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Responses of a Marl Lake to
Chelated Iron and Fertilizer

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Chelating and sequestering agents are organic compounds which form complexes with metallic ions. Many of these complexes are water soluble. Thus chelating agents may keep metallic elements in solution that would otherwise be precipitated. Trace metals complexed by chelating agents can be used to supply nutrients to living plant cells. The purpose of the present experiment was to observe how chelated iron influences the water chemistry and the photosynthetic rate of a small marl lake.

Microbiologists have recognized the value of the versenes in culture media as a complexing or chelating agent for metals. They found ethylenediaminetetraacetic acid to be valuable in mobilizing and solubilizing metals (Hutner et al. 1950). Provasoli and Mintner (1953) noted that the use of chelators in laboratory cultures parallels the situation in natural waters where humic acids are the non-metabolizable metal-buffering agents. Saunders (1957) pointed out that by acting as chelators dissolved organic substances may influence the concentration of trace elements in natural waters.

Many organic compounds, such as amino acids, polypeptides, and porphyrins, occur in natural waters (Valentyne 1957) and may act as metal binders. Domogalla, Juday and Peterson (1925) and Fogg and Westlake (1955) demonstrated that amino acids and polypeptides may be present in natural lakes. These compounds may be secreted into the water by living algae or may be released by decay of phytoplankton. Fogg and Westlake also demonstrated that laboratory cultures of diatoms and green and blue-green algae produced peptide nitrogen and showed that the extracellular

polypeptide nitrogen from Anabaena formed a soluble complex with cupric ion, zinc and iron. A solution of the Anabaena polypeptide brought solid tricalcium orthophosphate into solution. Titration curves for mixtures of the polypeptide and phosphate indicate that the two formed a soluble complex. Fogg and Westlake suggest that substances such as polypeptides might promote growth of algae in natural systems by maintaining phosphorus and iron in an available form in situations where precipitation might otherwise take place.

The phosphorus and iron in the water of marl lakes are in equilibrium with solid calcium carbonate. Marl is found not only on the lake bottom but is also suspended in the water. Thus the water is highly buffered and the pH is almost always between 8.0 and 8.4. Phosphorus and iron entering the epilimnion, therefore, may be precipitated and sedimented at all times of the year. Because marl lakes have been found to be deficient in phosphorus, several attempts have been made to enrich such lakes with inorganic fertilizers (Barrett 1953, Alexander 1956, Anton 1957 and Flosila 1958). In these trials the added phosphorus has been maintained in the epilimnion for only a short time. In most cases phytoplankton has been increased little, if at all, but the fertilizer has stimulated the growth of benthic algae (Flosila 1958).

Except when they contain suspended marl particles, Michigan marl lakes ordinarily are of high transparency. A characteristic of these ecosystems is that there is little organic matter in the bottom sediments and in the water. Since organic substances were lacking it was reasoned that marl lakes might be deficient in natural chelating agents and that an artificial chelate might make iron and phosphorus available for photosynthesis.

Schelske (1960) tested this hypothesis in Blind Lake, a southern Michigan marl lake. He added chelating agents and certain inorganic nutrients to bottles containing lake water and then suspended the bottles in the lake. After incubation in the lake for at least 4 days, the rate of carbon fixation was measured by means of the carbon-14 (C^{14}) method. Iron chelate alone increased the rate of carbon fixation fivefold and a combination of iron chelate and nutrients which included nitrogen and phosphorus, increased the rate of carbon fixation fiftyfold to a hundredfold. The addition of nutrients without chelated iron increased photosynthesis very little or not at all over the rate in the control bottles (bottles containing only lake water).

In the present investigation, we added chelated iron, NaFeHEDTA (iron sodium salt of N-hydroxyethylethylenediaminetriacetic acid), and inorganic fertilizer to a small marl lake to determine if these chemicals would increase the rate of primary productivity in the lake as a whole as it had in the bottle experiments mentioned above.

Experimental Lakes

Sand Lakes 1 and 2 are members of a series of five small marl lakes in Grand Traverse County, Michigan. During an earlier high-water stage these lakes were probably parts of a single lake. Today the lakes are not connected and have no inlets or outlets. Lake 1 has an area of 14.2 acres and a mean depth of 22 feet. Lake 2 has a greater area (17.3 acres) but is much shallower (mean depth of 11 feet). The shoreline of both lakes is soft white marl. At the time of the study one could not reach the water's edge without sinking a foot or more into marl. Marl sediments extend

throughout the littoral and sublittoral zones. Despite extensive marl deposits, these lakes are of only moderate hardness and calcium content for Michigan lakes (methyl orange alkalinity 75-105 ppm and calcium 15-30 ppm). The aquatic vegetation of the lakes is chiefly sparse stands of Chara.

Both lakes were stratified thermally and chemically when the experiment was begun and remained stratified throughout most of the investigation. In Lake 1, the epilimnion extended to a depth of 18 feet and the hypolimnion was below 34 feet. In Lake 2 the thermocline began at 12 feet and extended to the maximum depth.

Lake Treatments

For our study, Sand Lake 1 was the experimental lake and Sand Lake 2 was the "control" or reference lake. In order to simulate Schelske's bottle experiments as closely as possible we first added only chelated iron to Sand 1 and did not treat Sand 2. After a 10-day period in which the effect of the chelated iron in Sand Lake 1 was evaluated, we added inorganic fertilizer to both lakes. This enabled us to study the combined effect of chelated iron and fertilizer in Sand 1 and to study the effect of the fertilizer alone in Sand 2. The concentration of fertilizer after treatment was approximately the same in the two lakes if only the epilimnetic volumes are considered. Calculated on the basis of total lake volume the concentration was slightly greater in Sand 2 than in Sand 1 (Table 1).

On July 26, 1959, Sand 1 received a mixture of 491 pounds of NaHEDTA (Versenol 120, Dow) and 300 pounds of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). The Versenol, a liquid, was added to the solid ferrous sulfate. Mixing the

ingredients in this way maintained an acid solution and prevented precipitation of ferric hydroxide. The resulting mixture contained a chelated iron complex. The amount of ferrous sulfate added was less than the chemical equivalent of the Versenol. Hence a small fraction of the Versenol (approximately 10%) was not combined with iron.

Measurement of Photosynthetic Rates

Photosynthetic rates were measured using the C^{14} method. Water samples were collected at a selected series of depths with a Kemmerer water bottle. Four Pyrex glass-stoppered bottles of 250 ml capacity were filled with water from each depth. Approximately two microcuries of $NaHC^{14}O_3$ was added to each bottle. Two of the four bottles were wrapped in aluminum foil to exclude light. All four bottles were then suspended in the lake, at the depth from which the water sample was collected, for a period of from four to five hours. The four- to five-hour period ordinarily extended from 10:00 a. m. to 2:00 or 3:00 p. m. After exposure in the lake, two samples from each bottle were filtered through an HA Millipore filter. The filter was rinsed with 5 ml of 0.002 N HCl and 5 ml of distilled water and mounted on an aluminum counting planchet. The activity of samples was measured in a gas-flow counter equipped with a micromil window and an automatic sample changer. The net rate of uptake at each depth was then determined by subtracting the average activity of the dark bottle samples from the average activity of the light bottle samples. These results were plotted for each date and yielded a photosynthetic profile.

Since we were interested in detecting rate changes rather than absolute values, net C¹⁴ uptake rates were not converted to photosynthetic rates. Rodhe et al. (1960) points out that at present it is best to use C¹⁴ uptake values as relative rather than as absolute measurements.

Changes in Photosynthetic Rates

Comparison of photosynthetic profiles before (July 21-25) and after (July 30-August 5) treatment with chelated iron indicates that a change in photosynthetic rate occurred in Sand Lake 1 (Fig. 1). In the lower epilimnion (6- and 12-foot depths) and in the upper thermocline (18-foot depth), average rates were from four- to sixfold greater during the post-treatment period. Increases were smaller at 24 feet and little or no change occurred in the hypolimnion at 32 feet. As there is no evidence that the chelated iron penetrated the lower thermocline and hypolimnion, no increase would be expected at these depths. Despite considerable day to day variation, the range of values obtained at each depth for the four after-treatment profiles overlapped the ranges of the three pretreatment profiles only at the surface where photosynthesis appeared to be light inhibited after the chelate treatment and at the 24- and 32-foot depths which were not treated. The net effect of the chelate treatment is shown by the change in shape of the photosynthetic profiles before and after treatment. Before treatment the rate of photosynthesis in general increased with depth and the maximum photosynthetic rates were at the greatest depths, i. e., in the hypolimnion. After treatment, profiles showed light inhibition of photosynthesis at the surface, a maximum rate in the epilimnion, and rates decreasing with depth below the maximum.

In the case of the reference lake (Sand Lake 2), only two profiles were secured preceding fertilization, one before and one after the chelate addition to the experimental lake (Fig. 1). There was little difference in the shape of the profiles on these dates (July 26 and August 2); however, there was a higher rate at all depths on August 2 than on July 26. Although more data are needed, this suggests that a natural increase in photosynthetic rate occurred in the reference lake. Thus the increase in photosynthetic rates in Sand Lake 1 after the chelate treatment might also represent a natural increase. This seems unlikely because the photosynthetic increase after the chelate treatment was confined to the depths receiving iron chelate (Fig. 1) and because photosynthetic rates in Sand 1 were decreasing prior to the chelate treatment (Table 2).

Rates of C^{14} uptake before (control period) and after the chelate treatment of Sand Lake 1 were compared by an analysis of variance to determine whether or not the observed changes were significantly larger after the chelate treatment and therefore attributable to the chelate treatment (Table 3). Rates of C^{14} uptake were transformed to their logarithms before making analysis of variance tests. Transformed data fitted a normal distribution.

The effect of the chelate treatment was tested by comparing the treated depths (above) with the untreated depths (below). No significant difference exists between the rates of C^{14} uptake in the above and below depths before treatment whereas this comparison is highly significant after chelated iron was added to Sand Lake 1 (Table 3). Further evidence that the chelate

treatment increased photosynthetic rates comes from comparison of before treatment and after treatment F values. The F value is highly significant for above depths and not significant for below depths. This gives further indication that a change occurred in the zone which was treated with chelate.

Photosynthetic rates were so much greater after the addition of fertilizer than rates before fertilization that statistical analysis was not necessary. In Lake 1 photosynthetic rates increased markedly in the epilimnion a week after the addition of fertilizer. By August 14 and 15 C^{14} uptake was over 500 counts per minute at the surface and there had been similar large increases at the 6- and 12-foot depths. At this time the lake was clearly in bloom. Algal scum greatly reduced transparency of the water.

Two weeks after the addition of fertilizer (August 20-21) the bloom disappeared and water transparency increased (Fig. 4). Likewise, clearing was observed in the reference lake and it is believed that both lakes cleared in response to a week of exceptionally hot, calm weather. Photosynthetic rates increased again in Lake 1 the following week as the weather became cool and windy. On August 27 and 28 rates were the highest encountered during the experiment; C^{14} uptake was 1,000 counts per minute at the surface and 6-foot depth and similar large increases were detected at the 12- and 18-foot levels.

The response to fertilization of the reference lake was weak compared to the response of the experimental lake. From August 2 to August 15 the rate of C^{14} uptake increased from 48 to 154 counts per minute at the surface

and from 76 to 104 at the 6-foot depth (Fig. 1). Maximum values were on August 28; on this date rates as high as 300 counts per minute were recorded.

Transparency Changes

Light penetrating to various depths was plotted as percentage of surface light (Fig. 2). Transparency decreased in the experimental lake after addition of the chelate and there was a further fall in transparency after the fertilizer was applied. A spectacular increase in transparency occurred in both lakes during the week of August 15-19 when the weather was extremely calm and warm. Transparency was again low in both lakes on August 26 but photometer readings were not as low in Lake 1 as on August 14 even though the maximum photosynthetic rates were recorded the following 2 days (August 27-28). The fall in transparency of the reference lake between July 26 and July 29 supports results from C^{14} measurements which indicated that photosynthetic rates were increasing prior to fertilization.

Dissolved Oxygen

The unmodified Winkler method was used to determine the dissolved oxygen content of samples collected at selected depths. Samples for analyses of total phosphorus and total iron were taken at the same time and at the same depths.

Oxygen profiles in Sand Lake 1 were of the positive heterograde type. Supersaturation occurred in the upper thermocline on all dates except September 22 (Fig. 3). Little or no change occurred in the profiles after the addition of chelate. The influence of the chelated iron alone upon

production appeared to be small in comparison to its effect when combined with fertilizer; however, the period of time when only chelated iron was present was short and its effect might have intensified with time. The bloom of plankton which developed after fertilization produced supersaturation in the upper epilimnion (August 14). Later oxygen values decreased in the lower epilimnion producing a pronounced notch in the oxygen curve (August 26). The oxygen profile in Sand Lake 2 also changed after fertilization. Supersaturation was found on August 15 at the surface and at the 12-foot depth. Dissolved oxygen was soon depleted in the bottom waters and an oxygen deficiency appeared in the lower epilimnion.

Total Phosphorus

The addition of chelated iron had no effect upon the total phosphorus concentration in Sand Lake 1. The slight increase of phosphorus in the epilimnion between July 26 and July 31 appeared to be within the range of normal fluctuations. The fertilizer raised the phosphorus concentration of the epilimnion of Lake 1 from 5 to 20 ppb and from 5 to 30 ppb in Lake 2 (Fig. 4). It is clear, however, that the added phosphorus soon disappeared from the water of both lakes. Loss of phosphorus was more rapid in the reference lake than in the experimental lake. By August 21 the concentration in the epilimnion of Lake 2 was less than 10 ppb and by August 27 it had fallen to less than 5 ppb. A concentration greater than 15 ppb remained in the epilimnion of Lake 1 through August 27, suggesting that the chelate may have delayed the loss of phosphorus from the water. By September 22

phosphorus had fallen below 10 ppb in the epilimnion of Lake 1 and had increased substantially in the hypolimnion and lower thermocline.

Total Iron

Analyses for total iron measured with tripyridyl after oxidation of organic matter (American Public Health Association, 1955) give the only available information on the distribution of chelated iron in Sand Lake 1. Analytical methods for small amounts of chelate were not considered practical. Thus we must rely upon changes in total iron concentration for information on movements of the chelated iron. Interpretation of iron data in this manner leads to some uncertainty since changes in concentration of naturally occurring iron cannot be distinguished from changes in chelated iron. Also this interpretation assumes that the HEDTA remains complexed with iron and does not combine with other metals in the lake system. The latter assumption is believed to be sound because metals that might form a more stable complex than iron are present in very low concentrations.

The concentration of total iron in the epilimnion of Lake 1 before treatment with chelated iron (July 26) ranged from 49 to 54 ppb. Concentrations in the thermocline and hypolimnion were from 67 to 76 ppb. Large increases were detected in the epilimnion three days after treatment. On August 31, 189 ppb of iron was found in the surface water and 109 ppb at 13 feet (Fig. 5). In the thermocline and hypolimnion, no increase in iron concentration was noted indicating that the iron chelate had not penetrated the thermocline. While the lake contained only the chelating agent (before

fertilization) the concentration of iron in the epilimnion changed little and there was no indication that the iron chelate had moved into the thermocline and hypolimnion.

The first indication of a change in iron distribution was on August 15, 9 days after the fertilizer had been applied. On this date iron had fallen sharply in the epilimnion and ranged from 29 to 56 ppb. An increase was noted throughout the hypolimnion, but particularly in the bottom water where 180 ppb was recorded. This increase in the hypolimnion was not due to regeneration of iron from the bottom sediments because the hypolimnion still contained dissolved oxygen. Despite the iron increase in the hypolimnion, there was a net loss of iron from the lake as a whole.

The disappearance of iron from the water after August 15 coincided with the post-fertilization plankton bloom. The passage of iron into the hypolimnion noted above suggests that iron was removed from the water by organisms making up the bloom and settled into deeper layers as plankton detritus. Iron concentrations continued to decrease throughout the lake basin until August 27. On this date values ranged from 8 to 29 ppb in the epilimnion and thermocline (Fig. 5) and were the lowest encountered during the study. This stripping of iron from the water to concentrations considerably below those encountered before treatment suggests that the chelating agent made available new sources of iron to the planktonic organisms.

With the onset of anaerobiosis in the bottom waters after August 26, regeneration of iron occurred from the bottom sediments. On September 22, 531 ppb was encountered in the bottom waters and 94 ppb was found at 32 feet.

By September 22 the epilimnion had deepened due to autumnal cooling and it had an iron content in the range encountered before chelate treatment (Fig. 5). On October 21, during the fall circulation period, there was a rather uniform vertical distribution of iron. Values were slightly higher than those encountered before the chelate addition. This increase was not unexpected and was probably due to normal chemical cycles within the lake.

Discussion

The results of photosynthetic measurements tend to confirm the conclusions of Schelske (1960) that in marl lakes (1) iron chelate alone brings about a modest increase in photosynthetic rate and (2) iron chelate combined with other essential nutrients provides a powerful stimulus to photosynthesis. This effect is much greater than can be achieved with nutrients alone.

Because sulfate was added along with the chelated iron (Table 1) there is the possibility that the photosynthetic increase was caused by the addition of sulfate and not by the chelated iron. Bottle experiments conducted with water from Sand Lake and other marl lakes have demonstrated that sulfate has no influence upon this response (Schelske 1960).

The stripping of iron from the trophogenic waters by the algal bloom in Sand Lake 1 suggests that an iron fraction in the water previously unavailable to algae was utilized in the presence of chelate. If true, this would provide some support for Schelske's hypothesis that marl lakes in Michigan are deficient in available iron because they lack naturally occurring chelating materials. In our experiment at Sand Lake 1, HEDTA was added

in excess of its chemical equivalent of iron. The excess may have reacted with sestonic iron compounds making the iron available to plants.

The decrease in iron content of the water after fertilization suggests that chelate was also lost from the lake water. If chelate had remained, it would have reacted with iron in the sediments and maintained iron concentration at a high level. Schelske (1960) has demonstrated that HEDTA reacts with iron in sediments and brings it into solution. The mechanism by which chelate disappeared from the water is not clear. Hutner et al. (1950) found that EDTA is non-metabolizable, hence HEDTA is probably also non-metabolizable. Provasoli (1960) suggests that EDTA may be lost by photooxidation; if so, this may have been the process removing HEDTA from Sand Lake 1. Whatever the mechanism, it appears that to maintain HEDTA in lake water it will have to be added at frequent intervals. Alternatively, a more stable compound of similar function might be utilized.

Summary

An experiment was performed to determine the effect of chelated iron (iron salt of N-hydroxyethylethylenediaminetriacetic acid) and commercial fertilizer on the primary productivity of a 14-acre marl lake in Grand Traverse County, Michigan. Rate of C^{14} uptake by phytoplankton was measured at a selected series of depths to determine the effects of the lake treatments. After the addition of chelated iron, C^{14} uptake increased approximately fourfold over the rate during a pretreatment (control) period. Increases in the rate of C^{14} uptake were detected only in strata of the lake

which received chelated iron. Fertilizer was added to the experimental lake 10 days after the addition of chelated iron. This increased the rate of C^{14} uptake approximately 60 times over the rate during the control period. In a second marl lake (a reference lake) the addition of fertilizer did not increase the rate of C^{14} uptake greatly in comparison to the increase found in the experimental lake. Decreasing water transparency was accompanied by increasing rates of C^{14} uptake. The effects of the lake treatments and changes in the rate of C^{14} uptake are considered in relation to the distribution of total iron, total phosphorus and dissolved oxygen.

Acknowledgments

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Table 1. --Chemicals added to Sand Lakes in 1959

Chemical	Lake 1 (Experimental)			Lake 2 (Reference lake)		
	Pounds	Pounds per acre	Concen- tration ↓ (parts per billion)	Pounds	Pounds per acre	Concen- tration ↓ (parts per billion)
July 27						
Versenol 120 (Dow)	491	34.6	584			
Ferrous sulphate (FeSO ₄ · 7H ₂ O)	300	21.1	357			
Iron (Fe ⁺⁺)	60	4.2	72			
Sulphate (SO ₄ ⁻⁻)	135	9.5	161			
August 6						
Fertilizer (12-12-12)	2,000	141.0	2,384	1,520	88.4	3,036
Potassium (K)	240	16.9	285	182	10.6	363
Nitrogen (N)	240	16.9	285	182	10.6	363
Phosphorus (P)	106	7.5	126	80	4.7	160

↓ Calculated on basis of total lake volume.

Table 2. --Relative rates of C¹⁴ uptake before and after treatment
with chelated iron on July 27
(Counts per minute C¹⁴ activity per hour exposure
calculated for entire lake volume)

Before treatment			After treatment			
July			July		August	
21	22	25	30	31	2	5
168,000	34,000	34,000	215,000	387,000	293,000	589,000

Table 3. --Analysis of variance tests of treatment with
chelated iron

	df	Sum of squares	Mean square	"F"
Before Treatment (All depths)				
Within dates	2	9.597	4.799	12.0**
Above vs. below	1	1.481	1.481	3.70
Residual	<u>14</u>	<u>5.602</u>	0.400	
Total	17	16.680		
After Treatment (All depths)				
Within dates	3	21.65	7.22	3.13
Above vs. below	1	19.51	19.51	8.44
Residual	<u>19</u>	<u>45.96</u>	2.31	
Total	23	65.12		
Before vs. After (Depths treated)				
Within depths	3	17.601	5.867	4.46*
Before vs. after	1	49.288	49.288	37.5**
Residual	<u>23</u>	<u>80.247</u>	1.315	
Total	27	97.136		
Before vs. After (Depths not treated)				
Within depths	1	1.679	1.679	0.59
Before vs. after	1	0.081	0.081	0.03
Residual	<u>11</u>	<u>31.282</u>	2.84	
Total	13	33.042		

* Significant

** Highly significant

Photosynthesis

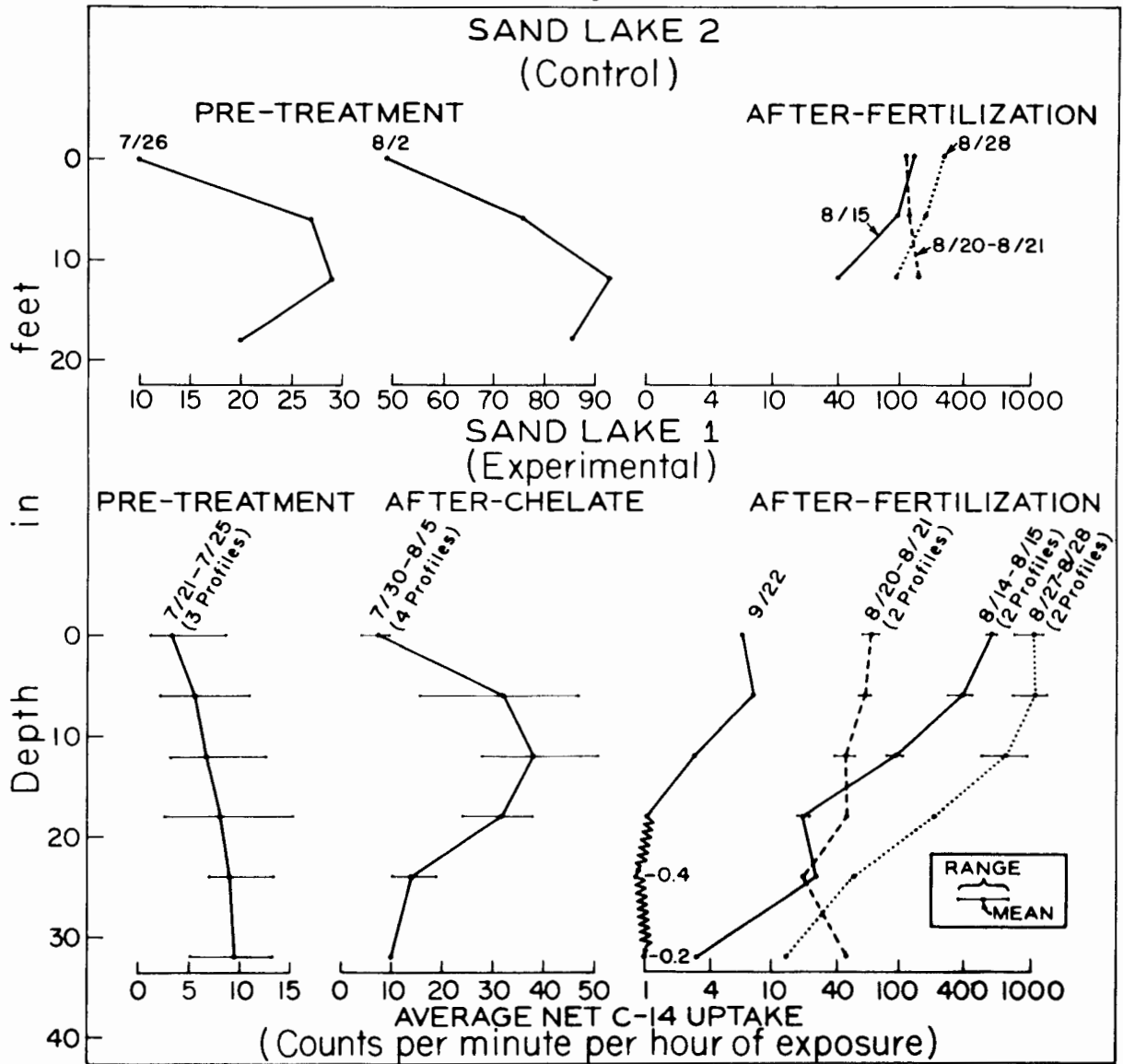


Fig. 1. --C¹⁴ uptake in Sand Lakes 1 and 2 before and after treatments with chelated iron and fertilizer.

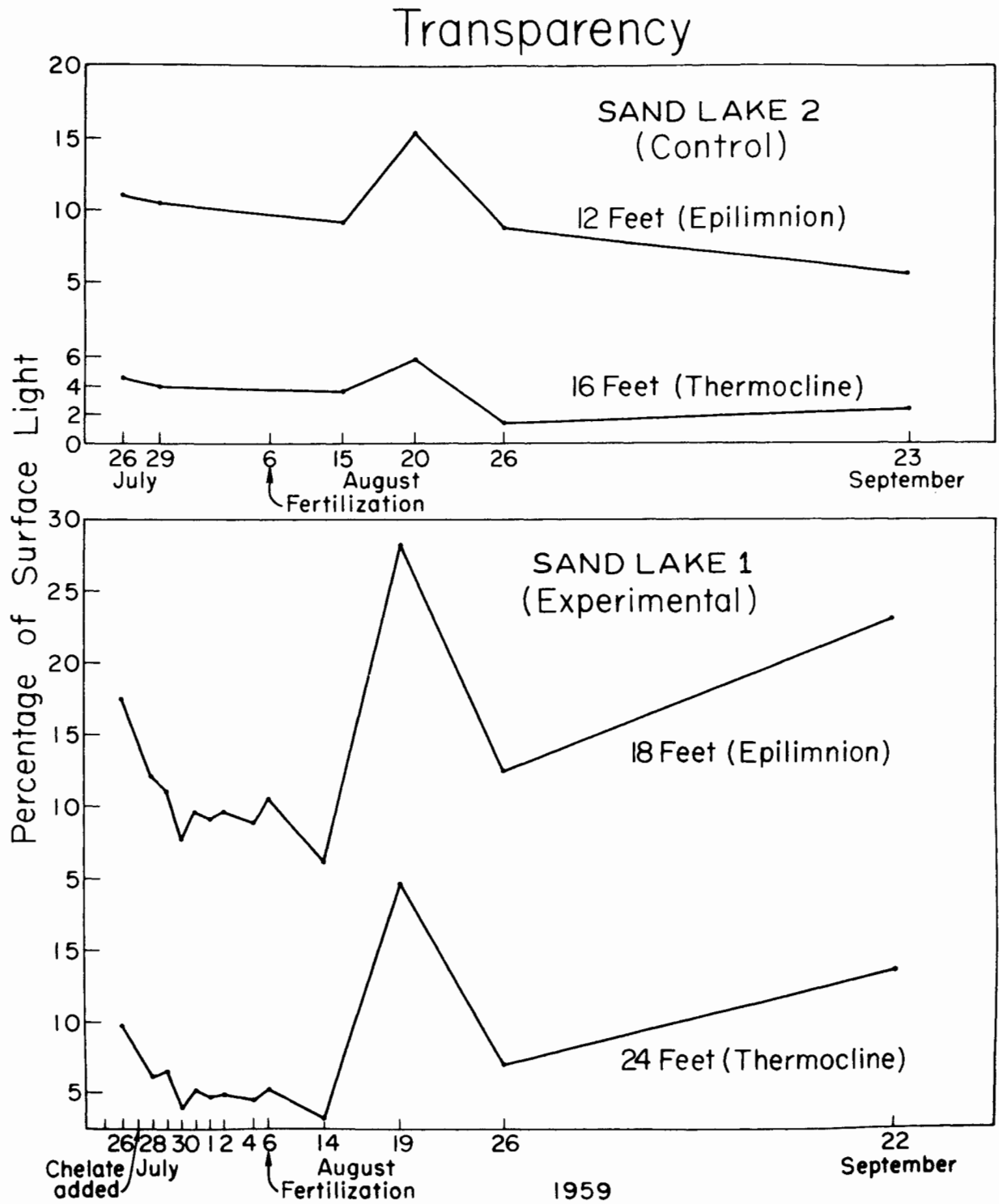


Fig. 2. --Percentage of light penetrating to selected depths in the epilimnion and thermocline of Sand lakes before and after treatments with chelated iron and fertilizer.

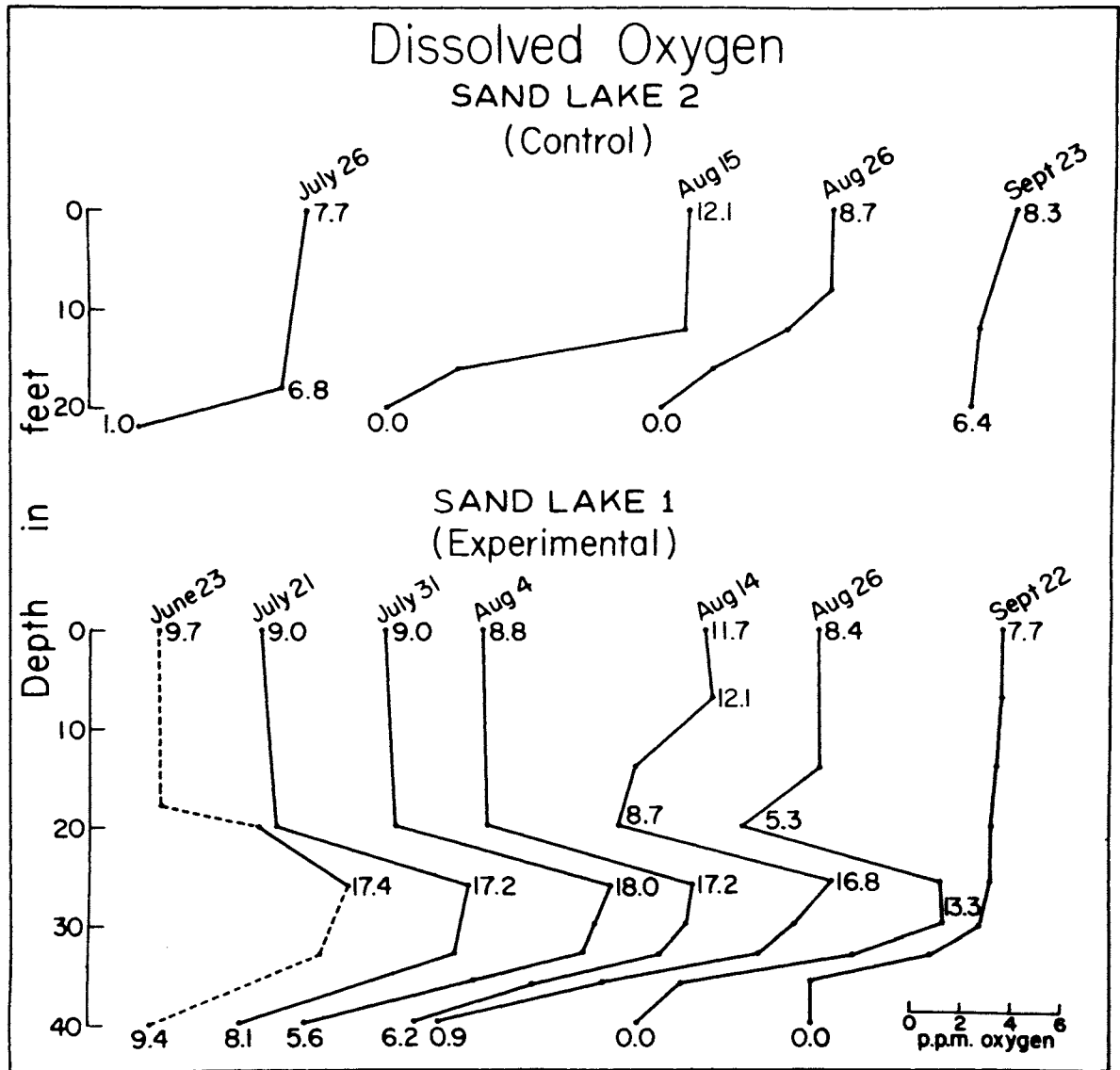


Fig. 3. --Dissolved oxygen concentration (parts per million) in Sand Lake 1.

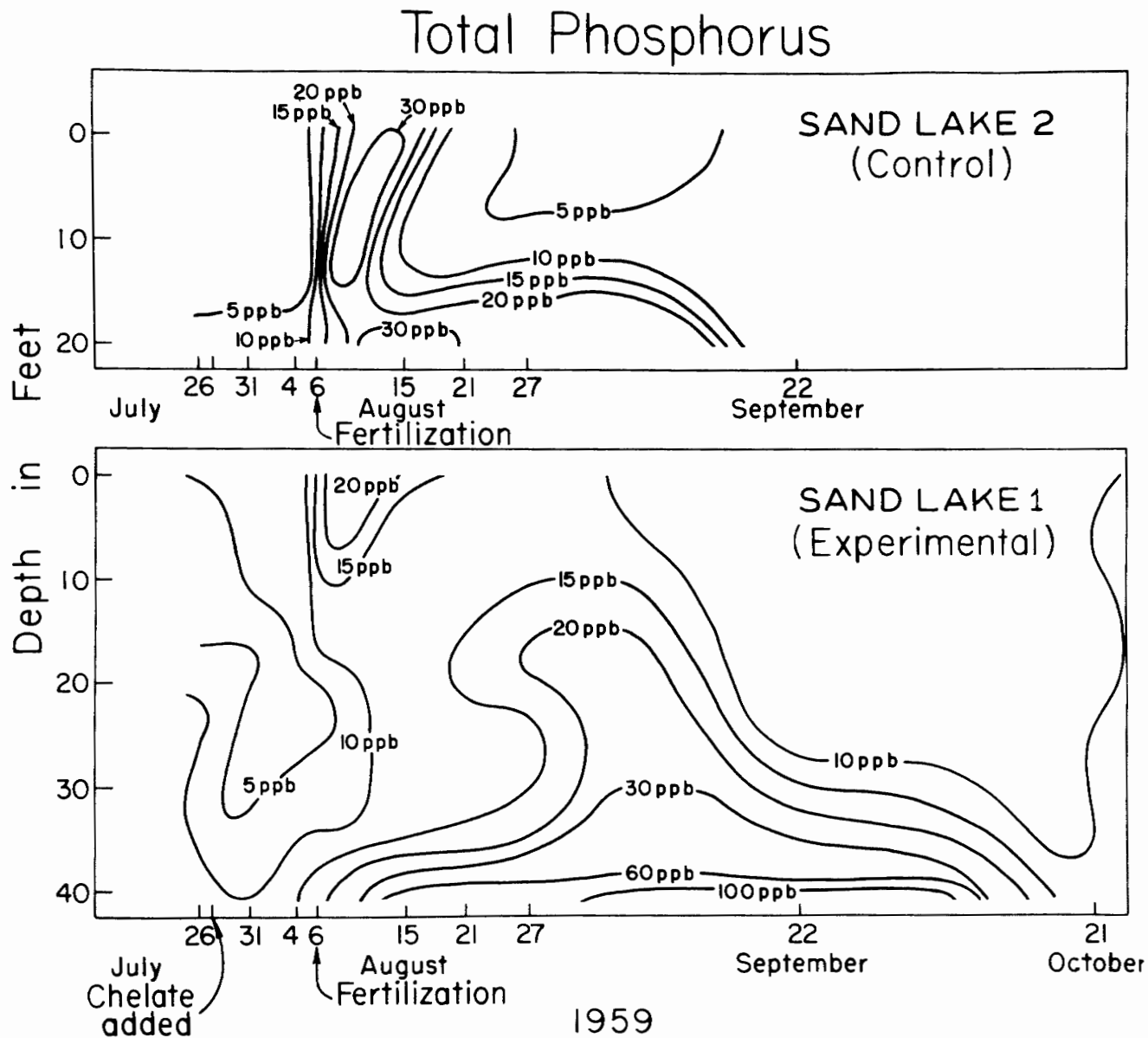


Fig. 4. --Depth-time diagram of total phosphorus in Sand lakes. Isopleths show total phosphorus in parts per billion.

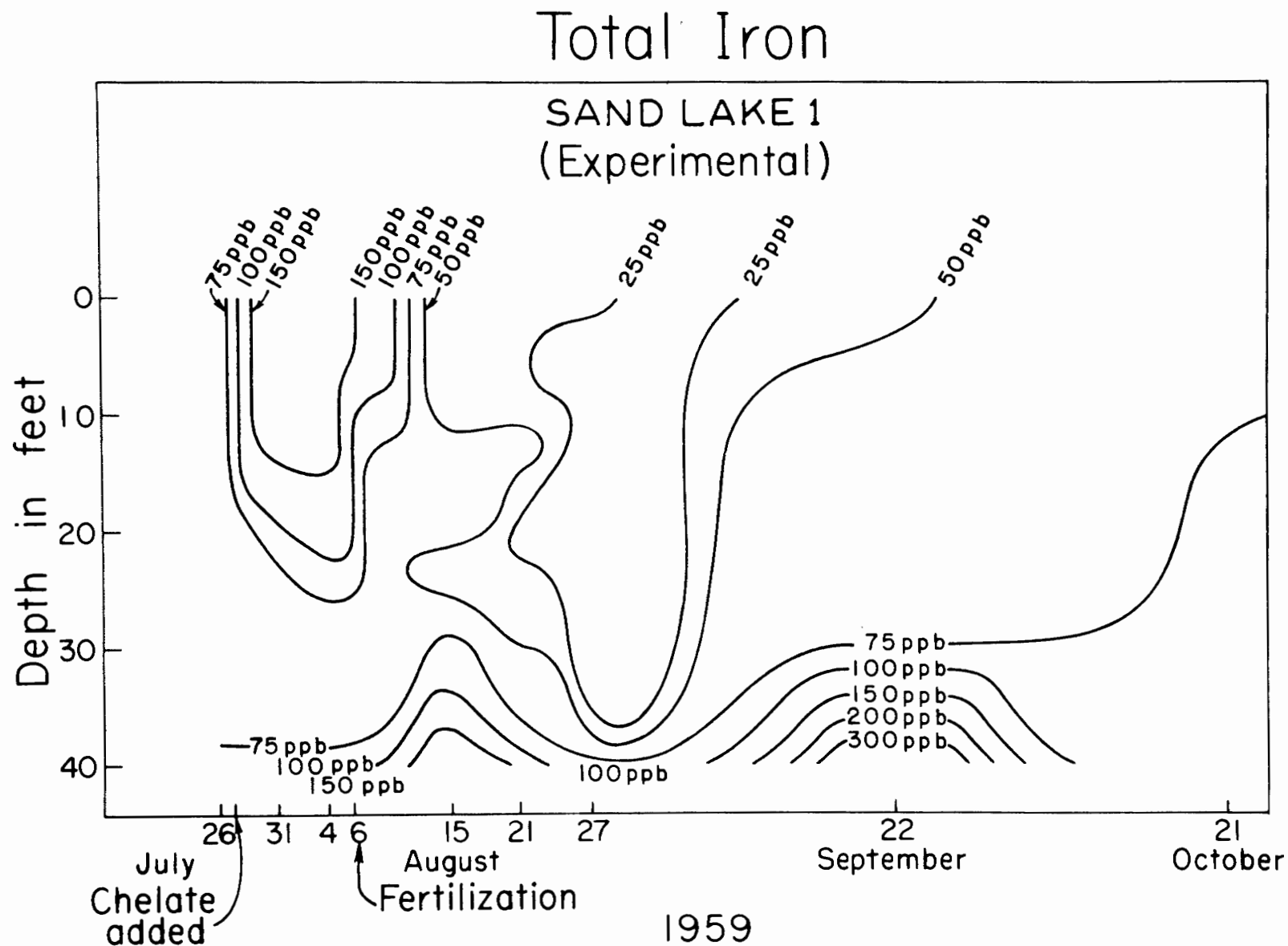


Fig. 5. --Depth-time diagram of total iron in Sand Lake 1. Isoleths show iron concentration in parts per billion.