THE AVAILABILITY OF IRON AS A FACTOR LIMITING PRIMARY PRODUCTIVITY IN A MARL LAKE

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THE AVAILABILITY OF IRON AS A FACTOR LIMITING PRIMARY PRODUCTIVITY IN A MARL LAKE

By

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INTRODUCTION

In Michigan, many unproductive or oligotrophic lakes are of the marl type; these lakes are characterized by an inorganic sediment composed chiefly of calcium carbonate. Because marl lakes are generally considered to be relatively low in productivity (Welch 1952, Ruttner 1953), it seemed desirable to investigate factors limiting primary productivity[1] in this type of a lake. The specific objectives of the study were to determine: (1) the influence of physical and chemical characteristics of marl lakes on primary productivity, (2) the possible effect of chelating agents on the iron cycle of marl lakes, (3) the magnitude of primary productivity in Blind Lake and (4) the effects of nutrients and synthetic chelating agents on primary productivity in marl lakes.

Blind Lake in Washtenaw County was selected as a marl lake to be studied intensively. Two other marl lakes were investigated to determine whether or not results from Blind Lake were representative of marl lakes. These were Hoffman Lake, Charlevoix County, and Titus Lake (Sand No. 1), Grand Traverse County. In addition, Whitmore Lake, a eutrophic lake in Washtenaw and Livingston counties, was studied so it could be compared with the marl lakes. The investigation was conducted from July 1957 to December 1959.

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[1] The definition of primary productivity used in this paper is that given by Strickland (1960).

Although it is generally accepted that marl lakes are unproductive, no adequate explanation has been proposed for the cause. Fish production in marl lakes has been studied and considered to be poor (Cooper 1937, Gerking 1950). Raymond (1937) attributed the low productivity of Bass Lake, Michigan, to the large quantities of marl, scarcity of free carbon dioxide and the paucity of rooted aquatic vegetation. Marl lake sediments are low in organic and mineral content (with the exception of calcium carbonate) when compared with more productive lakes (Roelofs 1940, 1944). Wohlschlag (1950) demonstrated that aquatic plants grew in a marl lake when the marl in the littoral zone was removed and replaced by peat. Thus it might be inferred that substrate and not nutrients was the limiting factor to rooted aquatics, although the possibility exists that the peat contains nutrients or that the organic material itself is important in mineral nutrition.

Although inorganic nutrients are generally considered to limit plant production in lakes, these generally cannot be determined by the chemical analysis of water (Lund 1950, Gerloff and Skoog 1957). It is also known that different phytoplankton populations require different nutrient conditions (Chu 1942, Rodhe 1948) and that there are seasonal changes in both phytoplankton populations and the concentration of nutrients in lakes. Because of these seasonal changes, it is difficult and not very fruitful to study the nutrients limiting phytoplankton or to infer the productive nature of waters solely from chemical studies. Investigators of production in aquatic habitats have circumvented the use of chemical means to determine limiting nutrients by the more functional biological means--bioassay (Schreiber 1927,

Potash 1956). The investigation of primary productivity in oligotrophic lakes has been impeded by methodology. However, it is now possible to measure primary productivity in extremely unproductive or oligotrophic lakes with the carbon-14 method because of its sensitivity (Steeman Nielsen 1952). Even more important, the carbon-14 method can be employed to measure experimentally the effect of various factors on primary productivity and to determine limiting environmental factors (Jones and Thomas 1958, Ryther and Guillard 1959, Goldman 1960a, Harvey 1960, Schelske, Hooper and Haertl 1960). In the present study, the author has used the carbon-14 method and bioassay to investigate nutrients limiting primary productivity in marl lakes.

It has been stated that phosphorus is more likely to limit lake productivity than any other mineral element (Hutchinson 1948, 1957). Unfortunately, as Provasoli (1960) points out, aquatic biologists have been guilty of studying productivity on the basis of inorganic nutrients alone. These workers have usually assumed that phosphorus, nitrogen and possibly potassium are limiting. Phosphorus has been considered a factor limiting algal growth in marl lakes and its deficiency has been associated with the formation of marl (Eyster 1958). However, in Michigan, the fertilization of marl lakes with phosphorus, nitrogen and potassium (Ball 1950, Ball and Tanner 1951, Barrett 1953, Tanner 1960) has not increased production to the extent indicated by artificial enrichment of waters in other parts of the United States and the world (Neess 1949, Maciolek 1954, Mortimer and Hickling 1954). In addition, drastic environmental changes have resulted from the fertilization of Michigan lakes, i.e., the creation of winterkills.

Iron is not generally considered as a limiting factor in freshwaters, but may be limiting in offshore oceanic waters. In these regions of the sea, waters become impoverished of iron because iron in the ionic form is insoluble and is precipitated (Gran 1933, Harvey 1960). Recently, Ryther and Guillard (1959) have indicated that iron might be a limiting nutrient in the Sargasso Sea. In many lakes, including marl lakes, iron in the ferric form is also insoluble because of high pH values. High iron content in lakes is usually associated with acid waters of considerable organic content. Because organic matter and iron are found together in such lakes, this is indirect evidence for a relationship between the two.

Since Putter's hypothesis on the use of dissolved organic matter as a source of nutrition for aquatic animals (Putter 1907, 1909), there has been investigation and speculation of the function of dissolved organic substances in water. It is now established that dissolved organic matter may affect phytoplankton in four ways (Saunders 1957): (1) as a nutritional source for heterotrophic metabolism, (2) as an accessory growth factor which is required for or stimulates growth, (3) as a toxic substance either inhibitory or lethal and (4) as a chelating agent. Chelation may be defined as a reversible chemical reaction between a polyvalent metal ion and an organic compound to form a soluble stable ring complex (Martell and Calvin 1952, Smith 1959).

Chelating agents in water may affect algal growth in four ways (Saunders 1957): (1) Chelating agents can lower the effective concentration of a trace metal ion in the water below the level at which it can be mobilized by algae and thus actually cause the ion to be

limiting to growth (Spencer 1957). (2) The formation of chelator complexes may reduce the metal ion concentration below the toxic level even though greater than toxic quantities of the metal are present. Spencer (1957) found that the cupric ion was toxic to a marine alga at concentrations in excess of 2×10^{-6} M while the Cu-EDTA complex was toxic only at concentrations more than a thousandfold greater. (3) Chelation may remove a metal ion which is antagonistic to a metal poison, effectively increasing the relative concentration of the poison to a toxic level (Albert 1951). (4) Trace metals, such as iron, which are precipitated under certain conditions, can be maintained in solution by chelating agents. Therefore, iron could be maintained in solution in lakes by reacting with chelating agents.

Chelating agents are known to occur in nature and could react in lake waters with iron to maintain it in solution. In fact, indirect evidence of the chelating function of organic matter in lakes comes from studies of the growth of algae in pure culture. It has been found that many species of algae cannot be grown on purely inorganic media but that these species will grow when certain organic compounds are added to the media (Provasoli and Pintner 1953). The purpose of soil extract in culture media is to maintain iron in solution (Pringsheim 1946). Humic acids and yellow organic acids are known to have properties of chelating agents, i.e., the formation of soluble complexes with iron (Shapiro 1957).

Synthetic chelating agents are now widely used in place of natural substances in various culture media (Provasoli and Pintner 1960). They

are used in these media for solubilization of iron and other trace metals (Heck and Bailey 1950, Hutner et al. 1950, Myers et al. 1951, Jacobson 1951, Gerloff and Skoog 1957). The practical applications of chelating agents in agriculture have been recognized (Stewart and Leonard 1952, 1955; Weinstein et al. 1954; de Kock 1955; Haertl and Martell 1956; Smith 1959; Wallace 1960). However, the effect of chelating agents on primary productivity in natural aquatic systems has been studied only in a marl lake by Schelske, Hooper and Haertl (1960).

METHODS

Physicochemical

The following physical and chemical characteristics of water from Blind Lake were determined: temperature, light penetration, dissolved oxygen, alkalinity, pH and total iron. Methods used in the analyses of water for dissolved oxygen, alkalinity and total iron are modifications of those given in Standard Methods for the Examination of Water and Sewage (American Public Health Association 1955). Samples for chemical analyses were taken at selected depths. During summer stratification, two samples were taken in the epilimnion; below the epilimnion samples were usually taken at intervals of 6 ft. The number of samples taken in the epilimnion was increased to three or four as the epilimnion deepened in the fall.

Water temperature.--Water temperature was measured in degrees Fahrenheit with a Whitney underwater thermometer of the resistance type which had been recently standardized. Measurements were usually made at depth intervals of two feet. As a check on the resistance thermometer, temperatures were also measured periodically with a mercury thermometer.

Light penetration.--A pair of 856 YR Photronic photoelectric cells, which had been matched for output and linearity, and a model 622 Weston microammeter were used to measure light penetration. One cell was used to measure surface light intensity and the other cell was used for subsurface intensity. This equipment was described and used by Beeton

(1958). Transparency measurements were made with a white Secchi disc, 20 cm in diameter.

Dissolved oxygen.--The unmodified Winkler method was used to determine the concentration of dissolved oxygen. Samples of 100 ml were titrated with a 0.010 N sodium thiosulfate solution which had been carefully standardized with 0.025 N potassium dichromate. Thyodene was used as an indicator.

Alkalinity.--Alkalinity was determined by titration of 100 ml samples with 0.020 N sulfuric acid. Phenolphthalein and methyl orange were used as indicators.

pH.--The pH was measured with a Beckman Model G pH meter.

Total iron.--Total iron was determined using the tripyridyl method. Two ml of concentrated hydrochloric acid was added to a 50 ml water sample. The sample was boiled for 10 minutes, cooled and diluted to 50 ml. One ml of hydroxylamine hydrochloride and 5.0 ml ethylenediamine were added and mixed with the sample. The sample was placed in a 4-cm Klett absorption cell and read in a Klett-Summerson colorimeter to determine the zero point. After this, 5.0 ml of tripyridyl solution was added and mixed with the sample. The color development in Klett units was read five minutes after the addition of the tripyridyl. The same procedure was followed using a sample of double distilled water for a reagent blank.

The relationship of colorimeter readings in terms of Klett units to iron concentration was established by the following procedure. A liter of standard iron solution was prepared by dissolving 1.322 mg of iron wire in 40 ml of concentrated hydrochloric acid. This solution was diluted to give solutions containing 1,322, 528.8, 264.4, 132.2 and

79.3 ppb of iron. Readings of color development were made for these solutions as described above. These data were plotted as Klett units against iron concentrations in ppb and the resulting curve indicated a linear relationship. The regression line calculated from these data had a slope and standard deviation of 4.58 ± .0637. The slope of the regression line (4.58) was the factor used to convert Klett readings to iron concentration by multiplying it times the corrected Klett reading. The corrected Klett reading was found by subtracting the reagent blank reading from the sample reading.

Mud-Water Experiments

To evaluate the effect of a chelating agent on the iron cycle in a lake, a series of experiments with mud and water were performed. The chelating agent was HEDTA (trisodium salt of N-hydroxyethylethylenediaminetriacetic acid). Mud and water from Blind, Hoffman and Weber lakes were utilized for this purpose. Experimental mud-water systems were established in one-gallon jars in the following manner. Several Ekman dredge samples of mud were taken from the deeper portions of each lake. Depths of collection were 56 ft in Blind Lake, 20 ft in Hoffman Lake and 34 ft in Weber Lake. In the laboratory, approximately one inch of mud was poured into each of the one-gallon jars. Mud which splattered on the sides of the jars was removed carefully with a damp cloth. Water was then siphoned slowly into the jar from a 500 ml beaker. Disturbance of the sediment was minimized by running the siphoned water onto a paper card (4 x 5 inches) placed on the surface of the mud, by having a U-shaped tube at the end of the siphon and by keeping the hydrostatic pressure of the siphon at a minimum. After 2 inches or more of water was siphoned into the jar, the paper card was

removed. The U-shaped tube allowed water entering below the water surface to flow perpendicular to the sediment surface. As the depth of water in the jar increased, the rate of siphoning could also be increased without agitation of the sediment. Siphoning was stopped when the depth of water in the jars was 6 inches. Using this procedure, mud-water systems could be established on the day of field collection and have the water phase contain no visible amount of mud. Mortimer (1941) was unable to do this unless ". . . the mud was allowed to stand exposed to the air for several days. After this, but not before, it was found possible to run lake water into the tanks . . . so that it remained clear."

Anaerobic mud-water systems were established by pouring one-half inch of mineral oil over the water surface. Glass covers were placed over both anaerobic and aerobic jars to exclude dust. The water samples for chemical analyses were taken three inches above the mud surface. Chemical analyses of water samples were made using the methods outlined above.

Measurement of the Rate of Carbon-14 Uptake

The vertical distribution of photosynthetic activity of the phytoplankton populations of Blind Lake was determined at selected depths ranging from the surface to 12 m. The carbon-14 method was used (Steeman Nielsen 1952). Water was collected in a Kemmerer water sampler with a capacity of 1200 ml. Three 250 ml glass-stoppered bottles were filled with water from each depth. Two bottles were used as "light bottles"; the other, a "dark bottle," was wrapped with aluminum foil to exclude light. A sealed ampoule containing carbon-14 (1.74 μC) was broken in each bottle and the contents of the bottles were mixed by shaking. The

bottles were then suspended in the lake at the depth from which the water sample was taken originally. These bottles were suspended on a line attached to an anchored buoy. After 6 to 8 hours of exposure, the bottles were removed from the lake. The exposure period was between the hours of 8 AM and 5 PM.

After the bottles were removed from the lake, they were stored in a wooden box with a hinged cover until filtering was started. Two subsamples from each bottle (50 or 100 ml aliquots) were filtered through HA filters[2] of 37 mm diameter, the effective filtering diameter was 25 mm. Each subsample was washed with 5.0 ml 0.002 N hydrochloric acid and 3.0 ml of distilled water. The filter papers were then cemented to aluminum counting planchets of 38 mm diameter. Filtration was usually completed within one hour after the bottles were taken from the lake.

Samples were counted with the following equipment:[3] a D-47 micromil end-window gas flow counter, a 183 scaler and a 110A automatic sample changer with a 111 printer-timer. A predetermined number of counts (at least 1280) was taken for each sample and the time required for these counts was recorded.

Photosynthetic fixation of carbon-14 by algae at a particular depth was determined from the counting rates by subtracting the average dark bottle rate from the average light bottle rate. Average counting rates were determined from the subsamples taken at each depth. This rate (carbon-14 fixed) was then converted to the total quantity of carbon-12 fixed per cubic meter per hour (mg $C/m^3/hr$) using the relationship:

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[2] Millipore Filter Corporation

[3] Nuclear Chicago

$$\text{Carbon-12 fixed} = \frac{\text{Carbon-14 fixed}}{\text{Carbon-14 available}} \text{ Carbon-12 available}$$

Calculations of available carbon-12 were made from alkalinity titrations, pH and an unpublished table prepared by Roger W. Bachmann from dissociation constants of carbon dioxide given by Hutchinson (1957). The quantity of carbon-14 available was determined by standardizing the activity of the carbon-14 in sealed ampoules with a sample of known activity (NBS 4294) obtained from the National Bureau of Standards, Washington, D. C. Activities were measured with a Packard Tri-Carb Liquid Scintillation Spectrometer.[4] Due to difficulties in establishing values for the unknown terms in the above equation and to other factors, at present, it is best to use photosynthetic data based on carbon-14 uptake on a relative rather than on an absolute basis (Rodhe et al. 1960).

Uptake rates for each depth were plotted and the area under each curve (photosynthetic profile) was determined by planimetry. A relationship was established between a unit of area and the rate of carbon fixation per square meter. This relationship was used to convert the area under the photosynthetic profile to rate of carbon fixation in mg C/m^2/hr.

Nutrient Experiments

Nutrients were added to lake water to study their effect on primary productivity. These experiments were conducted in the laboratory and in the field. Procedures in the laboratory were similar to those described below for the field experiments.

Various combinations of Chu 10 nutrients, chelating agents and minor elements were used in nutrient experiments. Chu 10 nutrients were five

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[4] Packard Instrument Co.

of the nutrient salts described for medium No. 10 by Chu (1942). The following Chu 10 stock solutions were prepared from analytical reagent grade chemicals: calcium nitrate, dibasic potassium phosphate, magnesium sulfate, sodium silicate and sodium carbonate. These five solutions were used collectively as Chu 10 nutrients and were used in various combinations for other nutrient additions. Measured quantities of each of these solutions were diluted (2.0 ml of each nutrient solution per liter of lake water) in the carboy to the following concentrations in ppm: calcium (Ca), 19.5; magnesium (Mg), 2.4; potassium (K), 9.0; sodium (Na), 18.1; phosphorus (P), 3.5; nitrogen (N), 13.7; silicon (Si), 5.8; sulfate (SO_4), 9.8 and carbonate (CO_3), 11.3. Minor elements consisted of one solution of "micrometabolic elements" (Chu 1942) and contained zinc, manganese, aluminum, boron, lithium and cobalt salts. Three solutions of Dow chelating agents were used. These were: Versenol, HEDTA, trisodium salt of N-hydroxyethylethylenediaminetriacetic acid; Versenol-F, NaFeEEDTA, iron salt of HEDTA and Versene, EDTA, tetrasodium salt of ethylenediaminetetraacetic acid. The concentration of HEDTA and EDTA used in experiments was 2.0 ppm unless otherwise indicated. The concentration of NaFeEEDTA was based on the iron content of the complex and was 2.0 ppm of iron unless otherwise stated. The above solutions were dissolved in double distilled water and were stored in polyethylene bottles. Fresh solutions were prepared every three or four weeks.

Glass-stoppered bottles of 250 ml capacity were used in most of the field experiments, 125 ml bottles were used in the remainder. Bottles were washed with Alconox, rinsed with tap water four times and cleaned with a chromic acid cleaning solution. Bottles were then rinsed five times with distilled water in the laboratory and two times with lake water in the field.

To insure uniformity of lake water and phytoplankton populations for experiments, all the lake water to be used on any one date was collected in a calibrated 5-gallon glass carboy. The carboy was filled with surface water using a 1200 ml Kemmerer water bottle or by allowing water to flow directly into the carboy. The results were not affected by either method of collection. After the lake water was collected in the carboy, bottles were filled with the untreated lake water to be used as the controls of the experiments. After the control bottles had been filled, nutrients from the stock solutions were added to the carboy. Two combinations of nutrients frequently used in these experiments were calcium nitrate and dibasic potassium phosphate (designated nitrogen and phosphorus) and the five Chu 10 nutrients (designated Chu 10 nutrients). The following procedure was used in setting up these experiments to insure uniformity of nutrients in all bottles. After the control bottles were filled, nitrogen and phosphorus were added to the carboy, and bottles for nitrogen and phosphorus experiments were filled. Then the remaining three solutions of Chu 10 nutrients were added so that bottles containing Chu 10 nutrients could be filled. Before bottles were filled, the contents of the carboy were always thoroughly mixed to insure uniformity of samples in the bottles. Chelating agents were added directly to the bottles after they were filled. Duplicate bottles were used for each of the various nutrient combinations.

After filling, the bottles were placed horizontally in racks and suspended in the lake at a depth of 1.5 m from an anchored buoy. Sufficient light was present at 1.5 m for a high rate of photosynthesis, but undesired effects caused by the inhibition of photosynthetic activity

at high light intensities were minimized. Bottles were incubated in the lake for varying lengths of time, usually 4 to 8 days. On the final day of the experiment, a sealed ampoule containing 1.74 μC of carbon-14 was broken in each bottle. The bottles were resuspended in the lake from four to eight hours. On removal from the lake, the sampling procedures described above for the measurement of carbon-14 uptake were followed with the exception that 25 or 50 ml aliquots were filtered from each bottle instead of 50 or 100 ml aliquots.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF BLIND LAKE

Some of the physical and chemical characteristics of Blind Lake are presented. Observations were made in 1957, 1958 and 1959, but were most extensive in 1959. Data are presented in the Appendix.

Morphometry

Blind Lake is a marl lake well protected from wind action by hills and trees. The lake has no inlet, but has an outlet which drains to the north into Half-Moon Lake (Fig. 1). At some previous time during a period of higher water levels undoubtedly these two lakes were connected. There are two basins in Blind Lake which are divided by a shoal area over which the water is less than 30 ft in depth. The shallow basin with a depth of 45 ft is located in the southern part of the lake; the deep basin is located north of the shallow basin in the western third of the lake. The morphological characteristics of the lake were determined from the contour map of the lake (Fig. 1). The lake has a maximum depth of 80 ft, a mean depth of 27.2 ft, a surface area of 68 acres and a volume of 1850 acre-feet (Table 1). The surface area of the lake is divided nearly equally between water of 20 ft or more and 20 ft or less in depth. The lake basin has a steep slope between the 20- and 30-ft contours which is indicated by the small surface area between these contours (4.27 acres or 6.3 per cent of the total area).

Water Temperature

During the three years of investigation, water temperature was measured on five dates from July 31 to September 6, 1957, on eight dates

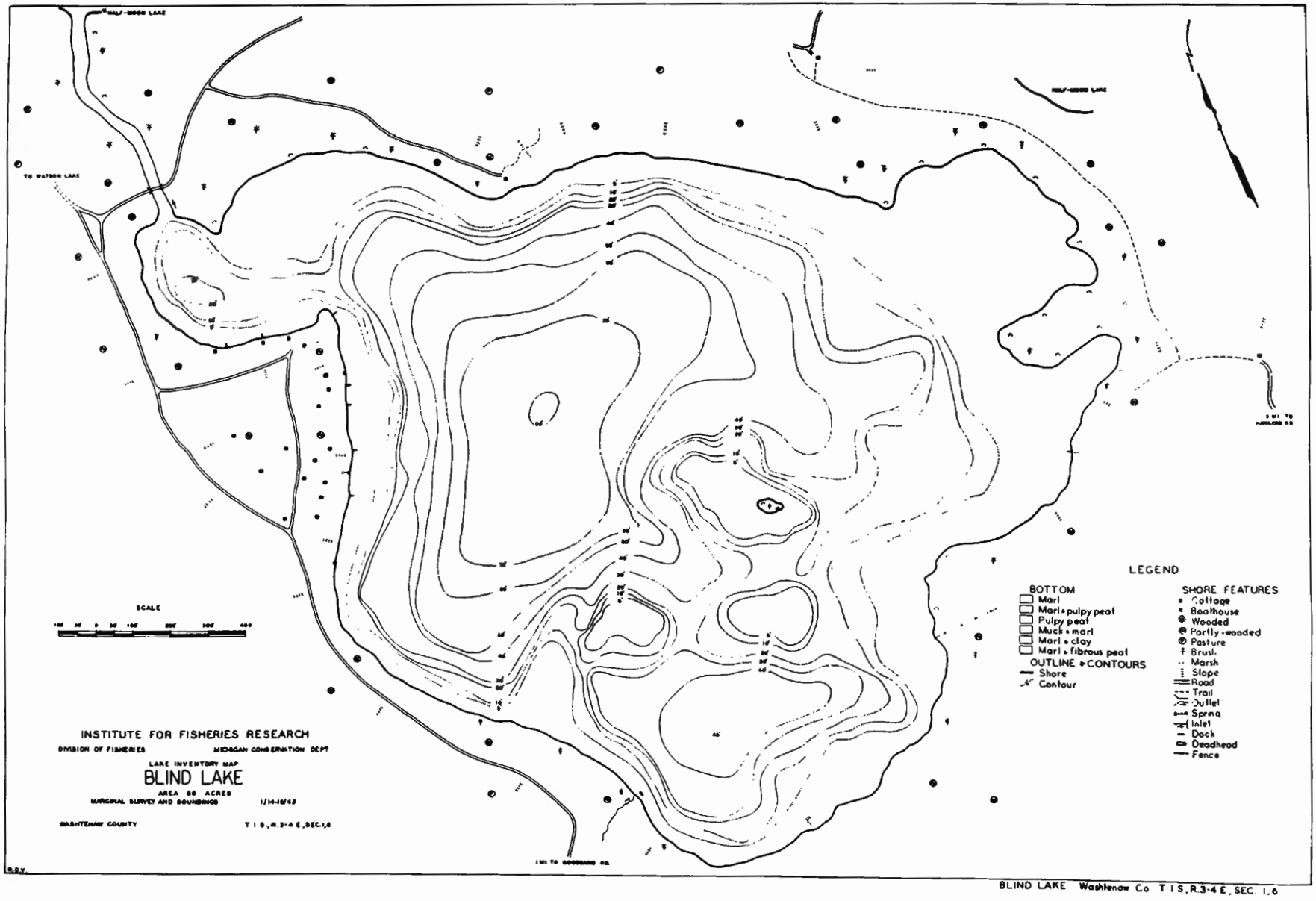


Fig. 1.--Contour map of Blind Lake, Washtenaw County, Michigan.

Table 1.--Area, volume and depth relationships in Blind Lake

Depth strata (feet)	Area (acres)	Per cent of total area	Cum. area	Volume (acre-feet)	Per cent of total volume	Cum. volume
0-5	19.73	29.0	19.73	289.32	15.6	289.32
5-10	7.30	10.7	27.03	222.63	12.0	511.95
10-20	6.94	10.2	33.97	373.93	20.2	885.88
20-30	4.27	6.3	38.24	318.67	17.2	1204.55
30-40	8.76	12.9	47.00	252.51	13.7	1457.06
40-50	6.44	9.5	53.44	176.82	9.6	1633.88
50-60	4.88	7.2	58.32	120.35	6.5	1754.23
60-70	4.23	6.2	62.55	74.63	4.0	1828.86
70-80	5.33	7.8	67.88	21.26	1.2	1850.12
80	0.12	0.2	68.00	0.08	0.0	1850.20
	68.00	100.0		1850.20	100.0	

from July 31 to December 5, 1958, and on 25 dates from March 20 to December 17, 1959. For the periods involved, at least one measurement was made each month.

The 1957 data were taken from July 31 to September 6 during the period of summer stratification and are not presented graphically. Measurements were made only in the deep basin. During the time of these measurements, the depth of the epilimnion increased from 10 to 18 ft and the lower limit of the metalimnion increased from 29 to 32 ft. The surface temperatures dropped from 81.3° to 70.6° F. Temperatures ranged from 45° to 75° F in the metalimnion and from 40.4° to 41.1° F at 75 ft in the hypolimnion.

In 1958, water temperature was measured in the shallow depression. The maximum surface temperature recorded was 79.4° F (August 8). The lowest bottom temperature of the shallow basin was 44.9° F at 44 ft on July 31. A depth-time diagram of the distribution of water temperature in 1958 was prepared by contouring isotherms at 5° F intervals (Fig. 2).

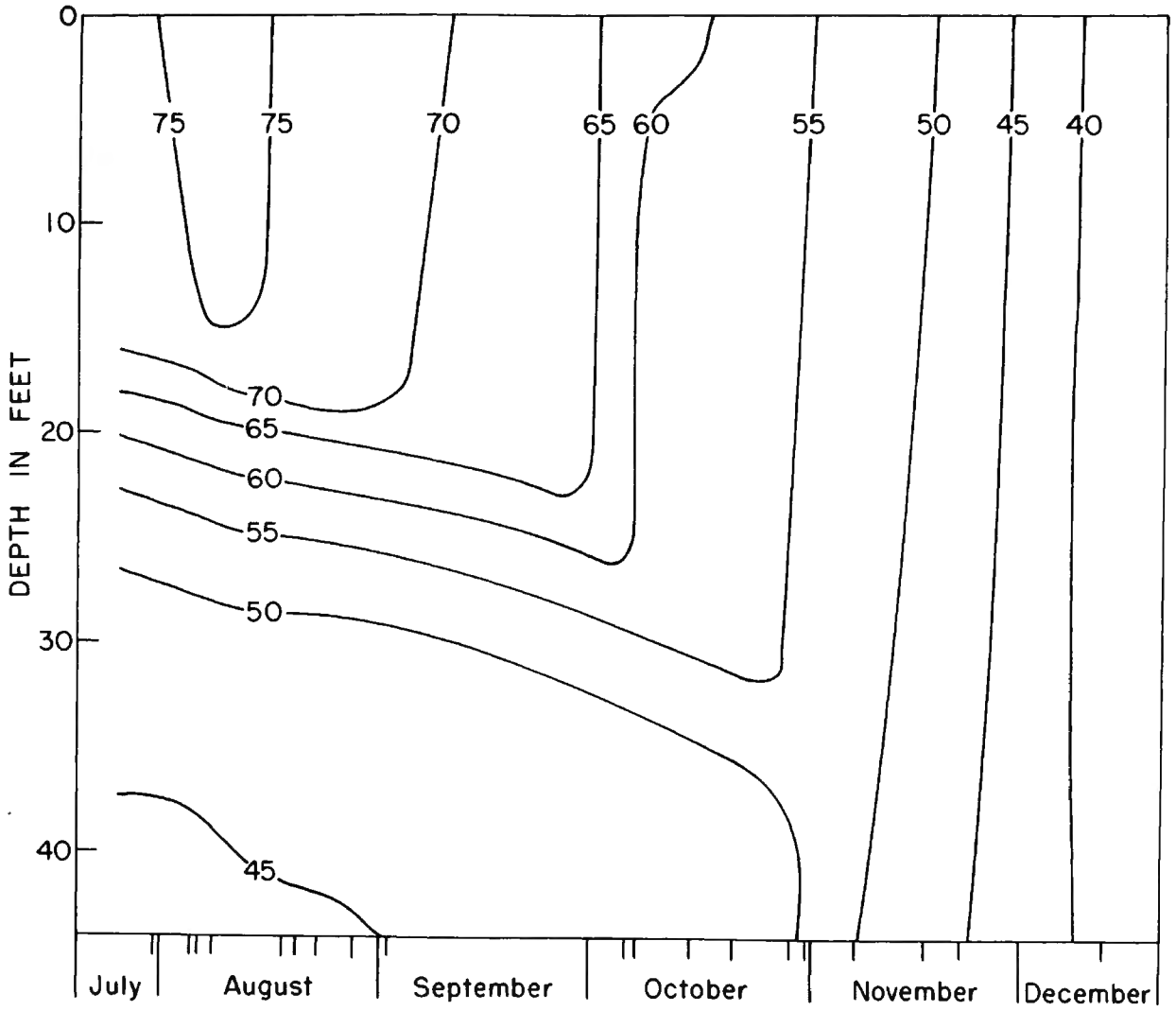


Fig. 2.--Depth-time diagram of water temperature (°F) in the shallow basin of Blind Lake in 1958.

A detailed interpretation or presentation of temperature data will not be presented in this paper although the temperature relations can be deduced from the depth-time diagrams and from the original data presented in the Appendix.

A depth-time diagram of the distribution of water temperatures in the deep depression in 1959 was prepared by contouring isotherms at 5° F intervals (Fig. 3). The ice cover on the lake was 13 inches thick on March 20 and 16 inches thick on March 28. The date on which the lake was ice free is not known exactly, but was not before April 5. By April 14 the water was thermally stratified. During summer stratification, the maximum surface temperature recorded was 79.4° F (August 28). The water in the lake was not homothermal until late November.

Light Penetration

On October 3, 1958, relative light transmission was determined photoelectrically using seven filters for wave lengths of light ranging from 300 to 750 millimicrons. Maximum transmission was found in the 540-590 millimicron wave lengths. The depth of penetration of one per cent of the surface light was 36 ft.

Secchi disc transparency measurements in 1957 and 1958 ranged from 8 to 10 ft.

Dissolved Oxygen

In 1957, dissolved oxygen was determined in the deep basin on only three dates. On August 27 and September 6, dissolved oxygen distributions were of the positive heterograde type (Hutchinson 1957) with metalimnetic

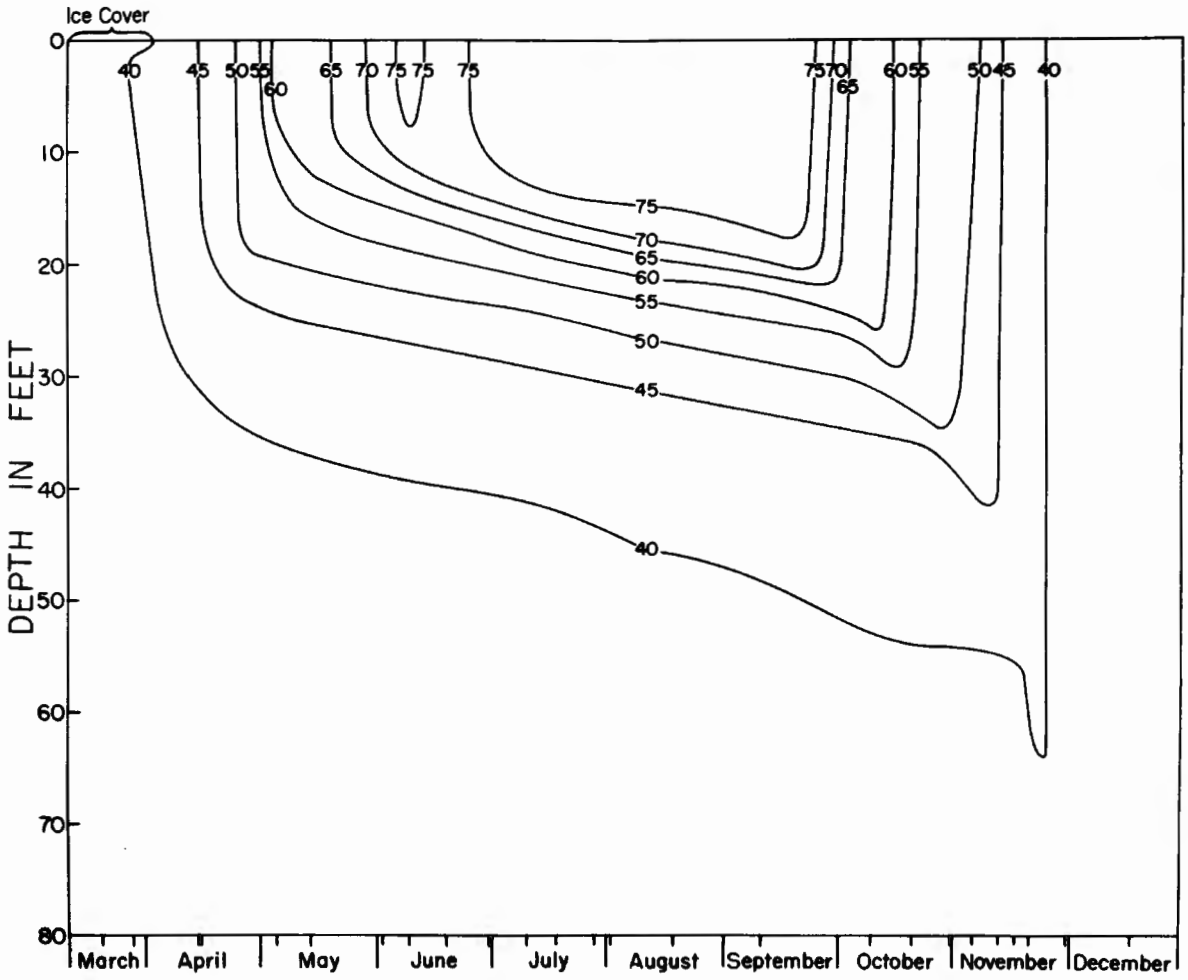


Fig. 3.--Depth-time diagram of water temperature (°F) in the deep basin of Blind Lake in 1959.

oxygen maxima at 23 ft of 19.0 and 18.2 ppm, respectively. No determinations were made at 23 ft on August 20. In the hypolimnion on September 6, oxygen depletion was restricted to depths greater than 66 ft.

In 1958, the oxygen distribution in the shallow basin was positive heterograde on August 5 and 28. There was a maximum of 13.2 ppm at 26 ft on August 28. The August 5 maximum was 11.5 ppm at 23 ft, no samples were collected at 26 ft on this date.

Dissolved oxygen was determined on 24 dates in 1959 and these data are contoured on isopleths at 1.0 ppm intervals (Fig. 4). The lack of vertical isopleths in early April indicates that mixing during spring circulation was not sufficient to distribute oxygen homogeneously in the entire water mass. Consequently, less than 1.0 ppm of dissolved oxygen was present below 75 ft in early June, and by the first week in November oxygen depletion had progressed to the extent that more than 1.0 ppm was found only above a depth of 45 ft. The closely grouped isopleths during November and December indicate that the oxygen content of the waters below 45 ft increased rapidly to more than 10 ppm. This increase in oxygen was associated with the mixing and deepening of the epilimnion during the fall overturn (Fig. 3). At the same time, the concentration of oxygen in the water above 40 ft increased from 9.80 to 10.7 ppm. The closed isopleths between 20 and 30 ft from June to September (Fig. 4) point out the presence of a positive heterograde oxygen distribution.

Alkalinity

Total alkalinity of epilimnetic waters ranged from 138 to 150 ppm in three years of this study and its vertical distribution was correlated

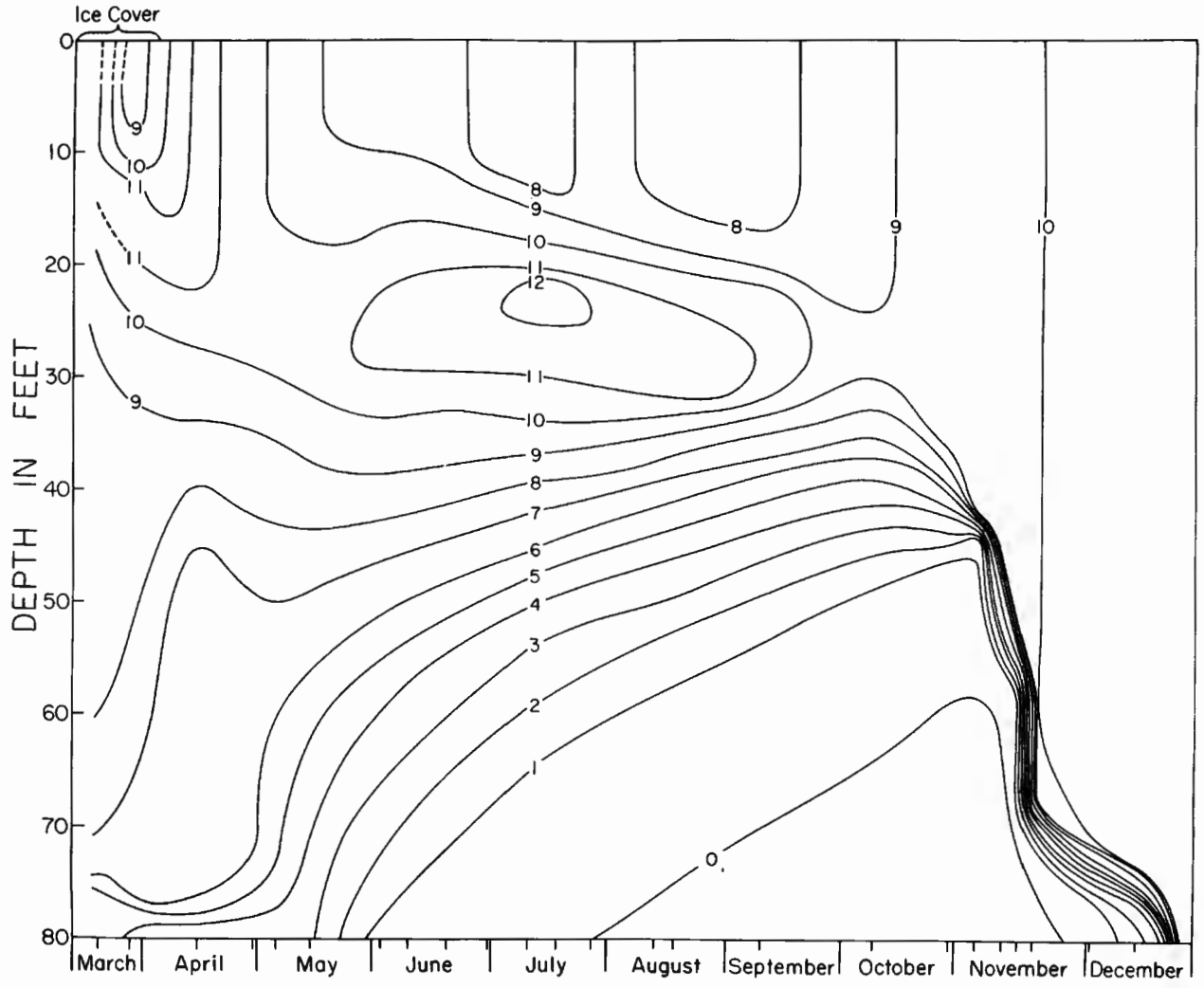


Fig. 4.--Depth-time diagram of dissolved oxygen (ppm) in the deep basin of Blind Lake in 1959.

with lake stratification. In the hypolimnion, alkalinity increased during summer stratification to a maximum of 195 ppm at 76 ft on October 20, 1959.

pH

The range of pH values observed was not great. In epilimnetic waters, pH ranged from 8.4 to 8.6. The hydrogen-ion concentration of the hypolimnion increased with the advance of stratification, the lowest pH found in the hypolimnion was 7.6.

Total Iron

Analyses for total iron were made in 1958 and 1959. In 1958, only three samples collected in October were analyzed, but a seasonal study was made of samples collected on 14 dates in 1959.

On October 7, 1958, iron was not detectable in a surface water sample, but a concentration of 207 ppb was found in a sample from the upper part of the hypolimnion (36 ft). By October 29, the epilimnion had deepened to 35 ft (Fig. 2) and the resultant mixing of waters increased the iron concentration of the surface waters to 57 ppb.

The results of total iron analyses in 1959 were contoured on a depth-time diagram (Fig. 5). In contrast to the 1958 results, all surface samples in 1959 contained detectable quantities of iron. Less than 30 ppb was present from June to November. In December when the water mass was nearly homothermal, the concentration increased to 40 ppb as a result of mixing of surface waters with waters from deeper strata of higher iron content. The maximum concentration in the surface waters was 50 ppb which was present in April after the melting of the

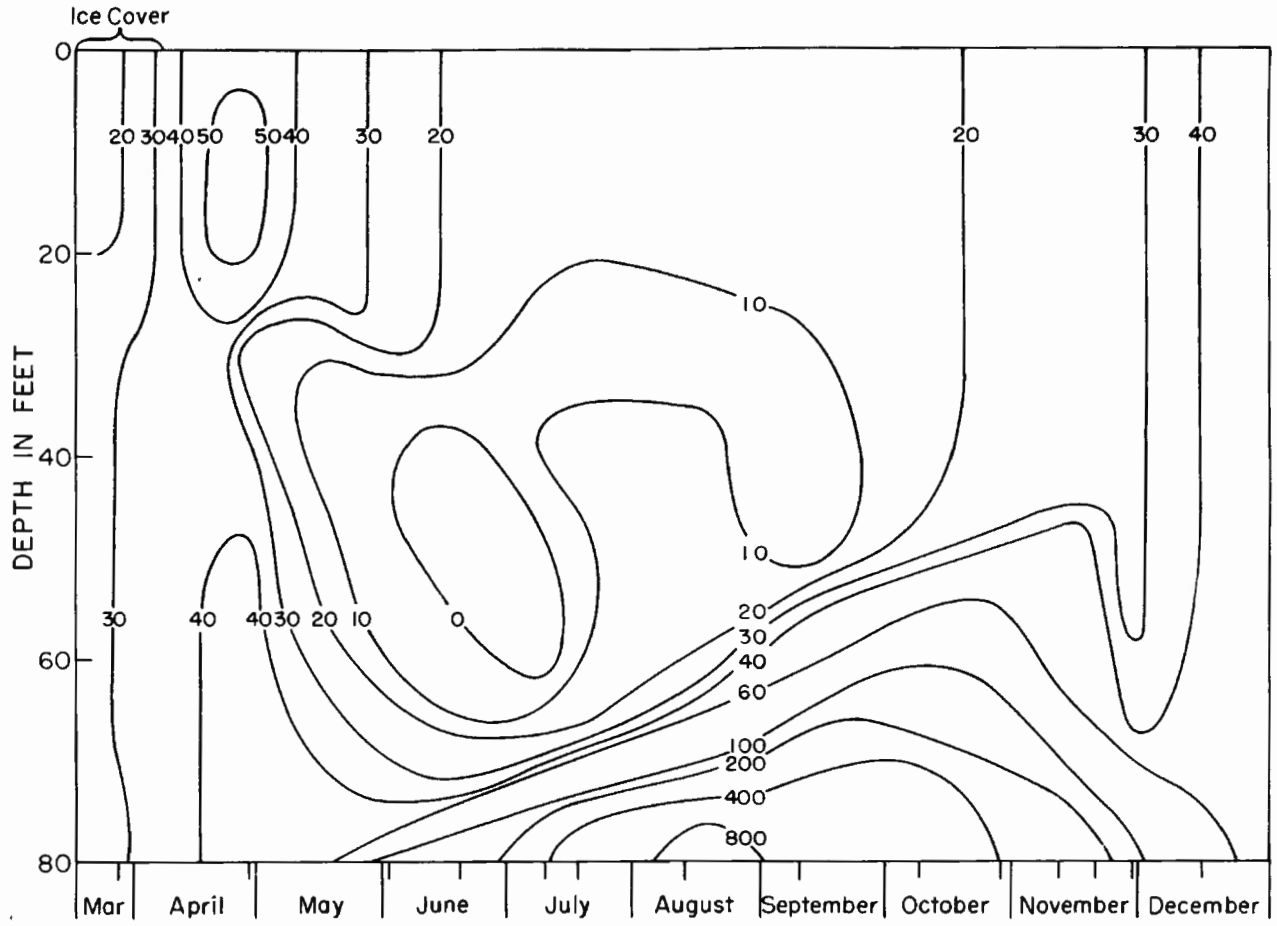


Fig. 5.--Depth-time diagram of total iron (ppb) in the deep basin of Blind Lake in 1959.

ice cover. Iron was not detectable between the depths of 40 and 60 ft in June and July. This may be attributed to the settling of various forms of particulate iron after the lake had stratified. Evidence for the loss of iron by settling can be seen from the configuration of the isopleths between the depths of 30 and 70 ft in May and June.

The increase in concentration of iron in the hypolimnion from June to November (Fig. 5) followed a pattern which was in general correlated with oxygen depletion (Fig. 4). Above 70 ft, the iron content increased up to the time of fall circulation. At 80 ft, the concentration of iron increased to 850 ppb on August 13 which was followed by a decrease to 76 ppb on December 17. The decrease in iron concentration at 80 ft was not related to oxygen content, but to the progressive stagnation of the hypolimnion. The odor of hydrogen sulfide was apparent at 80 ft which indicated the decrease was due to the precipitation of ferrous sulfide. In the fourth stage of hypolimnetic stagnation, ferrous iron is precipitated as ferrous sulfide (Hutchinson 1957). Hutchinson stated that "only in the cases of lakes with extremely reductive sediments and small hypolimnia will all four phases be observed." This is of interest because Blind Lake does not have a small hypolimnion in relation to the epilimnion and because the inorganic marl sediments probably are not extremely reductive. In Blind Lake, the fourth phase was restricted to the stratum of the lake below 70 ft, probably due to the lack of turbulence.

The Iron Cycle in Blind Lake

Iron is practically insoluble in the ionic (ferric) form at pH values and redox potentials of the epilimnetic waters of most lakes (Hutchinson 1957). The solubility of ferric iron in lake water at pH 6 is 10^{-9} ppm (Mydahl 1951). Iron in such waters may be present as (1) ferric hydroxide in suspension or adsorbed on seston particles, (2) various forms of both inorganic and organic sestonic iron and (3) soluble or colloidal iron organic complexes (Hutchinson 1957). Ferric iron may be lost from epilimnetic waters by settling of particulate forms, including precipitates. There is some question regarding the combined form of precipitates--whether they are ferric hydroxide or ferric phosphate. According to Einsele (1938), ferric phosphate is less soluble than ferric hydroxide and consequently will be precipitated completely before ferric hydroxide is formed. If Einsele is correct, the precipitation of iron robs the trophogenic waters of a very important nutrient--phosphorus. Iron in the reduced or ferrous state is soluble and is released from sediments along with phosphate, ammonia, silicate, sulfate and manganese (Mortimer 1942).

In the present study only total iron was determined because it is not known what fraction of the iron in water is available to algae (Pringsheim 1946, Lund 1950, Gerloff and Skoog 1957). Hutchinson (1957) stated that most of the published results on the fractionation of forms of iron in well-oxygenated lake waters "are incomplete and have been referred to meaningless categories." However, there is no doubt that iron is in the ferric form in the epilimnion of most lakes and that it

is in the ferrous or reduced form when released from the mud under anaerobic conditions (Mortimer 1942). It is presumed that ferric and ferrous iron would occur in Blind Lake under similar conditions.

The influences of weather and lake morphometry on the iron cycle in Blind Lake are shown by comparing physical and chemical data for 1957 and 1959. Due to hot, calm weather in the spring of 1959, the lake stratified shortly after the melting of the ice cover (Fig. 3) and an oxygen deficit in the hypolimnion resulted from the restricted period of spring circulation. There were greater quantities of oxygen in the hypolimnion in 1957 than in 1959, presumably because of a more extensive spring circulation in 1957. The distribution of oxygen in September 1957 and in July 1959 was approximately the same. If iron had been released under the same oxygen conditions in 1957 as in 1959, then less than 60 ppb of iron would have been present in the water above 70 ft and no more than 10 ppb would have been present in the water above 60 ft in September 1957 (Fig. 5). Although the concentration of iron below 70 ft appears to be great, it would not increase the iron concentration of the entire water mass greatly during an overturn because only about one per cent of the volume of the lake is contained in this stratum (Table 1). Likewise, the concentration of iron in the 60- to 70-ft stratum would be diluted about 25 times if circulation of the water mass were complete because this stratum represents only four per cent of the lake volume. During the overturn, waters are aerated causing insoluble ferric iron to be formed. The precipitation of ferric iron decreases the quantity of iron brought into the trophogenic layers at the turnover. Thus the concentration of iron in the surface waters probably was not

increased greatly in 1957 at the time of the fall overturn. However, in 1959, considerable quantities of iron were liberated from the sediments in the hypolimnion because of anaerobic conditions (Fig. 4) and the iron concentration of the surface waters increased more than 20 ppb during the fall overturn (Fig. 5). Because regeneration of iron from deep-water sediments may occur only in years when aeration of the water mass is incomplete, the supply of iron for the trophogenic waters may be irregular. A deficiency of iron might follow years in which the hypolimnion is well-aerated at the time of the spring turnover.

MUD-WATER EXPERIMENTS

The effect of a chelating agent on the iron cycle in a lake was investigated in the laboratory by establishing experimental systems of mud and water from three lakes. To do this, experiments of four types were set up for each lake. The four types were two aerobic experiments (aerobic chelate and aerobic control) and two anaerobic experiments (anaerobic chelate and anaerobic control). The experiments designated chelate contained HEDTA as a chelating agent.

The first group of four mud-water experiments were begun with Blind Lake mud and distilled water. To the jars designated chelate, 10.5 ppm of HEDTA was added. After 28 days, chemical characteristics of the water were determined (Table 2). The quantities of iron in the water of the experiments containing HEDTA were greater than in the corresponding controls. The aerobic experiment with HEDTA contained 0.45 ppm of iron; this was approximately 20 times that of the aerobic control. In the anaerobic experiments, the water with HEDTA contained 0.93 ppm of iron, about twice as much iron as the control, indicating that the anaerobic conditions caused the release of some iron from the sediments. The differences in iron content noted between the control and the experiment with HEDTA, under either aerobic or anaerobic conditions, do not appear to be related to dissolved oxygen, alkalinity or pH (Table 2). The differences, therefore, were attributed to the chelate function of HEDTA, i.e., the formation of soluble complexes with ferric iron.

In the second group of experiments, the effect of HEDTA on the exchange of iron between water and mud was studied during a period of 6

Table 2.--Chemical characteristics of water after 28 days of contact between Blind Lake mud and distilled water. Experiments designated chelate contained 10.5 ppm of HEDTA. Values in ppm.

Experiment	Total iron	Dissolved oxygen	Alkalinity Ph'th	Alkalinity M.O.	pH
Aerobic Control	0.02	5.4	2	178	8.6
Aerobic Chelate	0.45	5.2	2	160	8.3
Anaerobic Control	0.55	0.2	0	146	7.3
Anaerobic Chelate	0.93	0.2	0	138	7.3

days instead of the 28 days used in the first experiments. The experiments with Blind Lake mud and distilled water were repeated. In addition, experiments with mud and water from Blind, Hoffman and Weber lakes were included so that the effect of HEDTA could be studied in systems of natural lake waters and natural lake muds. Four days after these experiments were set up, 6.3 ppm of HEDTA was added to the jars designated chelate. Analyses of pH, alkalinity and dissolved oxygen were not made because the results of the first group of experiments show the effect of HEDTA was not related to these factors (Table 2).

The results of the second group of experiments show that iron was released from lake muds into water containing HEDTA in less than three days, whereas little or no iron was released from muds in the anaerobic control experiments (Figs. 6, 7 and 8). Thus anaerobic release of iron into the water was negligible in these short-term experiments in contrast to the results from the 28-day experiments (Table 2) and, therefore, the release of iron in the short-term experiments was attributed to the chelating properties of HEDTA. These results also show that the effect

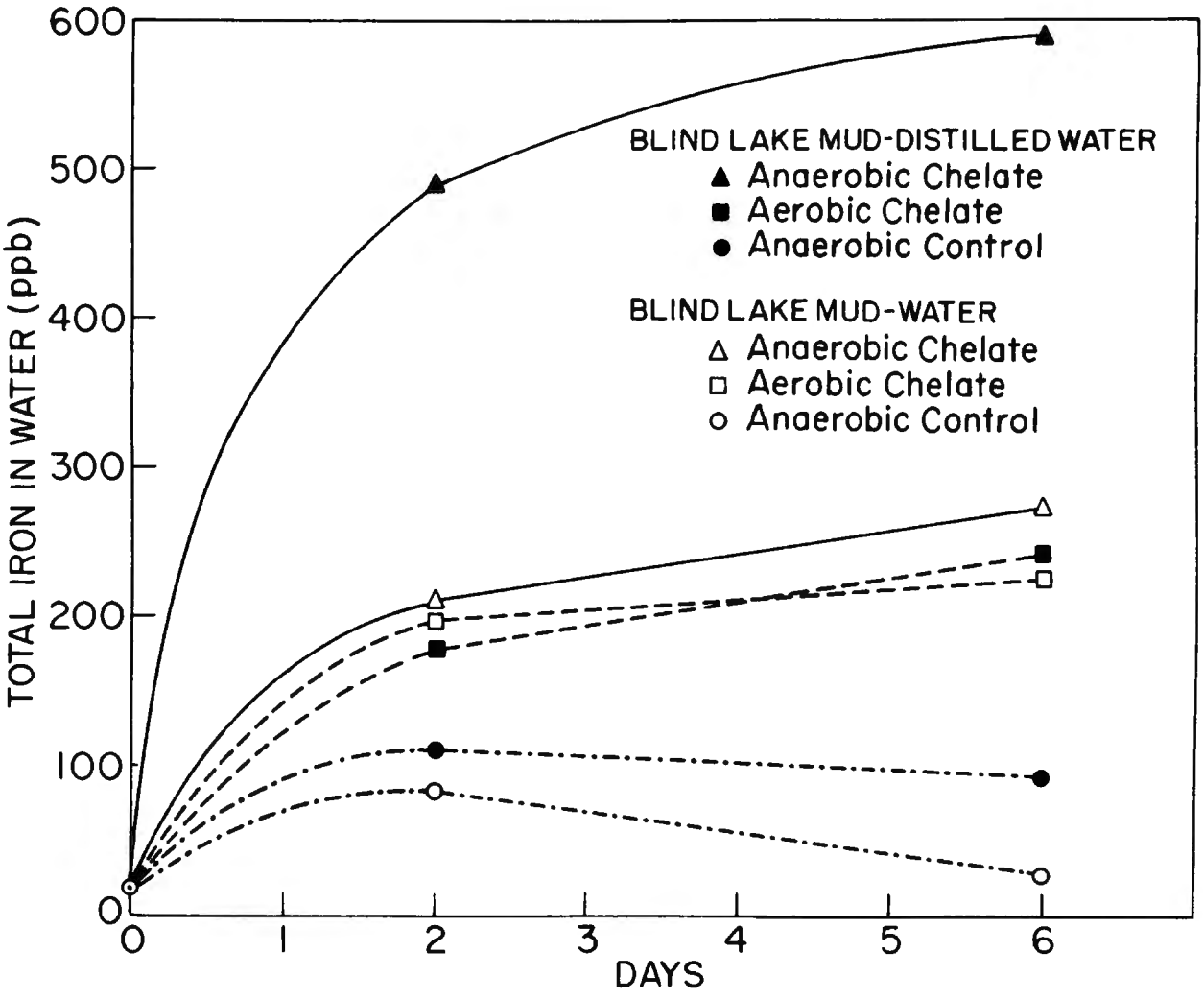


Fig. 6.--Total iron content (ppb) of water in Blind Lake mud-water experiments on days after the addition of 6.3 ppm of HEDTA to experiments designated chelate.

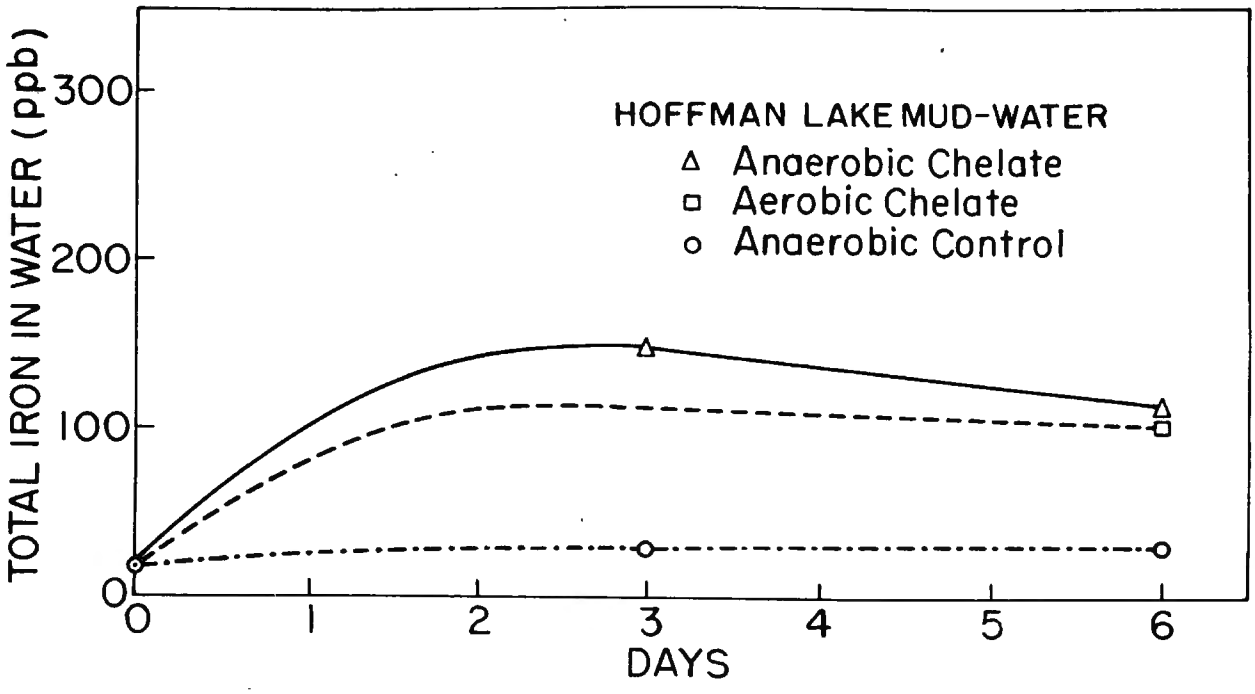


Fig. 7.--Total iron content (ppb) of water in Hoffman Lake mud-water experiments on days after the addition of 6.3 ppm of HEDTA to experiments designated chelate.

of HEDTA was similar when either Blind Lake water or distilled water was placed over Blind Lake mud, although in the anaerobic chelate experiments with distilled water two times more iron was chelated from the mud than in the corresponding jar containing Blind Lake water (Fig. 6).

More iron was chelated from Blind Lake muds than from muds of the other marl lake, Hoffman Lake (Figs. 6 and 7). In no instance did the quantity of iron in the water exceed 150 ppb in the Hoffman Lake experiments (Fig. 7), while only the anaerobic controls of the Blind Lake experiments contained less than 150 ppb (Fig. 6). Although this difference might be explained on the basis of different geological conditions in Washtenaw and Charlevoix counties, it may also be due to the morphometric features of the lakes. In shallow lakes, more suspended iron is carried from the lake basin with outflowing water than in deep lakes (Ruttner 1953). Because Hoffman Lake is shallow (maximum depth of 22 ft), suspended iron is more likely to be removed from the lake basin with outflowing water than in Blind Lake (maximum depth of 80 ft). Consequently, the iron content of Blind Lake sediments would be expected to be greater than that of Hoffman Lake sediments and more iron could be chelated from Blind Lake mud than from Hoffman Lake mud. Hoffman Lake mud was not analyzed for iron, but the iron content of Blind Lake mud was 3.5 per cent of the dry weight. This is not an excessive quantity. Ivlev found that the iron content of Lake Beloye sediments ranged from 4.55 to 13.35 per cent of the dry weight (Hutchinson 1957).

Iron was chelated from the muds of Weber Lake, which has highly organic sediments and soft water (Hooper 1954), at a more constant rate

(Fig. 8) than from Blind Lake (Fig. 6) or Hoffman Lake muds (Fig. 7). The iron concentrations in the water of Weber Lake experiments were increasing on the sixth day after the addition of HEDTA. In the marl lake experiments, the greatest increases in iron concentration occurred within three days after the addition of HEDTA. Because the iron content of the water in the Weber Lake experiments increased at a more constant rate than in the Blind Lake experiments, it is hypothesized that the organic material in the Weber Lake sediments retarded the chemical processes supplying iron to react with HEDTA. Consequently, less iron was available for the reaction with HEDTA in Weber Lake sediments. Mortimer (1942) stated that iron in the oxidized microzone may be in the form of a ferrilignoprotein complex. The fact that an oxidized microzone was not observed in any of the Weber Lake experiments, but was conspicuous in marl lake experiments, is an indication of iron being bound in some manner to the organic sediments of Weber Lake.

In most experiments, the quantity of iron chelated from the muds was not dependent on the quantity of HEDTA in the water. In the first group of experiments, the 10.5 ppm of HEDTA added to each jar could react with 1.67 ppm of iron, and 0.93 ppm of iron was found in the anaerobic chelate experiment (Table 2). Some of this iron may have been released due to the reduction of ferric iron during the 28 days of the experiment. In the second group of experiments, iron was not released into the water by reduction of ferric iron. In these experiments, the 6.3 ppm of HEDTA added to each jar could react with 1.02 ppm of iron and in the anaerobic jar of Blind Lake mud-distilled water approximately 0.60 ppm of iron was found in the water (Fig. 6). Thus no more than 60 per cent of the HEDTA added to the water reacted with iron to form soluble complexes.

The effect of a chelating agent on the exchange of iron between water and mud is evident because iron was released from inorganic sediments by the formation of a soluble complex with HEDTA under aerobic conditions. In lakes, iron is released from the sediments into the water only under reducing or anaerobic conditions (Mortimer 1941 and 1942) and reducing conditions do not occur in "extremely inorganic sediments" (Hutchinson 1957). In order to discuss the processes by which iron was available to react with HEDTA under aerobic conditions, the explanation given by Mortimer and Hutchinson for exchange of iron between water and mud under natural conditions should be considered. An oxidized microzone exists at the surface of the sediment when oxygen is present in the water. Below the oxidized microzone at some depth, sediments are reduced. In the reduced sediments, certain solutes including ferrous iron are present, but the oxidized microzone acts as a barrier to the passage of these solutes from the reduced portion of the sediment to the water. The solid ferric hydroxide of the oxidized microzone is in equilibrium with ionic ferric iron. The ionic ferric iron reacts with HEDTA. This reaction brings about further dissociation of ferric hydroxide and in this way iron is brought into solution in the water phase. Thus in the presence of a chelating agent (HEDTA), iron from an oxidized microzone can be brought into solution.

It was pointed out that the anaerobic release of iron from the muds into the water was negligible and therefore was not a factor to be considered in the second group of experiments (Figs. 6, 7 and 8). However, in these experiments which contained HEDTA, the water in the

anaerobic jars contained more iron than the water in the aerobic jars. An explanation for this is suggested when one considers that the HEDTA reacted with the ionic ferric iron in the oxidized microzone. The amount of iron released from the sediments into the water was governed by the law of mass action. Because the quantity of HEDTA was equal in all experiments, the amount released was dependent on the amount of ferric iron in the ionic form available for complex formation with HEDTA. The quantity of ionic ferric iron was in turn dependent both on the supply of iron and on the pH in the oxidized microzone. If the pH were lowered, greater quantities of iron would be brought into solution. The pH change in the anaerobic experiments apparently was not sufficient to reduce the iron in the oxidized microzone to the soluble ferrous state, but it was sufficient to allow more ionic ferric iron to be brought into equilibrium with the solid phase. This made more iron available to be chelated under anaerobic conditions than under aerobic conditions.

The supply of iron for the oxidized microzone and pH conditions can also be used to explain the greater iron content in the anaerobic chelate experiments of distilled water and Blind Lake mud as compared to the corresponding experiments with Blind Lake water (Fig. 6). The reduction of iron to the soluble ferrous state in the sediments maintained the supply of iron in the oxidized microzone of both experiments. Because the distilled water initially had a lower pH than the Blind Lake water, more ionic ferric iron was present to react with HEDTA in the distilled water and a greater quantity of iron was brought into solution by complex formation.

It can be concluded from these mud-water experiments that iron may be maintained in solution in lake waters by chelating agents.

Although synthetic chelating agents were used in these experiments, other investigators have indicated that naturally-occurring dissolved organic substances can function as chelating agents (Saunders 1957). Probably chelating agents are not present or are present in small quantities in water of marl lakes and their absence may be a factor affecting the availability of iron.

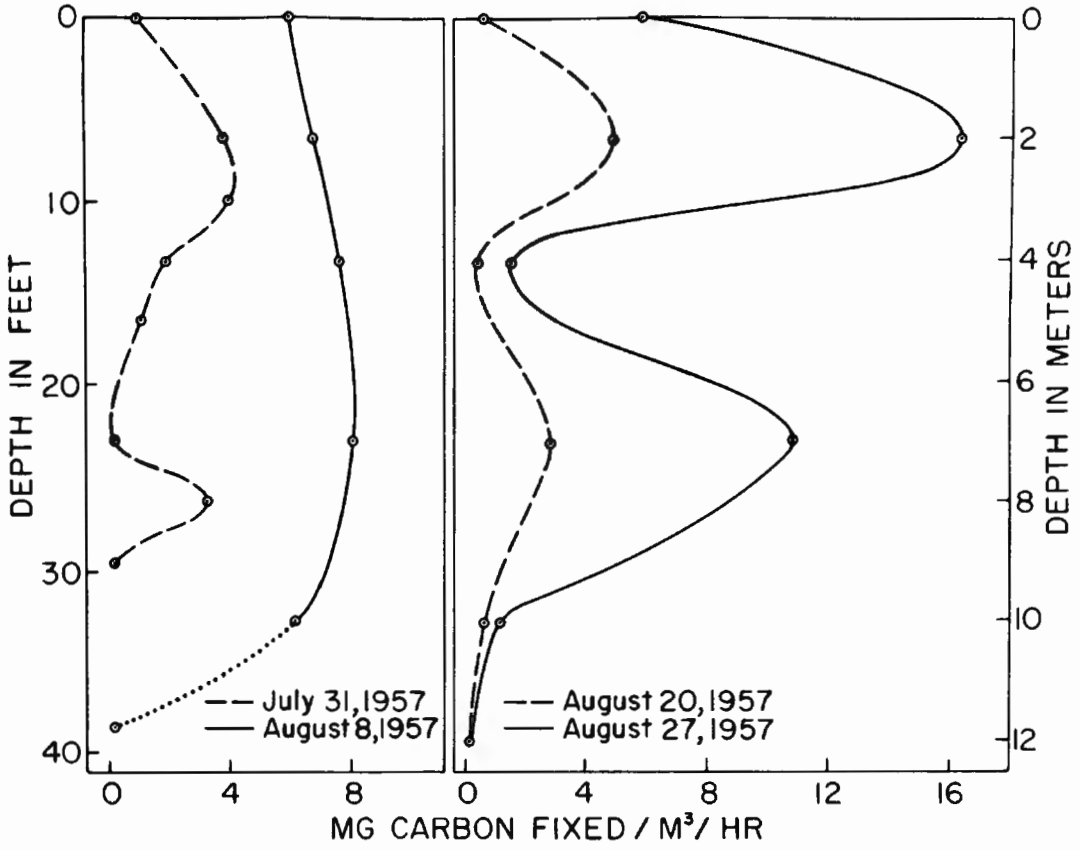
PRIMARY PRODUCTIVITY OF BLIND LAKE

The rate of photosynthetic fixation of carbon-14 was measured at selected depths in Blind Lake. From these measurements, the rate of carbon fixation per unit of water volume (mg C fixed/m^3/hr) was calculated and used to plot the photosynthetic profile for each day of measurement (Fig. 9). The rate of carbon fixation per unit area (mg C fixed/m^2/hr) was determined by planimetric integration of each photosynthetic profile (Table 3).

Table 3.--Rates of carbon fixation on an areal basis in Blind Lake in 1957 and 1958

Dates	Carbon fixed (mg/m^2/hr)	Duration of experiment (hrs)
July 31, 1957	18.3	6.3
August 8, 1957	80.6	7.0
August 20, 1957	22.9	7.5
August 27, 1957	81.3	8.0
September 6, 1957	71.4	6.3
June 26, 1958	50.1	6.5
July 31, 1958	35.0	6.0

The rates of carbon fixation per unit area ranged from 18.3 to 81.3 (Table 3) and had a mean and standard deviation of 51.4 ± 26.8 mg C/m^2/hr. There was considerable day to day variation in the amount of carbon fixed. The differences between duplicate measurements on each day were much too small to account for this variation. The variation was therefore attributed to day to day changes in phytoplankton populations and environmental conditions. Rodhe et al. (1960) stated



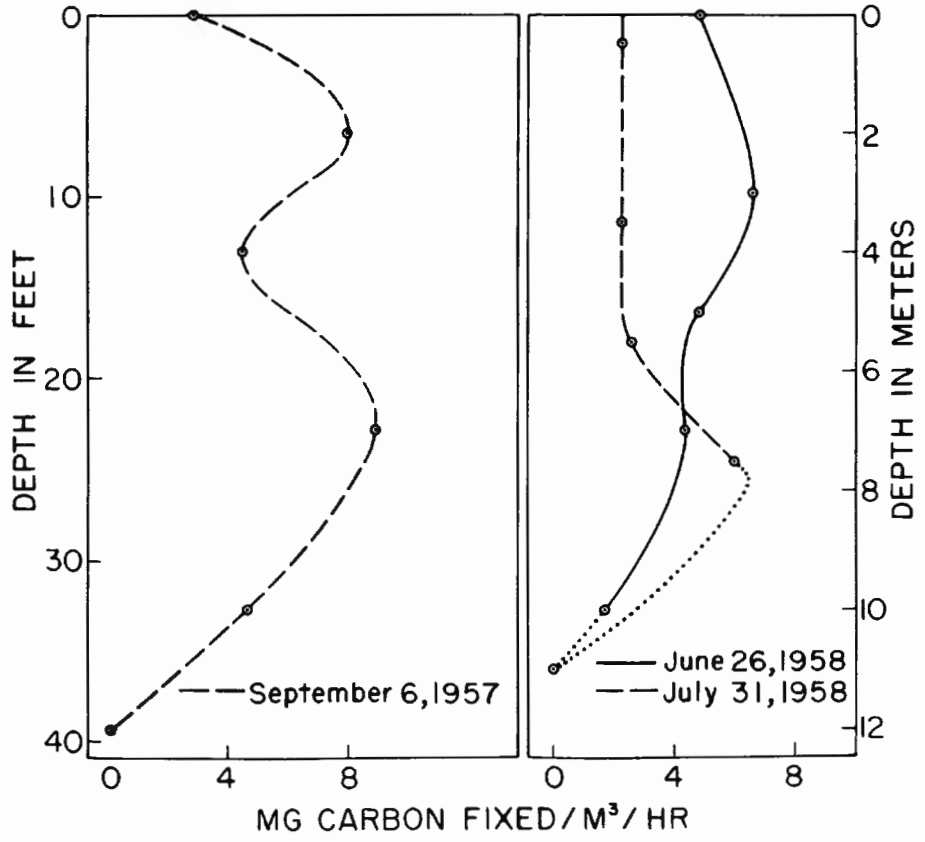


Fig. 9.--Seven vertical profiles of the rate of carbon fixation (mg $C/m^3/hr$) in Blind Lake in 1957 and 1958.

that variations from one day to the next in the rate of carbon-14 assimilation "were often as great as ± 100 per cent, in some cases even between ± 200 and ± 300 per cent."

The results of photosynthetic measurements in Blind Lake indicate that primary productivity was not great. In order to compare these results with those of other workers, they must be converted to the quantity of dry weight of organic matter produced per unit area per day. Assuming that production took place 12 hrs a day and that carbon made up 50 per cent of the dry weight of the organic matter produced, then the average primary productivity in Blind Lake was 1.2 g dry weight/m^2/day. This value has a standard deviation of approximately ± 50 per cent and represents production during the summer when rates would probably be higher than the yearly average. For these comparisons, it is assumed that net productivity was measured (Ryther 1959), although the author agrees that primary productivity measured by the carbon-14 method probably is intermediate between gross and net productivity (Strickland 1960). Therefore, it would seem that the average net primary productivity in Blind Lake on an annual basis would be less than 1.0 g dry weight/m^2/day. In the Sargasso Sea, a portion of the sea considered to be relatively unproductive, Ryther (1959) reported an average net primary productivity of 0.40 g dry weight/m^2/day. Odum (1959) listed an average net primary productivity of less than 0.8 g dry weight/m^2/day for deep lakes (usually considered as being oligotrophic) and for open oceans. Net primary productivity may be greater in other aquatic environments. Odum gave 15 g dry weight/m^2/day as a maximum value for estuaries and

coral reefs. Thus it is concluded on the basis of these comparisons that primary productivity in Blind Lake was low, but in accord with existing data from similar trophic environments.

An interesting characteristic of the Blind Lake photosynthetic profiles was the high photosynthetic rate in the metalimnion. Profiles for all seven dates, except June 26, 1958, had higher rates of photosynthetic activity in the metalimnion at 7 and 8 m than at depths sampled immediately above or below (Fig. 9). In three of the seven profiles, the maximum rate was at 7 or 8 m. As a result of the high photosynthetic activity, there was an oxygen maximum at these depths in 1957, 1958 and 1959. Eberly (1959) attributed the development of a metalimnetic oxygen maximum in a lake to: (1) transparent water, (2) stable thermal stratification and (3) optimal conditions for an alga in the metalimnion. The first two conditions are necessary for the existence of the metalimnetic oxygen maximum, the third may not be necessary because only a net gain in oxygen concentration is needed. In Blind Lake, the development of the metalimnetic oxygen maximum was first noticeable in 1959 during June (Fig. 4), although Blind Lake was thermally stratified in April (Fig. 3). This indicates that the conditions necessary for the development of the metalimnetic oxygen maximum were not established rapidly and suggests that the net gain in oxygen concentration was not rapid.

EXPERIMENTS ON NUTRIENTS LIMITING PRIMARY PRODUCTIVITY

To determine in general the nutrient conditions necessary to stimulate primary productivity in marl lakes, preliminary experiments were conducted in the laboratory. Nutrients were added to 125-ml Erlenmeyer flasks and to 3-gal aquaria containing water and phytoplankton collected at the surface of the lake. These containers were covered to exclude dust and were placed under continuous illumination. Results were evaluated by observing the amount of algal growth in the containers. Within two weeks, differences between certain experiments could be easily distinguished.

Chu 10 nutrients added to water from three marl lakes (Blind, Hoffman and Titus) had no observable effect on algal growth. However, an algal response was evident when either NaFeEEDTA (chelated iron) or ferric citrate-citric acid was added to these lake waters containing Chu 10 nutrients; the greatest and most rapid growth occurred in the presence of NaFeEEDTA. The chelating agents, HEDTA and EDTA, which contained no iron had little or no effect on algal growth. Water from Blind and Hoffman lakes to which Chu 10 nutrients and HEDTA had been added showed little algal growth in comparison to that in the corresponding experiments containing NaFeEEDTA; no algal growth was evident in water containing Chu 10 nutrients and EDTA. These results all pointed to one fact--algal growth was much greater in the presence of Chu 10 nutrients and a complex form of iron than under any other nutrient conditions.

Iron was not a factor limiting algal growth in preliminary nutrient experiments with water and phytoplankton populations from Whitmore Lake.

There was no observable difference between algal growth in the water containing either Chu 10 nutrients or Chu 10 nutrients and NaFeEEDTA. These results suggest that iron was present in Whitmore Lake water and was available to phytoplankton.

Evaluation of Responses to Nutrients

On the basis of the above results, an investigation was undertaken to study nutrients limiting the primary productivity of the surface waters of Blind Lake under the light and temperature conditions of the lake. The nutrients were added to lake water in bottles. The responses of algal populations in the lake water to the nutrients were evaluated from the rate of photosynthetic uptake of carbon-14. The problem of how and when to measure these responses was resolved by considering the rate of carbon-14 uptake in nutrient experiments at different times.

The rate of carbon-14 uptake in experiments containing Chu 10 nutrients and NaFeEEDTA (iron chelate) increased progressively with the passage of time in four-day experiments, indicating a time lag in the responses of phytoplankton populations to the added nutrients (Figs. 10 and 11). From the fourth to the seventh day after the addition of Chu 10 nutrients and NaFeEEDTA, the rate of uptake appeared to increase at a constant rate (Fig. 12). Because the maximum responses did not occur until the time lag was passed, no responses were measured until at least six days after the addition of nutrients. Responses were measured on one day only by determining the ratios of the rates of gross carbon-14 uptake of algal populations in the bottles containing nutrients to the rate of gross uptake in the untreated bottles (the control). For example, on day 4 (Fig. 11), the rate of gross uptake was 224 cpm/hr for

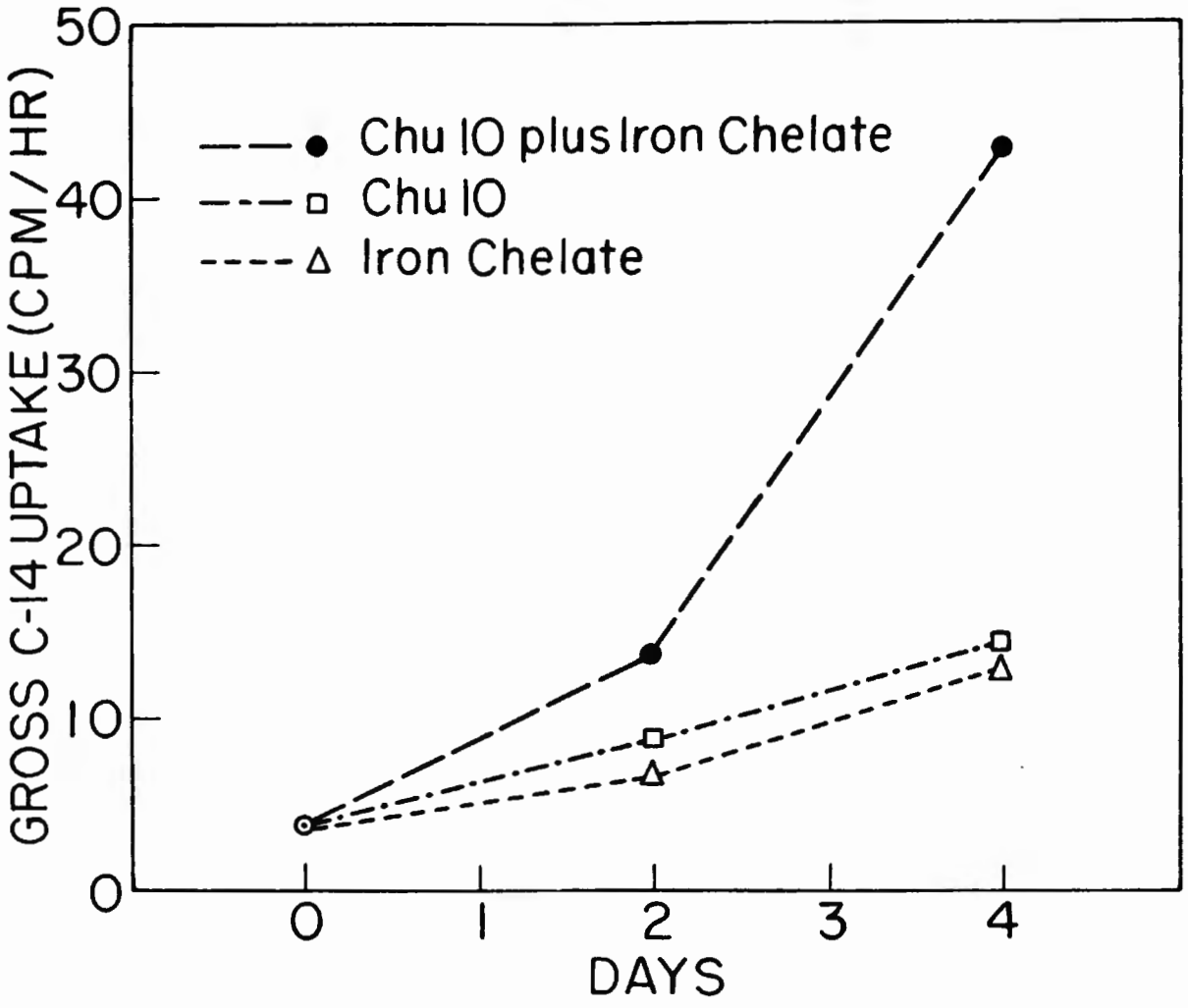


Fig. 10.--Changes in the rate of gross carbon-14 uptake with time (days) in bottles containing Blind Lake water to which nutrients were added, July 1 to 5, 1958. Iron chelate is NaFeEEDTA.

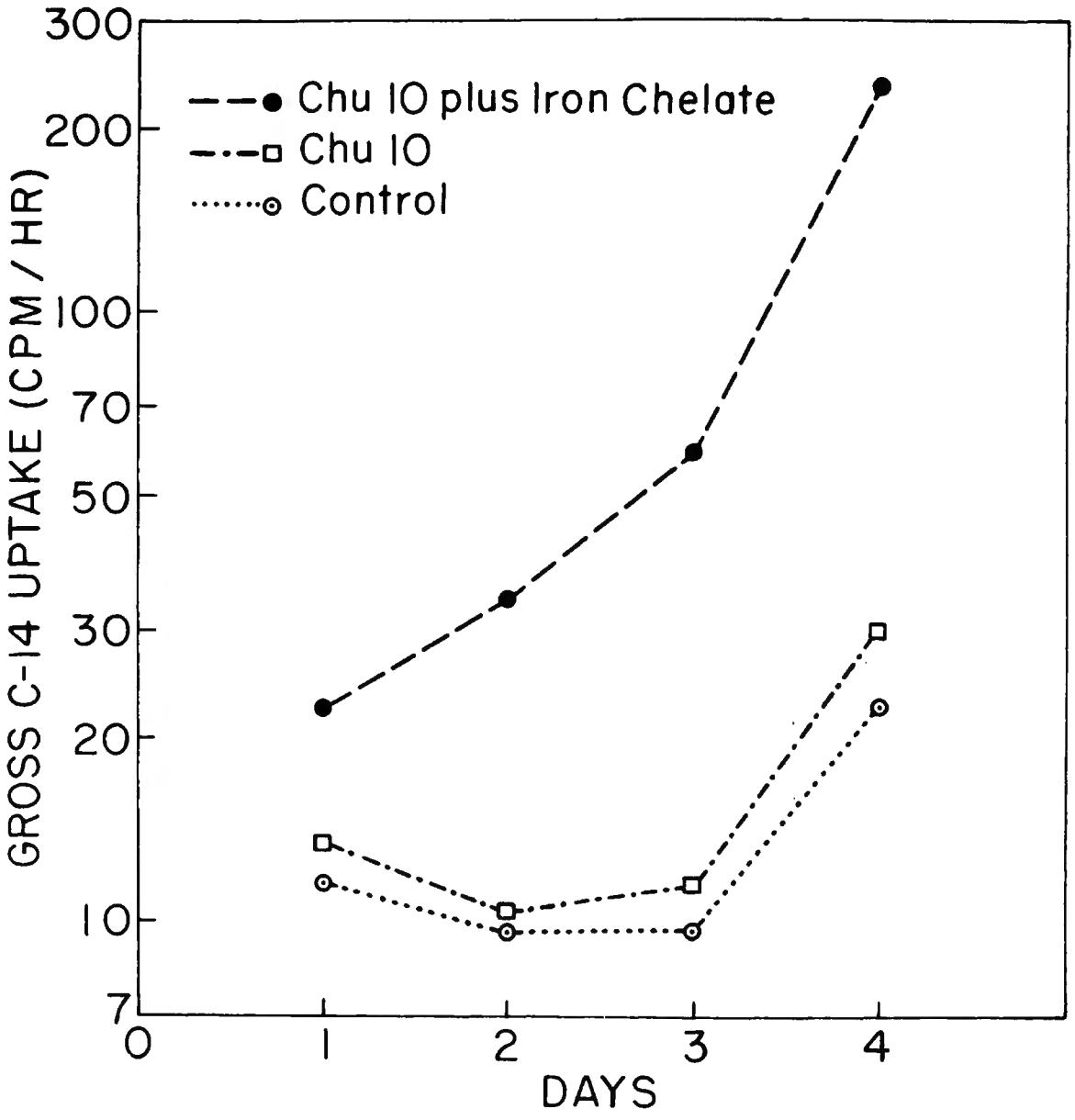


Fig. 11.--Changes in the rate of gross carbon-14 uptake with time (days) in bottles containing Blind Lake water to which nutrients were added, August 4 to 8, 1958. Iron chelate is NaFeEEDTA.

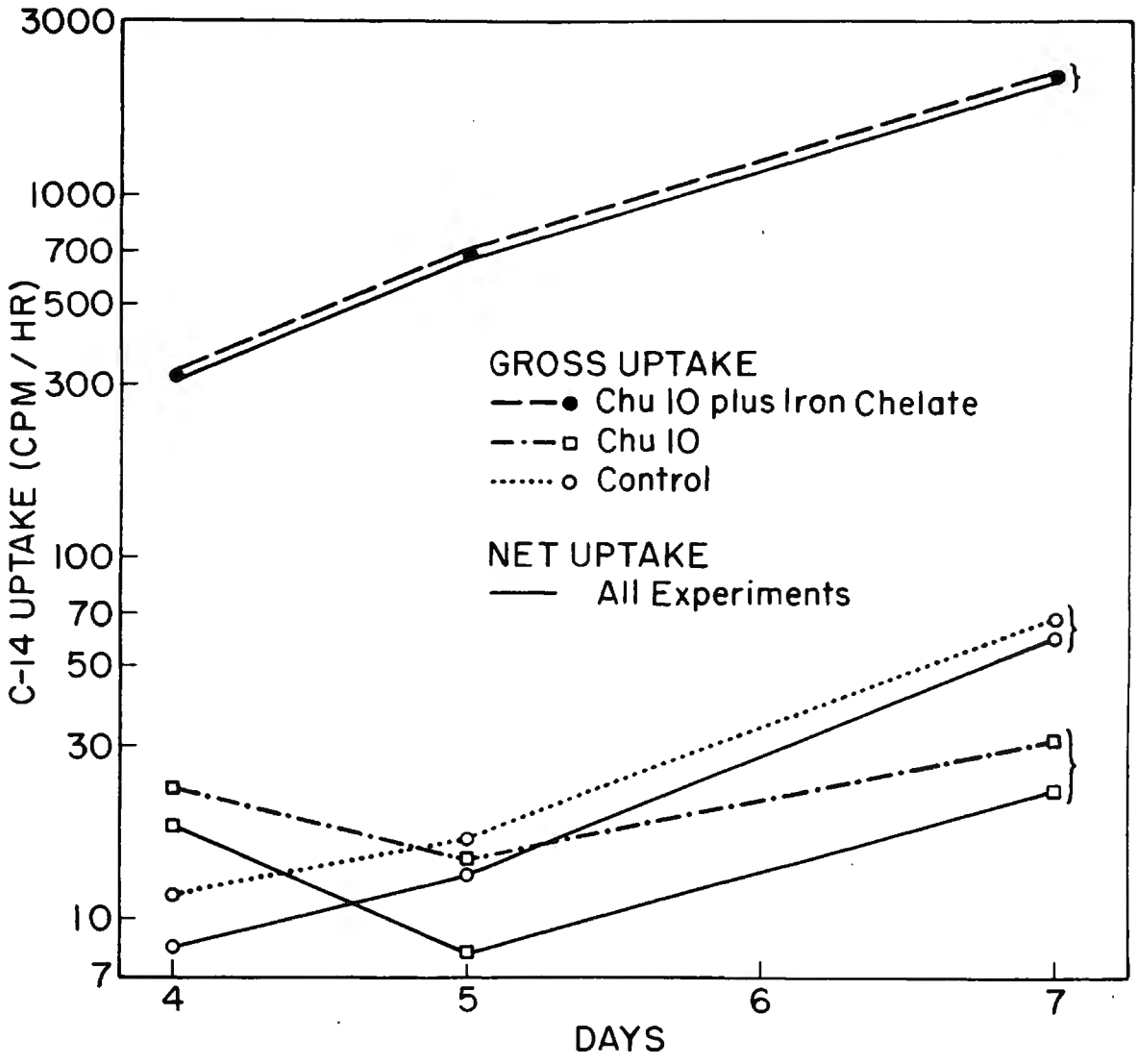


Fig. 12.--Changes in the rates of gross and net carbon-14 uptake with time (days) in bottles containing Blind Lake water to which nutrients were added, August 18 to 25, 1958. Iron chelate is NaFeEEDTA.

Chu 10 nutrients and NaFeEEDTA (iron chelate) and was 17.9 cpm/hr for the control. The ratio of the rates of uptake of the experimental to the control was 12.5 (224 divided by 17.9), indicating that the rate of uptake in the presence of Chu 10 nutrients and NaFeEEDTA was increased 12.5 times over the control rate. This ratio for the August 4 to 8 experiments, as well as ratios for the final day of other experiments in Figs. 10, 11 and 12, is plotted as part of the 1958 nutrient experiments (Fig. 13).

The rate of gross uptake (uptake in "light" bottles) rather than the rate of net uptake was used to measure responses for two reasons: (1) gross uptake indicated the responses of algal populations to nutrients as well as or better than net uptake (Fig. 12) and (2) it minimized the number of bottles used since "light" bottles but no "dark" bottles were needed. The rate of gross uptake tends to minimize responses more than the rate of net uptake because the greatest differences between gross and net rates of uptake were at the lowest rates (Fig. 12) and in general these occurred in the controls. Because responses were evaluated by dividing by the control rate, the ratios of the rates of gross uptake were smaller than ratios of the net rates of uptake.

The Effect of Chelates

The results of the preliminary nutrient experiments in the laboratory suggested that chelating agents were needed to increase algal growth in marl lake waters. Therefore, the effect of NaFeEEDTA and HEDTA on the primary productivity of the surface waters of Blind Lake was investigated under various nutrient conditions in bottles.

The greatest responses of phytoplankton populations to nutrients occurred in experiments in which NaFeEEDTA was added in combination with

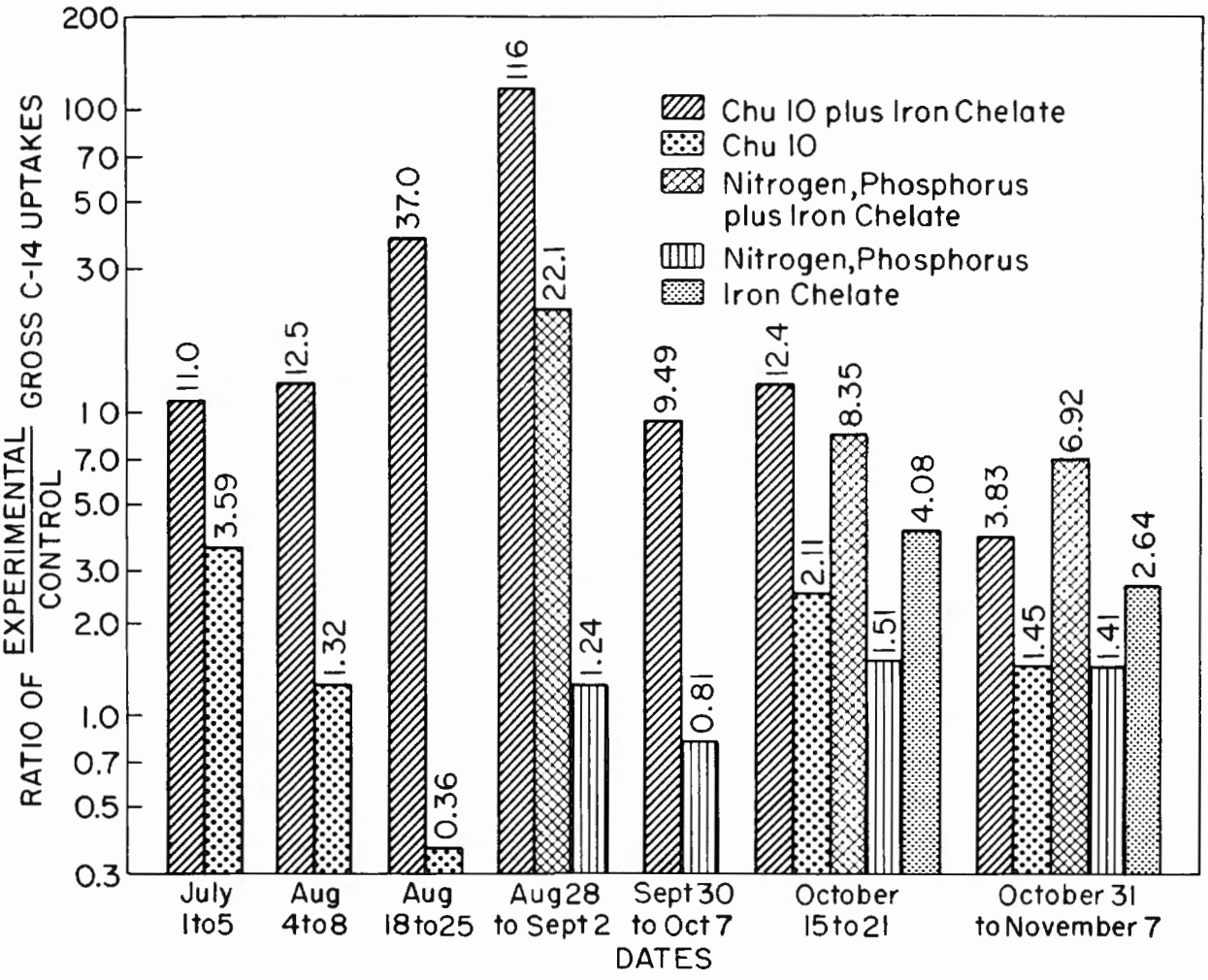


Fig. 13.--Responses (ratios of the rates of gross carbon-14 uptake of nutrient experiments to controls) of Blind Lake phytoplankton populations to nutrients added to Blind Lake water in bottles in 1958. Iron chelate is NaFeEEDTA.

Chu 10 nutrients or nitrogen and phosphorus. In 1958, the combination of Chu 10 nutrients and NaFeEEDTA gave the greatest responses on each date, except for the experiments conducted on October 31 in which the greatest response was with nitrogen, phosphorus and NaFeEEDTA (Fig. 13). The greatest responses in 1959 were found in experiments in which NaFeEEDTA was added in combination with either Chu 10 nutrients or nitrogen and phosphorus (Fig. 14). In 1958, the greatest response to NaFeEEDTA and Chu 10 nutrients increased the gross rate of carbon-14 uptake 116 times over the control rate (Fig. 13). In 1959, the gross rate of carbon-14 uptake was increased at least 40 times the rate of the control in the presence of NaFeEEDTA and either Chu 10 nutrients or nitrogen and phosphorus (Fig. 14).

The responses to HEDTA added in combination with either Chu 10 nutrients or nitrogen and phosphorus were greater than the responses in any experiments except those containing NaFeEEDTA and either Chu 10 nutrients or nitrogen and phosphorus (Fig. 14). The combination of HEDTA, nitrogen and phosphorus gave greater responses than HEDTA and Chu 10 nutrients except for experiments on June 19 and August 13. The addition of HEDTA, nitrogen and phosphorus increased the rate of carbon-14 uptake at least from 10 to 30 times that of the control rate. In only one experiment did the addition of HEDTA and Chu 10 nutrients increase the rate of carbon-14 uptake more than four times the control rate. The presence of HEDTA produced the greater responses in water containing nitrogen and phosphorus, presumably because smaller quantities of ions were present which made HEDTA more effective in supplying nutrients to phytoplankton.

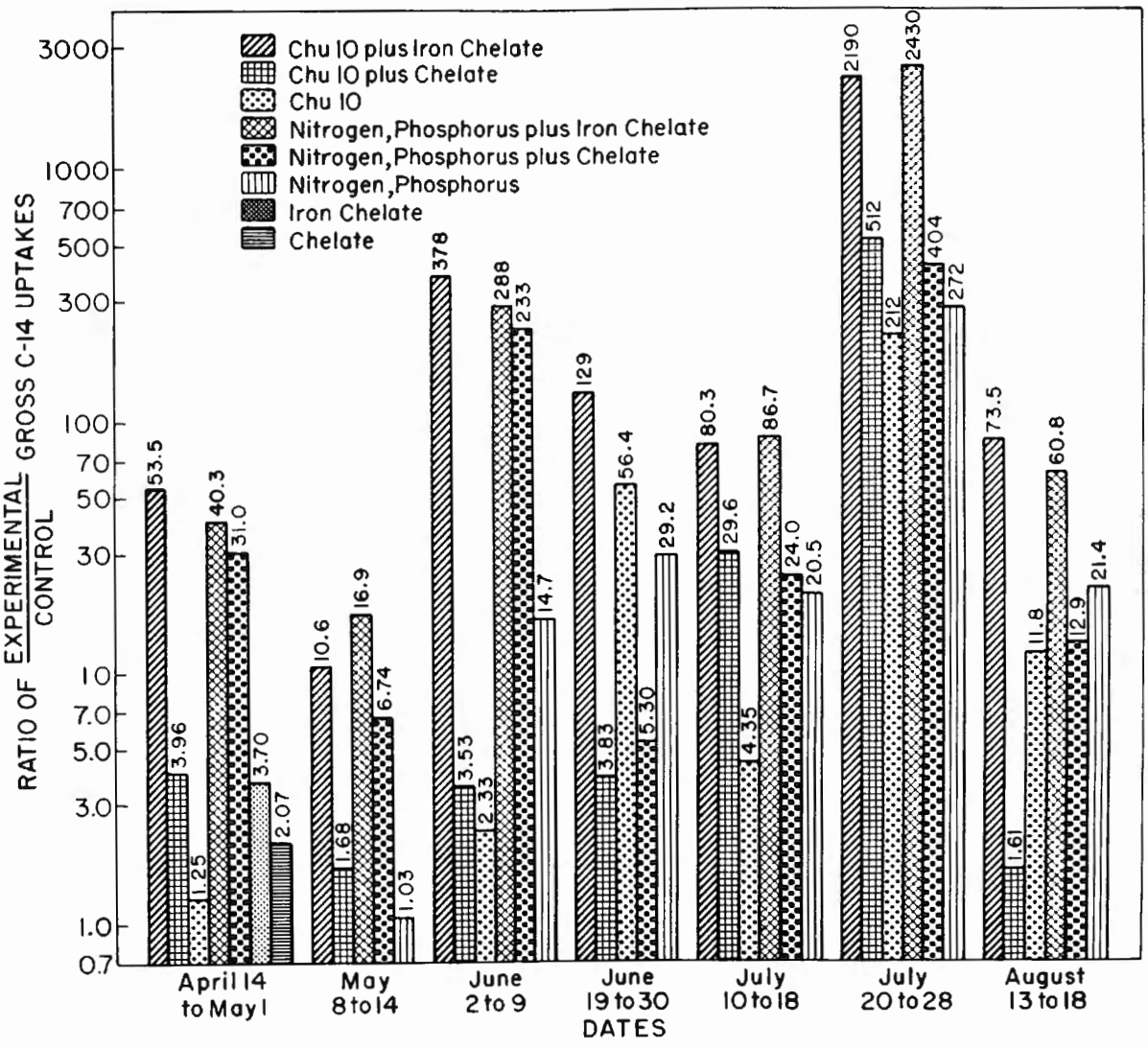


Fig. 14.--Responses (ratios of the rates of gross carbon-14 uptake of nutrient experiments to controls) of Blind Lake phytoplankton populations to nutrients added to Blind Lake water in bottles in 1959. Iron chelate is NaFeEEDTA and chelate is HEDTA.

The responses to Chu 10 nutrients or nitrogen and phosphorus were not great unless NaFeEEDTA or HEDTA were also present. In most experiments, the addition of Chu 10 nutrients increased the gross rate of uptake less than four times the control rate and in some cases did not increase the rate of uptake significantly over the control rate (Figs. 13 and 14). In a limited number of experiments, the presence of either HEDTA or NaFeEEDTA alone gave greater responses than the addition of Chu 10 nutrients alone. The gross rate of uptake was increased from 2 to 4 times over the control rate on the addition to bottles of NaFeEEDTA or HEDTA alone.

Some of the irregularities in the 1959 data (Fig. 14) can be explained. Because the series of experiments on June 19 and August 13 had been disturbed at Blind Lake, either accidentally or maliciously, the results are questionable. All of the July 20 experiments indicate higher ratios than were found in similar experiments on other dates. The gross rate of carbon-14 uptake for the control on July 20 was 3.9 cpm/hr (the lowest rate observed in any control) while the rate for the preceding date (July 10) was 26.2 cpm/hr or nearly seven times greater than the July 20 rate. If the July 20 ratios are divided by seven, they are similar in magnitude to those on other dates. Thus these high ratios are due, at least in part, to an exceptionally low rate of uptake in the control bottles.

The Effect of Chelate Concentration

Because it was demonstrated in the preceding section that the presence of chelates, especially NaFeEEDTA, greatly increased the rate of carbon-14 uptake, it seemed desirable to determine how the concentration

of chelate affected the responses. These experiments differed from those of the preceding section only in that the lake water in all bottles except the controls contained Chu 10 nutrients and in that different concentrations of chelates were added to the bottles containing Chu 10 nutrients.

The concentrations of NaFeEEDTA as ppm of iron which were used in these experiments ranged from 0.0020 to 20.0 ppm (Tables 4-7). In the single series of experiments in 1958, the rate of uptake did not differ from the control rate when 0.010 ppm was used. However, in the first series of experiments in 1959, the same concentration (0.010 ppm) gave a significant response in relation to the control. In comparing the experiments on these two dates (Tables 4 and 5), it can be seen that at least ten times more iron was required to obtain responses in 1958 than was required in 1959. These results may be related to the differences in iron concentrations of Blind Lake waters during the two years. In April 1959, 50 ppb of iron was present in the surface waters of Blind Lake (Fig. 5), but iron was not detectable in October 1958. The fact that the 1959 experiments were incubated for 17 days while those in 1958 were incubated only 7 days may also account for the differences in responses. In longer experiments, nutrients may be utilized at lower concentrations than in shorter experiments.

NaFeEEDTA was effective in producing responses over a wide range of concentrations (Tables 4-7). The lowest concentration was 0.010 ppm (Table 5) and the highest was 5.0 ppm (Table 4). A concentration of more than 0.020 ppm NaFeEEDTA was needed in experiments on only one date to produce a response greater than the control (Table 4). In one experiment at 20 ppm the rate of uptake was apparently inhibited (Table 6).

Table 4.--The effect of NaFeEEDTA concentrations on the gross rate of carbon-14 uptake (cpm/hr) in Blind Lake water to which Chu 10 nutrients were added, September 30 to October 7, 1958 (6.0 hr experiments)

NaFeEEDTA concentration (as ppm iron)	Time for 1280 counts (min)					Average gross uptake (cpm/hr)	Response relative to control
	Bottle 1 subsamples		Bottle 2 subsamples		Average		
	A	B	A	B			
0.0	19.69	17.63	19.56	17.90	18.70	9.5	0.81
0.010	14.48	14.50	18.93	17.77	16.42	11.3	0.96
0.10	3.73	3.82	4.74	4.70	4.25	53.3	4.52
1.0	2.69	2.60	3.14	2.90	2.83	81.8	6.93
2.0	1.79	1.71	2.51	2.36	2.09	112.0	9.49
5.0	1.78	1.81	2.13	2.01	1.93	125.0	10.6
Control	14.85	14.05	--	--	14.45	11.8	--

Table 5.--The effect of NaFeEEDTA concentrations on the gross rate of carbon-14 uptake (cpm/hr) in Blind Lake water to which Chu 10 nutrients were added, April 14 to May 1, 1959 (4.0 hr experiments)

NaFeEEDTA concentration (as ppm iron)	Time for 5120 counts (min)					Average gross uptake (cpm/hr)	Response relative to control
	Bottle 1 subsamples		Bottle 2 subsamples		Average		
	A	B	A	B			
0.0[1]	6.60	6.76	7.04	7.88	7.08	199	1.25
0.010[2]	0.20	0.20	0.22	0.20	0.20	7227	45.5
0.10	0.30	0.30	0.18	0.19	0.24	6021	37.9
1.0	0.20	0.20	0.17	0.16	0.18	8030	50.6
2.0	0.15	0.14	0.19	0.19	0.17	8503	53.5
Control[1]	12.40	12.60	3.04	3.32	7.84	159	53.5

[1] 1280 counts for samples

[2] 2560 counts for samples

Table 6.--The effect of NaFeEEDTA concentrations on the gross rate of carbon-14 uptake (cpm/hr) in Blind Lake water to which Chu 10 nutrients were added, May 8 to 14, 1959 (5.0 hr experiments)

NaFeEEDTA concentration (as ppm iron)	Time for 1280 counts (min)						Average	Average gross uptake (cpm/hr)	Response relative to control
	Bottle 1 subsamples		Bottle 2 subsamples		Bottle 3 subsamples				
	A	B	A	B	A	B			
0.0020	14.68	15.64	3.96	3.83	--	--	9.53	26.3	1.33
0.020	2.97	2.78	2.96	4.84	4.49	5.14	3.86	70.9	3.60
0.20	1.48	1.47	1.90	1.95	1.43	1.54	1.67	169.2	8.59
2.0[1]	1.24	1.35	1.52	1.32	--	--	1.36	208.6	10.6
20.0	7.52	13.03	--	--	--	--	10.28	24.1	1.22
Control	10.23	18.83	6.70	8.23	--	--	11.00	19.7	--

[1] 2560 counts for samples

Table 7.--The effect of NaFeEEDTA concentrations on the gross rate of carbon-14 uptake (cpm/hr) in Blind Lake water to which Chu 10 nutrients were added, June 2 to 9, 1959 (4.0 hr experiment)

NaFeEEDTA concentration (as ppm iron)	Time for 1280 counts (min)						Average	Average gross uptake (cpm/hr)	Response relative to control
	Bottle 1 subsamples		Bottle 2 subsamples		Bottle 3 subsamples				
	A	B	A	B	A	B			
0.0	16.52	15.28	9.24	8.38	14.41	14.05	12.98	22.8	2.33
0.020	4.53	3.06	--	--	--	--	3.91	87.5	8.93
0.20[1]	0.35	0.30	0.31	0.56	--	--	0.62	1161.0	118.00
2.0[2]	0.09	0.08	0.10	0.10	0.11	0.11	0.10	3703.0	378.00
Control	24.30	25.24	19.37	20.65	--	--	22.39	9.8	--

[1] 2560 counts for samples

[2] 5120 counts for samples

With the exception of the series of experiments started on April 14, 1959 (Table 5), the effect of NaFeEEDTA on carbon-14 uptake showed an increase with concentration. On two dates (Tables 4 and 6), the data fitted a straight line when the gross rate of uptake was plotted against the log of NaFeEEDTA concentration. As was pointed out above, the fact that the April 1959 experiment extended over a period of 17 days may have affected the results (Table 5).

Because NaFeEEDTA was effective in stimulating carbon-14 uptake over a wide range of concentrations, one series of experiments was conducted in which HEDTA concentration was varied in water to which Chu 10 nutrients had been added. HEDTA concentrations ranged from 0.0020 to 2.0 ppm and the rate of carbon-14 uptake increased with HEDTA concentration (Table 8). The two lower HEDTA concentrations showed rates of uptake lower than the control. Because there is no reason to expect an inhibition of carbon-14 uptake at the lower HEDTA concentrations, these results are believed to be due to: (1) a high control rate or (2) an inhibition of carbon-14 uptake by Chu 10 nutrients which was observed in the 1958 experiments (Fig. 13). At the lower HEDTA concentrations, the small effect due to HEDTA was not sufficient to overcome the inhibitory effect. It is concluded that the rate of carbon-14 uptake increased with increasing HEDTA concentration within the limits used in these experiments.

The effects of NaFeEEDTA and HEDTA in stimulating the rate of carbon-14 uptake cannot be strictly compared since HEDTA was used over a limited range of concentrations. The highest concentration of HEDTA which was used was equivalent to 0.32 ppm of iron and the next highest was 0.032 ppm (Table 8). However, in experiments

Table 8.--The effect of HEDTA concentrations on the gross rate of carbon-14 uptake (cpm/hr) in Blind Lake water to which Chu 10 nutrients had been added, May 8 to 14, 1959 (6.0 hr experiment)

HEDTA concentration (ppm)[1]	Iron equivalents of HEDTA (ppm)	Time for 1280 counts (min)					Average gross uptake (cpm/hr)	Response relative to control
		Bottle 1 subsamples		Bottle 2 subsamples		Average		
		A	B	A	B			
0.0020	0.00032	16.08	12.66	12.85	10.58	13.04	12.9	0.65
0.020	0.0032	9.71	7.40	10.44	13.05	10.14	17.4	0.88
0.20	0.032	7.31	7.68	7.17	5.73	6.97	26.7	1.36
2.0	0.32	6.07	5.08	6.21	5.58	5.74	33.0	1.68
Control	--	10.23	18.83	6.70	8.23	11.00	19.7	--

conducted at the same time, neither of these concentrations stimulated uptake as much as 0.020 ppm NaFeEEDTA (Table 6). It is apparent that an increase in HEDTA concentration increased the rate of carbon-14 uptake much less than an increase in NaFeEEDTA concentration under the same conditions. Because HEDTA could complex iron in the water to form NaFeEEDTA, these results indicate that iron or at least the availability of iron was limiting primary productivity in Blind Lake.

The Effect of Nutrient Concentration

It was pointed out above that Chu 10 nutrients may have slightly inhibited the rate of carbon-14 uptake. The effect of nutrient concentration on the rate of carbon-14 uptake was investigated in a series of Blind Lake experiments (June 19 to 30, 1959). The addition of NaFeEEDTA and one-half the quantities of Chu 10 nutrients ordinarily used increased the gross rate of carbon-14 uptake 225 times that of the control rate while NaFeEEDTA and the usual quantities of Chu 10 nutrients increased

the gross rate of carbon-14 uptake 129 times over the control rate (Fig. 14). The addition of the usual quantities of NaFeEEDTA, nitrogen, phosphorus and silicate increased the gross rate of uptake over 90 times that of the control. These results suggest that nutrients other than iron, phosphorus, nitrogen and silicate were limiting primary productivity in Blind Lake and that the quantities of Chu 10 nutrients may be somewhat inhibitory.

The nutrient concentrations used in experiments of the present study are greater than concentrations normally found in lake waters. However, in an experiment in which chelated iron and commercial fertilizer were added to Titus Lake, Grand Traverse County, there were increases in primary productivity even though the concentrations of iron, nitrogen and phosphorus in the lake were increased only slightly (Schelske, Hooper and Haertl 1960). These increases in primary productivity were comparable to the increases observed in bottles containing Blind Lake water and nutrients (Figs. 13 and 14). In less than 9 days the addition of chelated iron to Titus Lake increased primary productivity approximately four times that of a preceding control period. The addition of commercial fertilizer to Titus Lake, 10 days after the addition of the iron chelate, increased primary productivity approximately 60 times that of the control period. The results of the experiment at Titus Lake show that the high nutrient concentrations used in the bottle experiments at Blind Lake were not needed to increase primary productivity in Titus Lake.

Minor Elements

The addition of minor elements (Chu 1942) to Blind Lake water did not increase the rate of carbon-14 uptake when used in various nutrient experiments. It was assumed that these minor elements were not limiting primary productivity in Blind Lake. Rodhe (1948) found that trace elements ("A-Z solution") had no effect on the growth of Ankistrodesmus falcatus. Goldman (1960b) has reported molybdenum as a factor limiting primary productivity in Castle Lake, California.

Form of Iron

One experiment (July 10 to 18, 1959) at Blind Lake was used to evaluate the effect of inorganic iron on the rate of carbon-14 uptake. Two ppm of iron (Fe) added as a ferric chloride ($FeCl_3$) solution in combination with Chu 10 nutrients increased the rate of carbon-14 uptake over the control rate 48 times. Chu 10 nutrients and NaFeEEDTA (containing 2.0 ppm of iron) increased the rate of carbon-14 uptake over the control rate 80 times. Chu 10 nutrients alone increased the rate of carbon-14 uptake only four times that of the control rate (Fig. 14). Although it is evident that the rate of carbon-14 uptake was stimulated in the presence of inorganic iron, chelated iron (NaFeEEDTA) seemed to be more effective than ferric chloride.

Interpretation of Results

The responses to various nutrient conditions were determined in relation to the rate of carbon-14 uptake of the control (bottles containing only lake water) on each date. Therefore, these responses

cannot be used as absolute quantities. The responses, as evaluated, are relative to the experiments on the same date and should not be compared on an absolute basis with responses on other dates.

In the interpretation of the results of these nutrient experiments, the uncontrollable variables must be taken into account. Some of these are: seasonal and daily changes in water temperature, day length and light intensity, physical and chemical conditions of the water and composition of phytoplankton populations. From the 1958 experiments, it is clear that seasonal changes are a factor to be considered. As the fall season progressed, responses to nitrogen, phosphorus and NaFeEEDTA became larger when compared to the responses observed for Chu 10 nutrients and NaFeEEDTA (Fig. 13). The small responses to nutrient additions in the September and October experiments were probably a result of lower water temperature and less solar radiation in the fall as compared to the summer months. These seasonal influences are interrelated and in the present study their effects were not determined.

A subjective interpretation of differences between responses to nutrients was made in the present study. The great differences in responses and the conclusions made from the results warranted no further analysis of these data. A total of 72 nutrient experiments were performed in duplicate in 1958 and 1959. Two subsamples were taken from the two bottles used in each experiment, making a total of 4 subsamples for each experiment. The range of the rates of uptake in the subsamples was more than a factor of 2 in 14 of the 72 experiments and was more than a factor of 3 in only 7 of the 72 experiments. In the first case, this range in the rates of uptake of subsamples was

no more than ±33 per cent of the average in 58 of the 72 experiments. In the second case, this range in the rates of uptake of subsamples was no more than ±50 per cent of the average in 65 of the 72 samples. Because the responses were evaluated on the basis of the averages, it can be seen that the variation based on the range of subsamples was not great in comparison to the magnitude of differences in responses to nutrients.

DISCUSSION

The methods used to study limiting nutrients in the present study of marl lakes included the measurement of responses of existing phytoplankton populations in natural water to nutrient additions. Lund and Talling (1957) criticized both the direct use of natural populations and the addition of known quantities of nutrients to lake water, because these conditions are most favorable for the development of a restricted flora and the results of experiments under these conditions have included undetermined effects on the growth of bacteria. However, Lund and Talling also stated that the use of pure or bacteria-free cultures is "still in its infancy as regards true plankton algae . . . due to difficulties of culture and not of obtaining bacteria-free cells." Because cultures of plankton algae are difficult to maintain, practicality in itself may justify the addition of nutrients to lake water to study nutrients limiting phytoplankton productivity.

In the study of nutrients limiting primary productivity in natural systems, lake water containing natural phytoplankton populations can be used to better advantage than studies with pure or unialgal cultures. The chief advantage in the use of natural populations is that the "physiological state" has been determined by the complex of factors limiting productivity in the system. These are the same factors that make up the complex of the ecological environment and, since many are poorly understood, if not unknown, they are virtually impossible to reproduce artificially in cultures. Therefore, the advantage of using lake waters and phytoplankton populations is that the effect of known variables (i.e., those varied in experiments) can be studied without

concern for effects of unknown factors in the water or phytoplankton populations which might affect the results if an attempt were made to reproduce these conditions in cultures.

Theoretically, each species of algae has optimum conditions under which it grows best. If these conditions were known exactly, it is possible the use of cultures would prove valuable. At present, however, our knowledge of the nutrition, physiology and succession (ecology) of algae is too limited to use this approach. If this information were available, a great deal of work would be involved because experiments with more than one species would be necessary in a seasonal study because great changes occur in the natural system throughout the year.

When certain trace elements are concentrated in algal cells in quantities many times in excess of the amounts that the cells utilize, the excess nutrients may be "diluted" to limiting quantities by cell division (biological dilution). In the lake system, because of biological dilution, nutrient concentrations in algal cells are reduced over a period of time to limiting quantities if the rate of utilization and loss exceeds the rate of absorption (uptake from the water). In this case, a limiting nutrient is a reflection of not only present, but also previous environmental conditions within the lake. Consequently, cultures must be carried through many generations involving several successive transfers to media deficient in the nutrient being studied to determine whether a nutrient is limiting or not. Rodhe (1948) found that algae, which had previously been cultured in a medium containing iron, could be grown in an iron-deficient medium for six months. Hence, the advantages and importance of using natural phytoplankton populations

in the study of iron and other elements as factors limiting primary productivity cannot be overemphasized.

Even though natural populations were used in the present study, there are differences between the environmental conditions in experimental and natural systems (Table 9). In the bottle system, precipitated nutrients are not lost; in the lake, precipitates settle out of the trophogenic zone and are lost, at least temporarily. If conditions for nutrient regeneration are present in the sediments, nutrients will be mixed into the water mass during periods of overturn. The replenishment of nutrients is rapid in experimental systems due to equilibria existing between the dissolved and solid states (if present). In a lake, these equilibrium reactions are not so rapid, due to greater differences in diffusion distances and volume to area relationships. The water movements in a lake compensate partly, but may not provide efficient nutrient replenishment when a lake is stratified. There are no water movements in the bottle systems to give comparable changes in light or nutrients encountered by algal cells in turbulent lake waters, although movement of the buoy from which bottles were suspended caused a slight agitation.

Due to the lack of mixing in the closed bottle system, metabolic wastes are concentrated and high photosynthetic rates cause a supersaturation of oxygen (Table 9). Metabolic wastes are an important factor because they may stimulate or inhibit algal growth (Lucas 1947, Lefevre 1958, Hartman 1960). High oxygen concentrations may depress the rate of photosynthesis (Hill and Whittingham 1955) and in bottle experiments bubbles of oxygen were observed by the author on several occasions. In the lake, mixing of waters dilutes metabolic wastes and maintains the

Table 9.--A comparison of some environmental conditions of algal populations in bottles with conditions in the natural environment

FACTOR	EXPERIMENTAL	NATURAL
Precipitation of nutrients	Precipitates not lost in this type of system	Precipitates settle out, may be lost from trophogenic layer until periods of overturn
Replenishment of nutrients	Readily replenished by equilibria between dissolved and solid states (if present)	Not as readily replenished because of greater differences in diffusion distances and volume to area relationships
Mixing	Convection currents and slight agitation due to movement of floating buoy	Natural water movements
Metabolites	Concentrated	Diluted
Oxygen concentration	High values in enclosed system; may affect physiological processes adversely	Chance for equilibrium from agitation of surface waters; exchange between metalimnion and surface waters slow
Zooplankton	Samples collected during daylight hours; little chance for inclusion in this system; effect on algal population small	Grazing reduces algal population; may eliminate population under bloom conditions
Surface effect	Possibility of surface effect due to large ratios of surface area to volume in bottles	Characteristic of the lake; insignificant in comparison to that of bottles

dissolved oxygen in the epilimnion in equilibrium with atmospheric oxygen unless periods of high photosynthetic activity are associated with calm weather. In the metalimnion, this is not the case, because this stratum is essentially a closed system bounded by the lake basin, the epilimnion and the hypolimnion. In the bottle system, not only are metabolic wastes concentrated, but so are algal cells and other organisms. In the lake system, mixing of the water and grazing of the phytoplankton by the zooplankton reduce the concentration of algal cells and metabolic wastes. Zooplankton probably do not affect algal populations greatly in these bottle experiments because the water samples are collected from the surface during daylight hours. Water samples collected during daylight hours are not likely to contain zooplankton that undergo vertical migration.

There is always the question of whether or not experimental results are indicative of conditions in nature. The undetermined effect of bacterial activity in long-term bottle experiments has been questioned because of the large surface area to volume ratios in bottles (Lund and Talling 1957). In the field experiment discussed above in which nutrients were added to Titus Lake, the responses to nutrients were similar to those in bottle experiments in the laboratory. Thus it would seem that the results of the nutrient experiments of the present study are an indication of nutrients limiting primary productivity under natural conditions.

Iron as a Limiting Factor

As was pointed out above, aquatic biologists have usually considered phosphorus, nitrogen and possibly potassium, but not iron, as limiting

nutrients. However, in the present study of marl lake waters, additions of either phosphorus and nitrogen or Chu 10 nutrients were relatively ineffective in increasing primary productivity in comparison to additions of iron. When iron was used in combination with either nitrogen and phosphorus or Chu 10 nutrients, the greatest increases in rates of carbon-14 uptake were observed, indicating that iron was the limiting factor[5] to primary productivity in Blind Lake.

Rodhe (1948) concluded that iron would not be limiting to Scenedesmus quadricauda in most fresh-water lakes because of insufficient nitrogen and phosphorus. Rodhe stated that Uspenski had overemphasized the importance of iron as the dominant factor in determining the distribution of algae. There is some indication that the growth of planktonic algae in the open sea is limited by iron (Gran 1933, Ryther and Guillard 1959). In fresh-water lakes, iron has not been considered as a limiting factor (Lund 1950, Gerloff and Skoog 1957).

The question of whether or not iron is limiting stems from the fact that present knowledge of the quantity of iron necessary for growth of algae is insufficient and that chemical analyses of water cannot be used to determine available iron (Pringsheim 1946, Lund 1950, Gerloff and Skoog 1957). Only Rodhe (1948) has reported iron determinations which might be an indication of iron available to phytoplankton. He demonstrated that "reactive iron" determined by o-phenanthroline was a measure of iron available to phytoplankton, but judged the biological determination of assimilable iron with iron-starved cultures "more sensible than the phenanthroline analysis."

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[5] Limiting factor is used as defined by Clarke (1954)

The form of iron when added in large quantities was not a factor of major importance in glass-contained experiments. Similar results were obtained with 2.0 ppm of iron when ferric citrate-citric acid, NaFeEEDTA or ferric chloride was used. The latter was used in only one experiment. At lower concentrations, different results would have been expected with ferric chloride due to its limited solubility. The yield of algae was approximately the same when ferric citrate was used at iron concentrations ranging from 0.03 to 1.12 ppm, but the yield decreased with the same concentrations when ferric chloride was used as an iron source (Gerloff et al. 1950). Harvey (1937) stated that marine diatoms could utilize colloidal and larger particles of ferric hydroxide and ferric phosphate. Some marine diatoms can utilize only ferric hydroxide (Goldberg 1952).

Chelated iron used in the Blind Lake experiments stimulated photosynthetic activity in concentrations as low as 10 ppb when used with Chu 10 nutrients. Concentrations of phosphorus and nitrogen in these experiments were greater than those of lake waters. Rodhe (1948) found that the concentration of iron required by Scenedesmus quadricauda was dependent on the amount of phosphorus and nitrogen present. He stated that 0.5 ppb of iron was sufficient to stimulate growth of iron-starved cultures of Scenedesmus and that approximately 1.0 ppb of assimilable iron was needed by Scenedesmus for the nitrogen and phosphorus concentrations found in most lake waters. In the marl lake studies, the concentration of iron that was required probably would have been less if smaller quantities of nutrients had been added. However, these results indicate that the iron in NaFeEEDTA was in a form which was easily

mobilized because the concentrations of nitrogen and phosphorus were at least 100 times greater than those used by Rodhe.

Spencer (1957) suggested that algae mobilize ferric iron. If the phytoplankton in the experiments at Blind Lake mobilized the ferric iron in equilibrium with the NaFeEEDTA complex, the amount of iron available to the phytoplankton was many times less than the amount of iron in the iron chelate. The exact quantity of iron in equilibrium is not easily obtained since there is a possibility that many different equilibria are set up between the organic complex and cations in the water. Spencer (1958) has worked out equilibria occurring between EDTA and various cations in sea water.

Significance of Organic Material

Because ionic iron is practically insoluble in well-oxygenated, alkaline lake waters, iron if present, must be in other than the ionic form (Hutchinson 1957). This implies that iron may be present as an organic complex. That such complexes occur has been recognized by investigators of the nutrition of terrestrial plants and plankton algae (Burk et al. 1931, Gran 1933, Harvey 1937, Pringsheim 1946, de Kock 1955).

It is known that dissolved organic matter is present in fresh-waters (Birge and Juday 1934, Valentyne 1957, Fogg 1959) and the function of some of these organic materials has been investigated. Soluble organic matter isolated from the sea and from fresh-water increased growth of algae (Johnston 1955, Shapiro 1957). Accessory growth factors are needed by marine diatoms (Allen 1914, Harvey 1939). A polypeptide liberated by Anabaena has been shown to have some of the

functions of a chelating agent (Fogg and Westlake 1955). These are the formation of a soluble complex with cupric, zinc and ferric ions, the formation of a soluble complex with phosphate and the reduction of toxicity of copper sulfate. Domogalla et al. (1925) reported peptide concentrations in Lake Mendota that are high enough to be considered important in complex formation. Waris (1953) stated that chelating agents play an important role in determining the "nutritional value of waters." Thus naturally occurring organic substances can complex iron, can stimulate the growth of phytoplankton and may be present in some lake waters in quantities great enough for these functions.

A synthetic chelator complex, NaFeEEDTA, supplied iron to phytoplankton in an available form in experiments of the present study. There is evidence that substances in nature may function similarily. Harvey (1937) and Gran (1933) used an iron complex of a lignoprotein to grow marine diatoms. The function of soil extract in culture media is to form soluble complexes with iron (Pringsheim 1946). Yellow organic acids isolated from lake waters with chromatographic techniques formed soluble complexes with iron and stimulated the growth of three unialgal bacteria-free cultures (Shapiro 1957). Hutchinson (1957) has indicated that in some cases iron may be present as a ferric hydroxide sol protected by organic matter, but stated that iron in lakes certainly exists in the form of organic complexes although direct evidence is inadequate. Strong evidence for the existence of organic complexes of iron was found by Nydahl (1951). He reported that when lake water was passed through an ion exchanger only about 10 per cent of the iron was adsorbed. The iron which passed through the exchanger was considered to be bound in an organic complex.

Although the availability of iron was demonstrated to be a limiting factor in Blind Lake, the causal relations should also be considered. Because the results of the mud-water experiments showed iron was present in the lake basin in rather large quantities, the influence of iron on primary productivity was not due to edaphic conditions, but was related to the physical and chemical characteristics of the water, morphometry of the lake and meteorological conditions. The oligotrophic nature of the lake and its deep basin may result in iron being precipitated from the epilimnion to the extent that sufficient quantities of iron are not available to algae. The supply of iron is then dependent on that carried in by runoff or by that regenerated from the bottom muds. Regeneration of nutrients is to some extent dependent on meteorological conditions because it was shown that only in years when circulation of the lake is limited will reducing conditions exist for regeneration. Decomposition of one season's production is insufficient in these years to cause an oxygen deficit in the hypolimnion. Mud-water experiments demonstrated that iron could be brought into solution by complex formation with HEDTA under aerobic, alkaline conditions. It is assumed that natural organic materials might function similarily to maintain iron in solution in an available form in the trophogenic strata of the lake, but that such materials were not present in Blind Lake in sufficient quantities to supply iron to phytoplankton.

If more organic matter were present in Blind Lake water, iron could be maintained in suspension or solution. However, due to the low productivity of Blind Lake, the quantity of organic matter produced is not great and little iron can be complexed by organic matter. This may cause iron to limit primary productivity. It was shown that iron

was not a limiting factor in experiments in which nutrients were added to Whitmore Lake water. Whitmore Lake is an eutrophic lake and presumably the water in Whitmore Lake may contain sufficient quantities of dissolved organic matter to complex iron. Thus there is a possibility of a circular causal system (Hutchinson 1948) in Blind Lake which is limiting primary productivity. In other words, by a series of causal events, low productivity can be considered to be the factor causing iron to limit primary productivity. However, if the limiting effects of iron were removed, primary productivity probably would be increased no more than four times because phosphorus and/or nitrogen would then become limiting.

SUMMARY

1. Blind Lake, a marl lake in Washtenaw County, Michigan, was studied from July 1957 to December 1959 to determine factors limiting primary productivity in marl lakes.

2. The following physicochemical characteristics were determined: water temperature, light penetration, dissolved oxygen, alkalinity, pH and total iron.

3. The effect of a chelating agent, HEDTA, on the exchange of iron between water and mud was investigated in the laboratory by establishing experimental mud-water systems from Blind Lake and two other lakes. The results showed that in the presence of HEDTA, iron was released from muds into the water under aerobic conditions.

4. Blind Lake was considered to be relatively unproductive on the basis of measurements of primary productivity using the carbon-14 method and comparisons of primary productivity of other waters.

5. To study nutrients limiting primary productivity, nutrients were added to lake water in bottles and the responses of the natural phytoplankton populations were measured by determining the rate of carbon-14 uptake. The advantages of using this means of studying the nutrients limiting primary productivity in a lake are discussed.

6. The results of these nutrient experiments showed that the rate of carbon-14 uptake was increased to the greatest extent by the addition of either Chu 10 nutrients or nitrogen and phosphorus in the presence of chelating agents. This response was greater for a chelating agent which contained iron (NaFeEEDTA) than for a similar compound (HEDTA) which contained no iron.

7. Iron was considered to be the limiting nutrient in Blind Lake. Primary productivity was increased from two to four times over the control by the addition of NaFeEEDTA to lake water. Greater increases in primary productivity (at least 30 times more than the control) were obtained when either nitrogen and phosphorus or Chu 10 nutrients were added in combination with NaFeEEDTA to lake water.

8. The availability of iron for phytoplankton in the lake was affected by meteorological conditions and the morphological features of the lake basin. Only in years with a limited period of spring circulation was the summer oxygen depletion in the hypolimnion sufficient to release iron from the sediments into the water. When iron was not regenerated from the sediments, the renewal of the iron supply in the trophogenic layers was dependent on that carried in by runoff.

9. This study suggests that the low productivity of the lake was due to a lack of dissolved organic materials which could function as chelating agents. This may have caused iron to limit primary productivity in the lake. It is thought that the synthetic chelating agents, which supplied iron in an available form to phytoplankton in nutrient experiments, functioned similarly to naturally-occurring organic compounds in more productive waters.

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APPENDIX A.--Water temperatures in Blind Lake in 1957

(Temperature in °F)

July 31		August 8		August 20		August 27		September 6	
Depth (m)	Temp	Depth (m)	Temp	Depth (ft)	Temp	Depth (ft)	Temp	Depth (ft)	Temp
0	81.3	0	76.6	0	74.8	0	72.4	0	70.6
2	80.6	4	76.3	12	74.7	12	72.4	6	70.2
3	79.2	5	68.9	14	74.2	16	71.8	12	69.8
4	75.9	6	58.6	16	72.2	18	68.2	18	69.1
5	67.1	7	51.8	18	65.6	20	61.1	20	64.3
6	57.7	8	46.8	20	59.4	22	56.0	22	57.1
7	50.9	9	45.0	22	54.5	24	51.6	24	53.1
8	46.9	10	43.7	24	51.2	26	49.2	26	50.2
9	44.8	11	42.8	26	48.9	28	47.5	28	48.0
10	43.3	12	42.1	28	46.9	30	46.4	30	46.4
11	42.6	13	41.7	32	45.0	32	45.0	32	45.4
12	42.1	14	41.0	36	43.6	34	44.0	34	44.5
13	41.5	18	40.8	40	42.8	36	43.5	36	43.9
14	40.8	23	40.6	44	42.3	42	42.5	42	42.6
23	40.5	--	--	52	41.6	52	41.7	52	41.8
--	--	--	--	80	41.2	78	41.1	74	41.1

APPENDIX B.--Dissolved oxygen, total alkalinity and pH in Blind Lake in 1957 - Deep Basin

August 20				August 27			September 6		
Depth (m)	O_2 (ppm)	Alk (ppm)	pH	Depth (m)	O_2 (ppm)	Alk (ppm)	Depth (m)	O_2 (ppm)	Alk (ppm)
0	8.25	-	8.50	0	8.7	146	0	8.8	144
5	8.60	132	8.55	2	8.6	145	4	8.6	-
10	11.28	168	8.10	5	9.0	-	5	8.9	144
15	7.08	170	8.05	6	11.6	-	6	10.2	-
20	2.28	-	7.80	7	15.2	155	7	14.6	-
-	-	-	-	8	14.3	-	8	12.9	-
-	-	-	-	9	11.7	-	9	12.1	-
-	-	-	-	10	10.5	-	10	10.5	166
-	-	-	-	12	10.2	169	12	10.3	165
-	-	-	-	15	6.8	168	15	5.7	-
-	-	-	-	18	3.5	168	18	3.0	-
-	-	-	-	20	1.5	173	20	1.08	-
-	-	-	-	23	0.0	186	-	-	-

APPENDIX C.--Water temperatures in Blind Lake in 1958

(Depth in feet and temperature in °F)

July 31		August 8		August 23		September 2		September 30	
Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp
0	74.9	0	79.4	0	73.6	0	71.2	0	66.8
14	74.7	4	78.8	8	72.9	4	71.1	4	66.5
16	71.6	8	77.6	14	72.7	10	70.6	16	66.2
18	66.5	10	77.2	18	72.3	18	70.2	20	66.0
20	61.9	14	76.2	20	67.8	20	68.6	22	65.0
22	57.4	16	74.2	22	61.1	22	63.9	24	63.8
24	54.0	18	70.2	24	57.3	24	59.0	26	58.4
26	50.9	20	65.2	26	53.7	26	54.9	28	55.8
28	48.4	22	61.2	28	50.5	28	51.9	30	52.3
30	47.2	24	56.6	30	48.9	30	49.3	32	50.1
32	46.1	26	53.4	32	47.5	32	48.3	34	49.4
36	45.2	28	51.0	34	46.6	34	47.3	36	48.6
--	--	30	48.8	38	45.7	38	46.4	38	47.8
--	--	32	47.8	40	45.3	40	46.1	--	--
--	--	34	47.0	42	45.1	42	45.7	--	--
--	--	36	46.8	44	44.9	44	45.4	--	--

APPENDIX C (continued)

October 15		October 29		November 7		November 17		December 5	
Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp
0	60.7	0	55.5	0	51.6	0	51.0	0	40.0
2	60.3	16	55.2	4	51.5	4	50.5	40	40.0
4	59.9	24	55.0	12	51.3	12	50.2	--	--
8	59.4	28	54.8	20	51.3	20	49.9	--	--
12	58.9	32	54.6	28	51.2	28	49.4	--	--
20	58.6	34	54.5	36	51.1	36	49.0	--	--
26	58.2	36	52.2	40	50.9	40	49.0	--	--
28	58.0	38	50.1	41	50.7	45	48.8	--	--
30	56.6	40	48.7	--	--	--	--	--	--
32	53.6	41.	48.1	--	--	--	--	--	--
34	50.6	--	--	--	--	--	--	--	--
36	49.1	--	--	--	--	--	--	--	--
38	48.2	--	--	--	--	--	--	--	--
40	47.6	--	--	--	--	--	--	--	--
42	47.4	--	--	--	--	--	--	--	--

APPENDIX D.--Dissolved oxygen and total alkalinity in Blind Lake in 1958

August 5		August 20			August 28			December 12		
Depth (m)	O_2 (ppm)	Depth (m)	O_2 (ppm)	Alk (ppm)	Depth (m)	O_2 (ppm)	Alk (ppm)	Depth (m)	O_2 (ppm)	Alk (ppm)
0	8.0	0	8.2	137	0	8.4	137	0	10.2	151
4	8.0	4	-	135	2	8.5	139	18	-	150
7	11.5	7	12.8	145	4	8.7	138	20	10.0	151
9	10.8	10	7.5	161	6	8.7	140	-	-	-
12	2.8	-	-	-	7	12.1	146	-	-	-
-	-	-	-	-	8	13.2	149	-	-	-
-	-	-	-	-	9	10.8	159	-	-	-
-	-	-	-	-	10	6.6	164	-	-	-
-	-	-	-	-	11	7.0	161	-	-	-
-	-	-	-	-	11.5	2.0	-	-	-	-
-	-	-	-	-	12	0.3	173	-	-	-
-	-	-	-	-	13	0.0	178	-	-	-

APPENDIX E.--Water temperatures in Blind Lake in 1959

(Depth in feet and temperature in °F)

March 28		April 14		April 29		May 14		June 2	
Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp
0	34.9	0	44.7	0	51.6	0	62.3	0	72.2
2	40.8	10	44.4	4	51.5	8	62.3	4	72.0
4	42.1	20	43.1	16	51.3	10	62.2	8	71.2
6	41.4	22	42.0	18	50.6	12	60.3	10	68.1
8	40.8	24	41.5	19	49.7	14	56.6	12	64.0
10	40.2	28	40.9	20	49.0	16	54.1	14	61.2
12	39.6	30	40.2	22	48.1	18	52.6	16	58.7
14	39.5	32	39.4	23	45.5	20	50.2	18	55.9
18	38.9	36	38.7	24	43.8	22	48.8	20	53.0
28	38.4	44	38.3	26	42.3	24	46.9	22	50.0
40	37.9	64	38.0	28	41.6	26	44.3	24	47.4
56	37.7	--	--	32	40.6	28	42.6	26	45.4
76	37.8	--	--	36	39.9	30	41.9	28	44.0
78	37.9	--	--	46	38.7	34	40.8	30	42.8
80	38.4	--	--	58	38.3	42	39.2	34	41.2
--	--	--	--	71	38.3	54	38.7	38	40.2
--	--	--	--	--	--	75	38.5	48	39.0
--	--	--	--	--	--	--	--	78	38.8

APPENDIX E (continued)

June 19		July 10		July 28		August 18		September 10	
Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp
0	71.3	0	76.9	0	79.4	0	78.9	0	78.2
4	71.2	4	76.6	4	79.2	2	78.5	12	78.2
12	70.2	12	75.8	6	78.5	10	77.6	14	77.5
14	68.5	14	73.7	8	77.6	12	77.0	16	76.5
16	62.8	16	69.0	12	76.6	14	76.0	18	74.5
18	58.5	18	62.0	14	75.6	16	74.3	20	68.4
20	54.2	20	57.2	16	72.5	18	70.5	22	61.4
22	50.9	22	53.2	18	65.3	20	64.8	24	56.6
24	48.9	24	50.0	20	59.6	22	58.7	26	52.6
26	46.5	26	47.9	22	55.6	24	54.3	28	50.7
28	44.5	28	46.3	24	52.1	26	51.4	30	48.0
30	43.4	30	44.5	26	49.0	28	48.8	32	46.2
32	42.5	32	43.5	28	47.1	30	46.3	34	44.8
34	41.7	36	41.6	30	45.2	34	43.6	38	42.8
38	40.5	40	40.2	34	43.1	38	41.7	42	41.5
48	39.3	44	39.4	38	41.7	42	40.8	46	40.8
54	38.9	50	38.9	42	40.5	46	40.0	48	40.4
79	38.9	78	39.0	46	39.2	50	39.5	52	40.4
--	--	--	--	78	39.0	76	39.2	--	--

APPENDIX E (continued)

October 9		October 20		November 12		November 30		December 17	
Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp	Depth	Temp
0	64.8	0	55.7	0	45.5	0	37.2	0	36.5
8	64.7	20	55.5	10	45.5	30	37.4	2	36.7
12	64.6	24	55.2	30	45.2	32	37.6	4	36.7
18	64.5	28	54.8	40	45.0	38	37.8	6	36.8
20	63.9	30	54.7	44	44.9	46	37.9	12	36.8
22	63.5	32	51.8	46	44.1	56	38.0	78	38.0
24	62.7	34	48.3	48	42.7	66	38.2	80	38.0
26	58.1	36	44.7	50	41.2	70	38.4	--	--
28	52.8	38	43.3	52	40.5	72	38.8	--	--
30	50.3	40	42.7	56	40.0	74	39.0	--	--
32	48.8	42	42.0	60	39.8	--	--	--	--
34	46.8	44	41.3	74	39.8	--	--	--	--
36	45.2	48	40.6	--	--	--	--	--	--
38	43.4	54	40.0	--	--	--	--	--	--
42	42.0	62	39.6	--	--	--	--	--	--
46	40.8	77	39.6	--	--	--	--	--	--
50	40.2	--	--	--	--	--	--	--	--
56	39.7	--	--	--	--	--	--	--	--
76	39.4	--	--	--	--	--	--	--	--

APPENDIX F.--Total alkalinity (ppm) in Blind Lake in 1959

Depth (ft)	April 15	April 29	May 5	June 2	June 19	June 30	July 10	July 18	Aug 18	Oct 20	Oct 29	Nov 12	Nov 16	Nov 20
0	149	154	153	143	146	147	148	147	138	140	142	148	146	152
19	149	154	151	146	146	147	149	148	140	-	-	-	-	-
26	-	154	152	149	151	156	153	158	156	-	-	-	-	154
32	-	157	-	153	156	-	-	-	162	-	144	-	-	-
38	-	161	-	-	159	164	164	164	165	-	154	148	-	-
44	161	-	163	159	-	166	168	168	166	-	170	150	-	-
50	-	162	-	163	163	168	171	170	168	-	171	160	149	-
56	-	-	-	163	164	-	172	172	170	-	-	162	165	153
62	-	-	169	-	165	170	173	171	172	-	174	-	-	-
70	-	162	-	-	168	171	175	174	172	-	-	-	-	153
75	-	-	171	176	-	-	-	-	-	-	-	182	-	184
79	-	-	-	-	169	174	178	178	191	195	190	-	-	-

APPENDIX G.--pH in Blind Lake in 1959

Depth (ft)	June 19	June 30	July 18	October 20	October 29
0	8.43	8.49	8.52	8.50	8.50
19	8.45	8.42	8.47	-	-
26	8.28	8.31	8.32	8.52	-
32	8.16	-	-	8.15	8.50
38	8.03	8.05	8.03	7.90	8.20
44	-	7.93	7.85	7.75	7.75
50	7.80	7.79	7.78	7.70	-
56	7.75	-	7.71	-	-
62	7.71	7.67	7.66	-	7.72
70	7.68	7.68	7.62	-	-
79	7.64	7.68	-	7.60	7.65

APPENDIX H.--Dissolved oxygen (ppm) in Blind Lake in 1959

Depth (ft)	March 28	April 15	April 29	May 5	May 14	June 2	June 9	June 19	June 30	July 10	July 18	July 28
0	8.4	11.1	10.3	9.8	9.1	8.4	8.3	8.5	7.6	7.9	7.9	8.2
12	11.2	-	-	9.6	-	9.4	12.4	-	-	-	-	-
15	11.2	-	-	-	9.8	-	-	-	-	-	-	-
19	10.4	11.1	10.4	10.4	10.6	10.7	11.2	-	11.7	12.5	12.4	-
26	9.7	-	10.3	10.7	-	11.1	10.9	10.9	10.9	11.1	10.9	11.1
32	8.7	-	9.3	8.8	9.5	10.0	9.8	9.6	-	-	-	-
38	-	-	8.6	8.8	-	8.8	8.7	8.6	8.7	8.0	8.3	8.0
44	-	7.0	-	7.2	-	7.2	7.0	6.7	6.9	6.7	5.4	-
50	8.1	-	6.7	-	6.4	6.0	5.6	5.2	4.6	3.5	3.3	-
56	-	-	-	-	-	-	4.5	3.3	-	2.4	2.0	-
62	7.6	-	-	5.2	-	3.5	2.8	3.0	1.8	1.4	0.6	-
70	6.3	-	6.7	5.3	-	-	1.9	1.5	0.6	0.2	0.2	-
75	6.2	-	-	4.2	3.3	1.4	1.4	0.5	-	-	-	-
79	4.9	-	-	-	-	0.8	-	-	0.2	0.1	0.1	-

APPENDIX H (continued)

Depth (ft)	Aug 13	Aug 18	Sept 10	Oct 9	Oct 29	Nov 5	Nov 12	Nov 16	Nov 20	Nov 30	Dec 17
0	7.9	7.6	7.8	8.7	9.5	9.8	9.9	9.7	9.8	10.9	-
12	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	9.9	-	-	-	-
19	10.6	9.4	10.4	8.8	-	9.7	9.9	9.7	9.8	10.7	10.7
26	11.6	11.4	10.9	9.3	-	9.6	-	-	-	-	-
32	-	9.2	8.5	7.6	9.6	9.6	-	-	-	-	-
38	6.9	6.6	5.8	4.8	7.0	9.6	9.5	9.8	9.9	-	-
44	4.6	4.3	3.3	2.1	1.5	1.6	9.1	-	-	-	-
50	2.7	2.8	1.5	0.2	0.1	0.2	4.6	9.8	-	-	-
56	1.0	1.4	0.3	-	-	-	-	0.3	-	-	-
62	0.1	0.3	0.2	0.2	-	-	-	-	9.9	-	-
70	0.1	0.1	0.0	-	-	-	-	-	2.7	10.2	-
75	-	-	-	0.0	-	-	0.0	0.0	-	0.7	2.3
79	0.0	0.0	0.0	-	-	-	-	-	0.0	-	-

APPENDIX I.--Total iron (ppb) in Blind Lake in 1959

Depth (ft)	Mar 28	April 29	June 2	June 19	July 10	July 18	July 28	Aug 13	Sept 10	Oct 10	Oct 29	Nov 12	Nov 20	Nov 30	Dec 17
0	--	44.0	25.0	10.0	21.9	9.6	21.9	40.0	27.6	27.6	33.3	28.6	--	28.6	38.1
15	18.6	--	--	--	--	--	--	--	--	--	--	--	28.6	--	--
19	--	53.0	--	18.6	12.4	--	--	22.0	12.4	12.4	--	28.6	--	--	--
26	--	--	31.0	18.6	--	--	--	9.6	15.7	--	23.8	--	--	28.6	52.4
38	31.0	32.0	12.4	0.0	12.4	--	--	12.4	--	--	--	23.8	--	--	--
43	--	--	--	--	--	--	--	--	--	--	23.8	--	--	28.6	--
50	--	45.2	6.2	3.4	0.0	--	--	15.7	7.1	--	--	52.4	--	--	33.3
60	31.0	--	12.4	12.4	0.0	18.6	--	21.9	--	--	76.2	52.4	--	--	--
70	--	38.1	--	--	28.1	92.8	--	93.8	216	191	--	--	71.4	38.1	--
75	24.8	--	--	--	216	--	--	--	--	480	--	260	--	95.2	76.2
79	--	--	93	119	--	476	--	851	690	488	357	--	262	--	--

APPENDIX J.--Water temperature and dissolved oxygen in Blind Lake

October 1, 1960

(Data collected by limnology class supervised by K. W. Cummins and G. R. Marzolf)

Depth (m)	Temp (°F)	O_2 (ppm)
0	61.7	8.84
6	61.2	-
7	59.4	-
7.5	51.8	9.7
8	45.1	-
9	43.5	-
10	42.3	-
11	41.5	-
12	41.0	5.46
19	41.0	3.30

APPENDIX K.--Summary of nutrient experiments, Blind Lake

Date and experiment (hours of C-14 in parentheses)	Time for 1280 counts (min)					Average gross uptake* (cpm/hr)	Response relative to control
	Bottle 1 subsamples		Bottle 2 subsamples		Average		
	A	B	A	B			
8/27-9/2/58 (6.0)							
Control	10.16	10.29	12.16	12.20	11.20	16.0	-
Chu 10 and NaFeEEDTA	0.17	0.18	0.09	0.08	0.13	1851	116
Nitrogen, phosphorus	4.76	5.13	13.20	14.31	9.35	19.8	1.24
Nitrogen, phosphorus and NaFeEEDTA	0.78	0.77	0.41	0.45	0.60	353	22.1
9/30-10/7 (6.0)							
Control	14.85	14.05	-	-	14.45	11.8	-
Chu 10	19.69	17.63	19.56	17.90	18.70	9.5	0.81
Chu 10 and HEDTA	9.09	9.06	9.66	9.45	9.32	22.5	1.91
Chu 10 and NaFeEEDTA	1.79	1.71	2.51	2.36	2.09	112	9.49
10/15-10/21 (5.0)							
Control	17.23	15.60	25.91	20.88	19.91	9.3	-
Chu 10	10.81	10.35	12.61	14.94	12.18	19.6	2.11
Chu 10 and NaFeEEDTA	2.33	2.16	2.64	2.68	2.45	115	12.4
Nitrogen, phosphorus	9.53	10.70	20.44	17.38	14.51	14.0	1.51
Nitrogen, phosphorus and NaFeEEDTA	3.89	3.07	2.91	2.74	3.15	77.7	8.35
NaFeEEDTA	6.70	6.55	6.15	5.26	6.17	37.9	4.08
10/31-11/7 (5.0)							
Control	25.36	16.26	-	-	20.81	8.7	-
Chu 10	16.99	17.51	-	-	17.25	12.6	1.45
Chu 10 and HEDTA	9.03	9.27	-	-	9.15	27.5	3.16
Chu 10 and NaFeEEDTA	8.04	7.43	-	-	7.74	33.3	3.83
Nitrogen, phosphorus	17.08	15.20	-	-	16.14	12.3	1.41
Nitrogen, phosphorus and HEDTA	5.12	3.92	-	-	4.52	53.0	6.09

* Corrected for background activity and added carbonate

APPENDIX K (continued)

Date and experiment (hours of C-14 in parentheses)	Time for 1280 counts (min) Bottle 1 subsamples A	Bottle 1 subsamples B	Bottle 2 subsamples A	Bottle 2 subsamples B	Average	Average gross uptake* (cpm/hr)	Response relative to control
10/31-11/7 (5.0)							
Nitrogen, phosphorus and NaFeEEDTA	4.16	3.85	-	-	4.01	60.2	6.92
HEDTA	9.73	13.80	-	-	11.77	18.2	2.09
NaFeEEDTA	11.47	7.74	-	-	9.61	23.0	2.64
4/14-5/1/59 (4.0)							
Control	3.10	3.15	0.76	0.83	1.96	159	-
Chu 10	1.65	1.69	1.76	1.97	1.77	199	1.25
Chu 10 and HEDTA	0.85	0.93	0.26	0.24	0.57	629	3.96
Chu 10 and NaFeEEDTA	0.15	0.14	0.19	0.19	0.17[1]	8503	53.5
Nitrogen, phosphorus and HEDTA	0.14	0.15	0.12	0.12	0.13[2]	4918	31.0
Nitrogen, phosphorus and NaFeEEDTA	0.11	0.10	0.08	0.09	0.10[2]	6395	40.3
HEDTA	1.12	0.99	0.78	0.96	0.96	329	2.07
NaFeEEDTA	0.51	0.49	0.60	0.54	0.54	588	3.70
5/8-5/14 (7.0)							
Control[3]	10.23	18.83	6.70	8.23	11.00	19.7[3]	-
Chu 10 and HEDTA	6.07	5.08	6.21	5.58	5.74	33.0	1.68
Chu 10 and NaFeEEDTA[3]	2.48	2.69	3.04	2.65	2.72[2]	208.6[3]	10.6
Nitrogen, phosphorus	11.50	10.75	4.94	4.68	7.97	20.3	1.03
Nitrogen, phosphorus and HEDTA	1.25	1.08	1.71	1.34	1.35	132.8	6.74
Nitrogen, phosphorus and NaFeEEDTA	1.17	1.08	1.15	0.94	1.09[2]	333.0	16.9

* Corrected for background activity and added carbonate

[1] Time for 5120 counts

[2] Time for 2560 counts

[3] 5.0 hr experiment

APPENDIX K (continued)

Date and experiment (hours of C-14 in parentheses)	Time for 1280 counts (min)					Average gross uptake* (cpm/hr)	Response relative to control
	Bottle 1 subsamples		Bottle 2 subsamples		Average		
	A	B	A	B			
6/2-6.9 (4.0)							
Control	24.30	25.24	19.37	20.65	22.39	9.8	-
Chu 10	(See Table 7)						
Chu 10 and NaFeEEDTA	(See Table 7)						
Nitrogen, phosphorus	2.36	1.95	2.19	2.10	2.15	144	14.7
Nitrogen, phosphorus and HEDTA	0.56	0.59	0.53	0.57	0.56[1]	2281	233
Nitrogen, phosphorus and NaFeEEDTA	0.65	0.65	0.29	0.26	0.46[1]	2820	288

* Corrected for background activity and added carbonate

[1] Time for 5120 counts

APPENDIX K (continued)

Date and experiment (hours of C-14 in parentheses)	Time for 2560 counts (min) Bottle 1 subsamples A	B	Bottle 2 subsamples A	B	Average	Average gross uptake* (cpm/hr)	Response relative to control
6/19-6/30 (4.0)							
Control	7.09	7.63	3.55	3.87	5.54[1]	53.3	-
Chu 10	0.14	0.17	0.32	0.33	0.24[1]	3009	56.4
Chu 10 and HEDTA	2.81	3.18	0.48	0.46	1.73	203.9	3.83
Chu 10 and NaFeEEDTA	0.10	0.11	-	-	0.11	6881	129
Nitrogen, phosphorus	0.40	0.42	-	-	0.41	1556	29.2
Nitrogen, phosphorus and HEDTA	1.25	3.21	-	-	2.23	282.5	5.30
HEDTA	5.75	7.38	8.04	14.81	9.00[1]	31.1	0.58
NaFeEEDTA	6.42	6.08	-	-	6.25[1]	46.7	0.88

* Corrected for background activity and added carbonate

[1] Time for 1280 counts

Date and experiment (hours of C-14 in parentheses)	Time for 2560 counts (min) Bottle 1 subsamples A	B	Bottle 2 subsamples A	B	Average	Average gross uptake* (cpm/hr)	Response relative to control
7/10-7/18 (4.5)							
Control	5.27	11.17	14.49	6.72	9.41[1]	26.2	-
Chu 10	1.41	1.50	3.02	3.69	2.41[1]	114.0	4.35
Chu 10 and HEDTA	0.66	0.66	0.81	0.80	0.73	775.3	29.6
Chu 10 and NaFeEEDTA	0.45	0.45	0.09	0.07	0.27	2103	80.3
Nitrogen, phosphorus	1.30	1.33	0.78	0.80	1.05	537.8	20.5
Nitrogen, phosphorus and HEDTA	0.95	0.99	0.84	0.82	0.90	628.0	24.0
Nitrogen, phosphorus and NaFeEEDTA	0.23	0.27	0.23	0.27	0.25	2272	86.7
Chu 10 and $FeCl_3$	0.52	0.41	-	-	0.47	1263	48.2

* Corrected for background activity

[1] Time for 1280 counts

APPENDIX K (continued)

Date and experiment (hours of C-14 in parentheses)	Time for 2560 counts (min) Bottle 1 subsamples A	B	Bottle 2 subsamples A	B	Average	Average gross uptake* (cpm/hr)	Response relative to control
7/20-7/28 (4.0)							
Control	36.31	34.59	44.34	37.12	38.09[1]	3.9	-
Chu 10	0.79	0.75	0	0	0.77	826.8	212
Chu 10 and HEDTA	0.28	0.36	-	-	0.32	1995	512
Chu 10 and NaFeEEDTA	0.16	0.14	-	-	0.15[2]	8528	2190
Nitrogen, phosphorus	0.56	0.53	0.62	0.69	0.60	1062	272
Nitrogen, phosphorus and HEDTA	0.40	0.41	-	-	0.41	1575	404
Nitrogen, phosphorus and NaFeEEDTA	0.13	0.14	-	-	0.14[2]	9477	2430

* Corrected for background activity

[1] Time for 1280 counts

[2] Time for 5120 counts

Date and experiment (hours of C-14 in parentheses)	Time for 1280 counts (min) Bottle 1 subsamples A	B	Bottle 2 subsamples A	B	Average	Average gross uptake* (cpm/hr)	Response relative to control
8/13-8/18 (4.0)							
Control	22.84	20.37	-	-	21.61	10.3	-
Chu 10	2.52	2.55	-	-	2.54	121.5	11.8
Chu 10 and HEDTA	9.67	9.78	19.56	21.73	15.19	16.6	1.61
Chu 10 and NaFeEEDTA	2.73	2.71	0.62	0.64	1.68[1]	757.5	73.5
Nitrogen, phosphorus	1.47	1.57	1.32	1.30	1.42	220.9	21.4
Nitrogen, phosphorus and HEDTA	1.47	1.45	3.10	3.30	2.33	132.9	12.9
Nitrogen, phosphorus and NaFeEEDTA	1.34	1.37	2.68	2.74	2.03[1]	626.0	60.8

* Corrected for background activity

[1] Time for 5120 counts

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