of dormancy occur in nature and spores may be found on the bottom of the jar. After the water has evaporated and the jar has stood for a period, more water may be added and the cultures may be found again.

Refrigeration after collection will make it possible to study material for a week or two instead of a day or two. Collection in metal containers, especially in warm weather, is detrimental to some species.

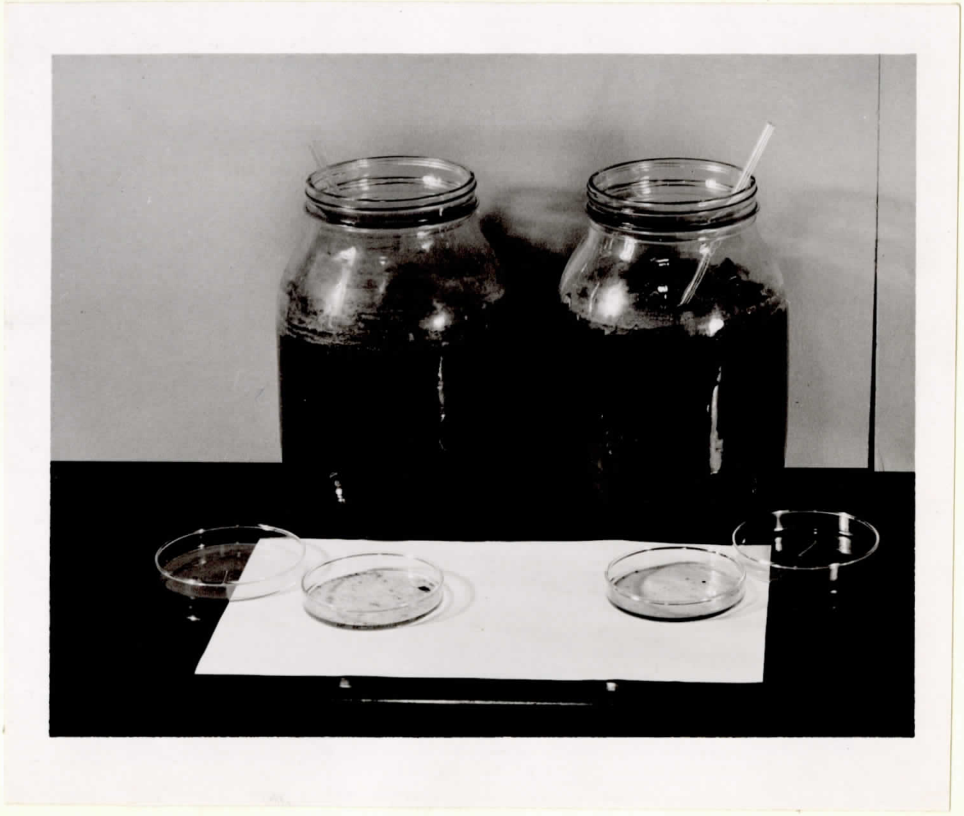

Figure 26

PLANKTON CULTURE JARS AND PETRI DISHES

Directions for culture methods of freshwater algae are given by Snow (1918). Culture methods are necessary for identification, ie. procedures for separation and observation at all stages of growth.

The methods of the bacteriologist in handling sterile petri dishes and agar plates are used. Several transfers from plate colonies to other sterile plates may be made to further "purify" or to separate the organisms, such as the Chlorella, from other larger forms. Transferring the algae cell to another water plate by a sterile pipette, then sterilizing the pipette with boiling water, allowing it to cool, and transferring the cell from the plate of distilled water to an agar plate will aid in selective transfer.

Loosely fitted lids which admit a little air seem to work best on the small receptacles which can be placed directly on the stage of the microscope.

The agar-agar or gelatine is mixed with a good nutrient solution over the bottom of the dish. After sterilization the inoculation with diluted water from the pond is made. The time required may be a few days or three weeks.

No one medium is favorable to all species of algae, and the form must be taken into consideration before a medium is prepared. If the species is a new form, various different media must often be tried before the right one is found. If a quantity of different forms from any collection be placed in one medium and a second quantity in another, the probabilities are that in the course of three or four weeks, few of the same species will be found in both cultures. Certain forms will have died in one, while these same forms have found in the other medium the substances and conditions for their development.

The media to which the greatest number of forms are adapted are Moore's solution and Knop's solution.

Moore's Solution.

Ammonium nitrate	0.5 gram
Potassium phosphate	0.2 gram
Magnesium sulphate	0.2 gram
Calcium chloride	0.1 gram
Iron Sulphate	trace

These amounts should be dissolved in one liter of distilled water.

Knop's Solution.

Potassium nitrate	1 gram
Potassium phosphate	1 gram
Magnesium sulphate	1 gram
Calcium nitrate	4 grams
Iron chloride	trace

The first three substances are dissolved in the required amount of water to make from 1 to 5 percent of the solution, then the calcium nitrate is added. This solution may then be diluted as needed; usually a 0.2 per cent or a 0.4 per cent solution is favorable for ordinary cultures.

It should be borne in mind that among the plankton forms there may some which will not develop in either. For the forms in this catagory, a solution made from the organisms of the plankton itself has been found to be the most satisfactory. Although even in this solution some forms fail to develop, many forms develop perfectly normally. For the cultivation of the unicellular algae, bouillon; earth decoctions; moist, finely pulverized earth; bits of bark and cubes of sterilized peat form good substances. However, the filamentous algae are more difficult to cultivate. In order to learn the life history of a plankton form, an observation should be made at different times and under different conditions and cultures.

Cultures may also be made of the "animal" plankton, normally called the zooplankton. Of particular interest are the protozoa. Culture material may be made of hay infusion or of decaying pond weeds. However, after inoculation with pond water, all cultures usually require several weeks to develop to a point at which it is best for laboratory study.

Bottles or jars containing cultures can usually be kept with loose tops at a temperature of not more than 70° F. and out of direct sunlight.

In searching for the protozoan in the culture bottle, the characteristics of the species should be kept in mind, and much fruitless searching with pipette can be eliminated. Amoeba settle to the bottom or on the sides of the bottle. Paramecium, Euglena, Didinium, Blepharisma, Euplotes, and Colpidium are found swimming freely throughout the entire culture. Stentor and Vorticella attach themselves to food, and the sides and bottom of the jar.

In culturing Protozoa the important factors to consider are temperature, light, kind and quantity of food, type of culture jar, and freedom from contamination.

Amoeba may be cultured in shallow dishes from an inoculation of decaying waterplants and pond water, on a medium made by boiling wheat kernels and pieces of timothy hay for about five minutes. After about two weeks time the amoeba can be seen plainly, and after six weeks a heavy culture should be ready for class use.

The culturing of Paramecium is not so difficult as culturing Amoeba. Ordinary room temperature is satisfactory for good growth, but for maximum growth in a shorter time, a temperature of 80° F. to 85° F. is best. Dishes with shallow depth and wide surface, containing timothy, rice, and wheat boiled about five minutes, are best for growing the Paramecium. Indirect light is best for this culture.

Euglena cultures may be inoculated from the greenish scum of stagnant pools or along the shallows of some ponds. Euglena requires plenty of food, such as boiled wheat, rice, or timothy hay, and grows best in direct sunlight. The temperature can range from room temperature to 90° F. with little damage to the culture.

Cultures may be made of the other Protozoa in a similar manner. Many forms will require sub-culturing or transfer to new containers.of media. Several of the larger forms will require sub-culturing about every three weeks to media which have been inoculated with smaller Protozoa earlier so that these rich growths provide food for the larger forms.

For classroom demonstration a large pipette may be used to transfer samples of each culture to small watch glasses or to clean slides. The microscope slides may be used in conjunction with microscopes which have pointer oculars, or with microscope projectors such as the bioscope.

Futher use may be made of the bioscope to have members of the class to draw charts for classroom display or for individual notebooks.

Individual containers filled with media may be inoculated from a master culture, so that reports may be written or given orally by class members, with an added advantage of comparison, perhaps, under different conditions of temperature, light, or media.

For microscope observation a small drop of dilute formaldehyde will kill the organisms. Special techniques for making permanent slides vary with the species and may be found by consulting books on microtechniques. Some forms are too sensitive for ordinary harsh preservatives, which may disintegrate as well as discolor the specimens.

APPENDIX B.

POND CULTURE

<u>Dynamics of the Pond</u>. The pond waters should contain enough inorganic nutrients, organic matter, and sunlight to provide the food supply for bacteria and the green algae. Nitrogen, carbon dioxide, sodium, potassium, and phosphates are some of the inorganic elements needed.

The algae and bacteria provide food for copepods, cladocerans, ostracods, and rotifers in the ordinary ponds, which have water teeming with these forms.

Larvae of insects, adult insects, zooplankton (larger animal plankton forms), and other animals feed upon the copepods and other animals mentioned in the above paragraph, and then are themselves the food for bluegill bream, the best of the pond forage fish.

The small bream provide food for the largemouth bass, which are the fighting, good-tasting fish that the pond owner or fisherman desires.

In short, the plankton are at the bottom of the food pyramid which leads up to the fish that the pond owner eats. Without an ample supply of plankton, the fish will be stunted and will upset the whole aquatic balance.