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# PREDICTED EFFECTS OF FERTILIZERS UPON THE ALGAE PRODUCTION IN FERN LAKE[1]

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## INTRODUCTION

Fern Lake is situated about 60 km south-west of Seattle in Kitsap County, Washington, U.S.A. (Fig. 1). The lake was, in 1958, made the object of a 10-year research project by the Laboratory of Radiation Biology, University of Washington, with the Washington State Department of Game and the U.S. Atomic Energy Commission as sponsors. The objective was to study the mineral metabolism in the entire watershed in an attempt to accumulate knowledge for the improvement of natural resources (DONALDSON, OLSON and DONALDSON 1959).

Limnological descriptions of the lake are given by GELDIAY, DONALDSON and OLSON (1959) and by OLSEN and OLSON (1966). Details of the metabolism of certain elements have been gathered through tracing radioactive isotopes such as P-32, Ca-45, I-131 and Mo-99, using the whole lake as experimental field (DONALDSON 1962), (DONALDSON 1964), (PALUMBO *et al.* 1963).

The lake has been extensively studied both with respect to the rate of gross production by phytoplankton, composition and quantities of zooplankton and insects, as well as the growth of the fish. Originally the fish population consisted of eight species. To simplify the whole ecosystem to better control the fish production, the lake was depopulated in 1957 and again in 1960. Restocking has annually been made with 7,000–20,000 steelhead broods (*Salmo gairdneri*). The production of the lake is low, for fish in the order of 1/10 of the nearby Bay Lake, which has been used for comparison. OLSEN, CHAKRAVARTI and OLSON

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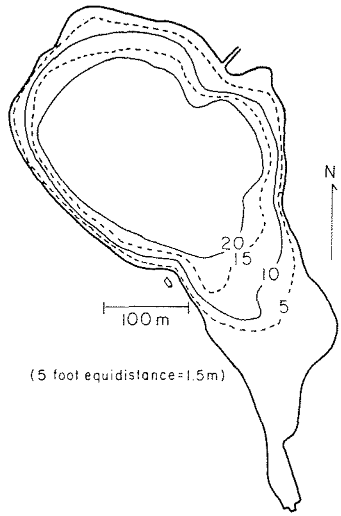
Contribution given in honour of Gunnar Rollefsen at his 70th birthday.




| | |
|---|---|
| Height above sea level | Appr. 64 m |
| Area of watershed incl. lake | Appr. 800 ha |
| Area of lake | 9.65 ha |
| Depth, max. | 7.6 m |
| Depth, mean | 4.3 m |
| Volume | 416,170 $m^3$ |
| Length | 625 m |
| Breadth, mean | 154 m |
| Shoreline | 1683 m |
| Development of shoreline | 1.5 |
| Development of volume | 1.7 |
| Variation of water level | ±0.3 m |

Fig. 1. Fern Lake. Morphometric data (OLSEN and OLSON 1966).

(1967) analyzed the spectrum of macro- and micro-element composition of the water, and demonstrated extremely low levels for most of the plant nutrients. They concluded that a nutrient deficiency was seriously limiting the production of the lake. By comparing the mineral composition of Fern Lake with that of the productive Bay Lake it seemed reasonable that the productivity of Fern Lake could be improved by fertilizing the water with the whole spectrum of elements, bringing them to the levels of Bay Lake. On the basis of such evaluations, OLSEN (1965) suggested the composition and amounts of fertilizers to be used (Table I).

Before the fertilization of the lake was initiated, pilot experiments were carried out in two of four plastic silos anchored in the lake. The silos each enclosed a water column of 154 $m^3$ lake water with a surface area of 30 $m^2$. They were both treated with fertilizers, one with the proposed concentration exclusive of iron and the other with iron included. The experiments revealed slowly increasing chemical reactions in the iron-treated silo, forming colloids resulting in a 75% decrease in transparency after a week (OLSON, personal communication). In the other silo no such effects were observed within the first week. A strong growth increase in the phytoplankton was recorded in both silos, after a short period of inhibition. About nine weeks after application the maximum rate of gross production was recorded in the iron-treated silo with a value in surface waters of 180 times the original gross production (OLSON, personal communication). Some doubt existed as to what degree the responses in the silos could be indicative of the lake itself due to the prehistory of the silo waters since the implantation of the silo in 1964 (OLSEN 1965). Also, since no information concerning optimum fertilizer concentrations could be obtained from the pilot experiment, a bioassay analyzing effects of different quantities of fertilizers and specifying the importance of iron upon the rate of gross production in lake water was scheduled.

Different approaches have been used in measuring limiting nutrient factors in natural phytoplankton populations since RHYTER and GUILLARD (1959) and GOLDMAN (1960) applied the C-14 technique on similar problems for seawater and lakes, respectively. A review of this literature is given by WETZEL (1965) and GOLDMAN (1965). The present investigation is in principle similar to the bioassays of limiting factors made by RYTHER and GUILLARD (1959), since the analyzes are made on water enriched with different degrees of the whole spectrum of fertilizers and deals with the effect of removing one of the trace elements. It deviates, however, in that respect that a large volume of water was applied and that the analyzes of effects were carried out until a maximum level in the rate of primary production seemed to be reached.

## MATERIALS AND METHODS

The bioassay was carried out in Fern Lake during the period 9 August–1 September 1966 in a series of fourteen 5-gallon glass bottles. Previous to the experiment the bottles were washed and rinsed in hot 2% $Na_2CO_3$ solution followed by hot 1% HCl solution and finally rinsed several times in lake water. Fifteen litres of lake water, collected 10 cm below surface from a raft anchored in the lake was filled into the bottles, and fertilizers added as follows:

| | | | | | |
|---|---|---|---|---|---|
| Container No. | 1 and 5 | ........ | No | fertilizers | added |
| | 2 and 9 | ........ | $\frac{1}{4}$ (c-Fe) | » | » |
| | 3 and 10 | ........ | $\frac{1}{2}$ (c-Fe) | » | » |
| | 4 and 11 | ........ | 1 (c-Fe) | » | » |
| | 6 and 12 | ........ | $\frac{1}{4}$ c | » | » |
| | 7 and 13 | ........ | $\frac{1}{2}$ c | » | » |
| | 8 and 14 | ........ | 1 c | » | » |

where c refers to the composition and concentrations given in Table I, c-Fe to the same concentrations omitting iron.

The fertilizers were made from p.a. grade chemicals in five separate solutions in order to avoid precipitations:

| Sol. 1 | Sol. 2 | Sol. 3 | Sol. 4 | Sol. 5 |
|---|---|---|---|---|
| $MgSO_4$ | $Ca(NO_3)_2$ | $H_3BO_3$ | $NH_4H_2PO_4$ | $FeSO_4$ |
| $K_2SO_4$ | $CaCl_3$ | $MnCl_2$ | $NaHCO_3$ | |
| $ZnSO_4$ | | $CoCl_2$ | | |
| | | $H_3MoO_4$ | | |
| | | KI | | |

The containers were plugged with rubber stoppers perforated with 1 cm glass tubing (30 cm long) with a bent plastic tube at the upper end. This system allowed free gas exchange and acted, at the same time, to guard the cultures from accidental contamination. Arranged in numbered sequence, the containers were left floating in the lake attached to a line anchored between the raft and the western shore (Fig. 2).

At intervals as given in Table II, parallel samples were collected from the containers into 125 ml Pyrex glass bottles for productivity measurements. Sampling was made with a squeeze-ball pump, after a thorough mixing of the content was secured by squeezing air into the bottom of the containers. The samples were placed in an incubator, injected with 2,5 microcuries C-14 as $NaHCO_3$, and incubated under artificial light for four hours (Steemann-Nilsen 1952). Similarly, bottles wrapped in aluminium foil were used to evaluate C-14 dark fixation during the same time. The temperature in the incubator was controlled to that of the lake. After four hours, the samples were filtered on Millipore filters (HA), preserved and removed of inorganic $^{14}CO_3^{--}$ by afterfiltering of 10 ml 10% formalin. Following drying in a desiccator, $\beta$ counting

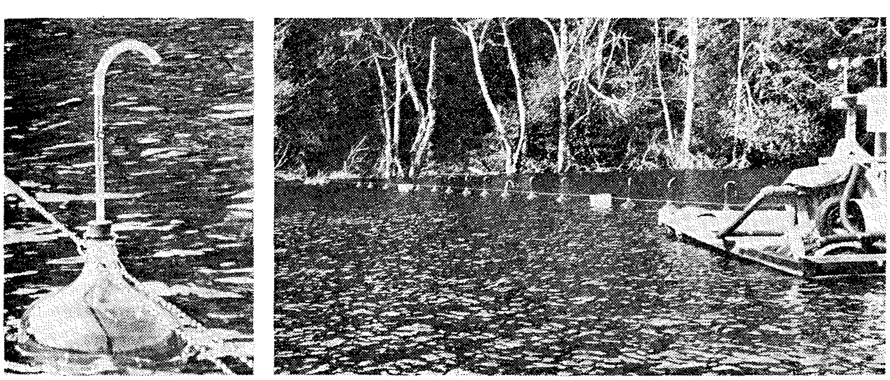


Fig. 2. Fern Lake bioassay. Experimental set-up.

of the MP filters was made on a Nuclear Chicago low-level $\beta$ counter. The efficiency of the system was 6%.

Total alkalinity was determined from titrations with 0.02 N HCl in 200 ml samples, and the total available $CO_2$ was calculated as mgC/l using the conversion tables given by SAUNDERS, TRAMA and BACHMANN (1962). The rate of gross production under the standardized condition was calculated from the net count of the light and dark bottles as follows:

$$P = \frac{(\text{cpm}_L - \text{cpm}_D) \cdot 1.1 \cdot \text{total } CO_2}{R}$$

where $\text{cpm}_L$ and $\text{cpm}_D$ = counts per min. net from light- and dark-exposed samples,
R = dpm C-14 added times counter efficiency times correction for MP filter absorption (SAUNDERS *et al.*, 1962),
1.1 = correction for isotope discrimination and respiration.

Estimates of *in situ* rates of gross production were made according to BERGE (1958).

## RESULTS AND DISCUSSION

The net cpm from the duplicate C-14 light incubator measurements are listed in Table II. Dark bottles were run only for one of the controls and for containers 13 and 14 (half- and full-fertilized lake water). These results are listed in Table III as net count per minute.

The data demonstrate generally good agreement between the duplicate C-14 measurements. On the 96% confidence level the average of the C-14 measurements are accurate to 14.8% on the whole, and for the first four days better than 7.7%. Within these errors of measurement, the results from the parallel series of experiments mostly agree very well. An exception demonstrate the duplicates with ½ (c-Fe) fertilizer by the end of the experiment where the deviation from their mean is greater than 60%. The container with the higher of the values had, by the tenth day of observation, lost the stopper and glass tube protection. During the application of fertilizers to the lake initiated that day, contamination of this container likely has happened. In the following evaluations, this value has therefore been omitted.

No serious bacterial activity occurred in the samples, the dark fixation of C-14 being rather constant throughout the experiment (Table II), and constituting a decreasing significance in the C-14 measurements. It appears that day to day variations in the rate of photosyntesis occurred and had the same trend in the controls and in the other containers. Such variations were expected as effects of day to day changes in environmental conditions. Since the effects presumably act proportionally on the activities in the cultures, they are eliminated in analyzing changes in the rate of gross production for each of the containers relative to those of the controls at the same time. Such data have been calculated as percentages of the average from the two controls and are listed in Table 1.

Table 1. C-14 fixation in the fertilized containers as percentages of that in non-fertilized lake water. Values are averages of duplicate measurements in four-hour light box incubations.

c = full proposed concentration of fertilizer in lake water.

c-Fe = full proposed concentration of fertilizer less iron in lake water.

| | Time after fertilization | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4 hrs | 24 hrs | 2 days | 4 days | 7 days | 11 days | 17 days |
| $\frac{1}{4}\cdot$(c-Fe) | 80 | 98 | 123 | 236 | 673 | 1223 | 1533 |
| $\frac{1}{4}\cdot$(c-Fe) | 85 | 111 | 147 | 200 | 455 | 1400 | 1356 |
| $\frac{1}{4}\cdot$c | 84 | 119 | 164 | 242 | 392 | 1688 | 2101 |
| $\frac{1}{4}\cdot$c | 79 | 109 | 152 | 226 | 748 | 1647 | 2072 |
| $\frac{1}{2}\cdot$(c-Fe) | 75 | 87 | 99 | 188 | 607 | 1804 | 10019 |
| $\frac{1}{2}\cdot$(c-Fe) | 75 | 86 | 117 | 187 | 413 | 3231 | 2484 |
| $\frac{1}{2}\cdot$c | 76 | 103 | 143 | 268 | 617 | 4131 | 5841 |
| $\frac{1}{2}\cdot$c | 65 | 81 | 105 | 244 | 397 | 3038 | 3335 |
| 1.(c-Fe) | 56 | 63 | 83 | 208 | 490 | 2817 | 7445 |
| 1.(c-Fe) | 56 | 61 | 73 | 205 | 493 | 2505 | 8061 |
| 1.c | 61 | 84 | 112 | 211 | 414 | 1227 | 20732 |
| 1.c | 57 | 70 | 87 | 291 | 423 | 2583 | 15317 |

It is evident that no increase in the C-14 fixations was obtained in the fertilized containers before closely one day after application and in some instances, not before the second and third day after application (Fig. 3). On the contrary, inhibitions in the rate of C-14 fixations after four hours were evident in all the fertilized containers. The inhibitions, demonstrated by the maximum depressions in the curves are highly significant as confirmed by a t-test, and are increasing with increasing amounts of fertilizer applied. However, they are not significantly different for the series with and without iron as demonstrated by Table 2 and the diagram in Fig. 4 (left).

The intervals before the cultures have recovered increased with the amount of fertilizers used, but they were also significantly different for the series with and without iron: consistently, the iron-treated containers showed more rapid recovery (Fig. 4, right).

The inhibition was probably due both to sudden changes in the osmotic pressure of the water and to toxic effects from one or more of the elements introduced (GOLDMAN 1965) and (WETZEL 1965). The difference

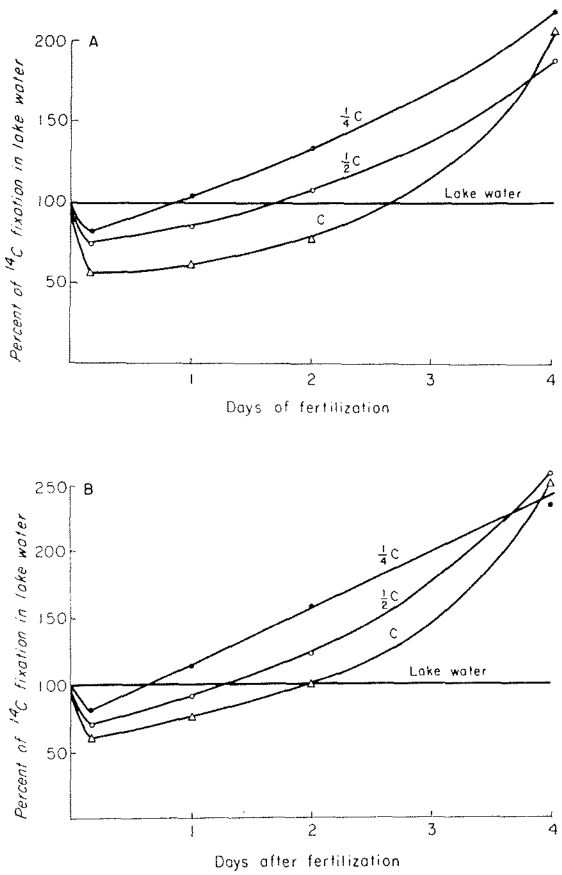



Fig. 3. Effects of fertilizers upon the rate of gross production during the first four days of the experiment. (A) without iron, (B) with iron, c = proposed concentration of fertilizers.

Table 2. Inhibition in C-14 fixation four hours after fertilization (%) and the time until recovery (hrs).

| Concentration of fertilizer | Fertilizer inclusive of iron | | Fertilizer exclusive of iron | |
|---|---|---|---|---|
| | % inhibition at 4 hrs. | Duration in hrs. | % inhibition at 4 hrs. | Duration in hrs. |
| ¼ c | 17.6 | 20 | 18.5 | 16 |
| ½ c | 25.1 | 40 | 29.4 | 31 |
| c | 43.9 | 64 | 41.0 | 46 |

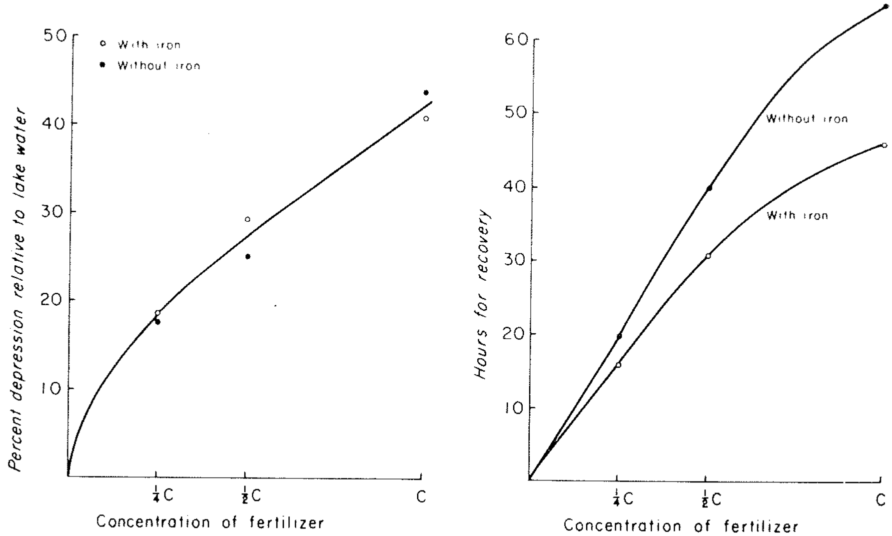



Fig. 4. Maximum inhibition of $^{14}C$ assimilation (left) and time before recovery (right) for different degrees of fertilization.
c = proposed concentration of fertilizers.

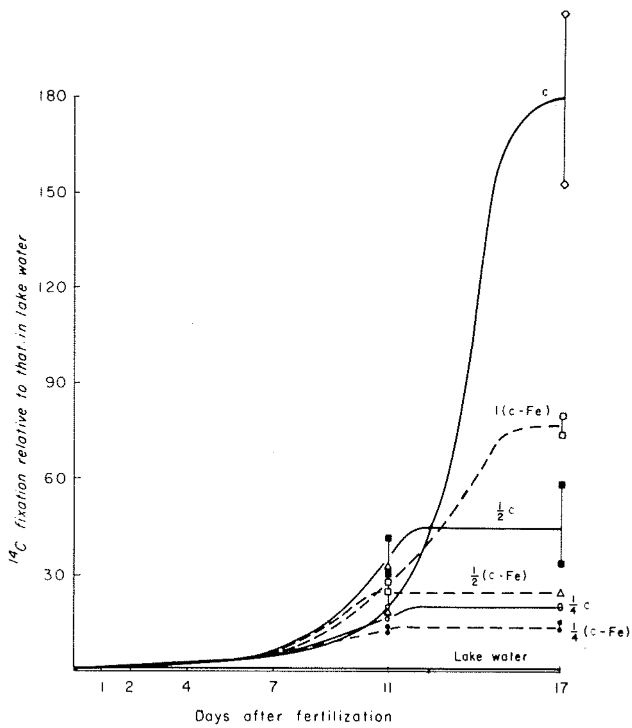



Fig. 5. Effects of fertilizers upon the rate of gross production.
c = proposed concentration of fertilizers, c-Fe = proposed concentration of fertilizers less iron.

in times when the inhibitions were effective in containers with and without iron, can be explained by the chemical reactions iron created in lake water, slowly forming colloidal materials less available to the plankton and with reduced effect on the osmotic pressure.

The data from Table 1 have been plotted in the diagram of Fig. 5 and growth curves are matched to the average of each of the duplicate series. It is evident that very high increases occurred in the rate of production in all the fertilized containers following a rather long lag phase. The final effects increased with increasing amounts and were higher in the series with iron than without. By the end of the experiment the production in the containers had apparently reached a steady state. For $\frac{1}{4}$ (c-Fe), $\frac{1}{2}$ (c-Fe) and c-Fe these levels seem to be limited by iron deficiency, since the corresponding series with iron all reached considerably higher values (Fig. 6). The highest effect was demonstrated in the container with full proposed fertilizers, where the rate of gross production had reached levels 180 times higher than in the non-treated lake water. Similarly, the rate of production in the fully fertilized containers without iron was 75 times that of lake water. The trend in the curves (Fig. 6) further indicates that stronger fertilizations than made both with and without iron would still increase the rate of production. The necessary quantities to reach maximum effects are not predictable from this experiment.

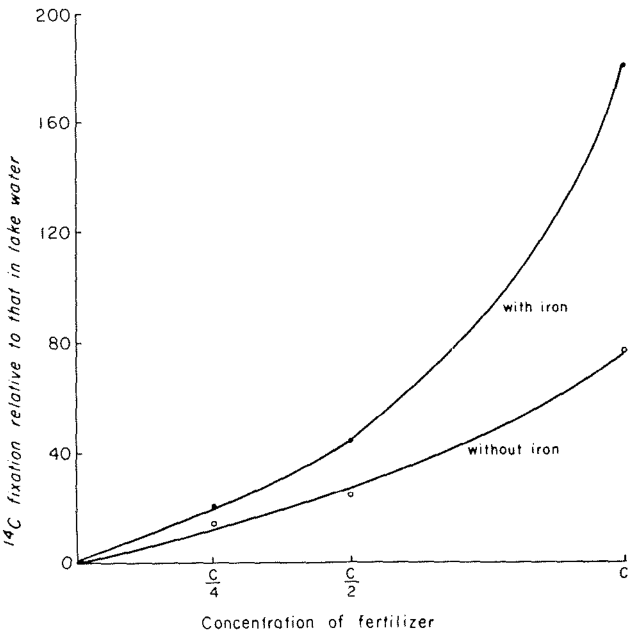



Fig. 6. Relations between rates of gross production in fertilized lake water with and without iron, seventeen days after treatment.

Using the average cpm for the duplicate experiments and 2.3 mg C/l as a representative average value for the total available $CO_2$ (Table IV), the ultimate rates of production reached in the differently treated containers have been calculated and are shown in Table 3.

Table 3. Rates of gross production in the fertilized and non-fertilized containers at the end of experiment. Values are average of the series.

| | Av. cpm at 17 days | P mg C/m³ day | Rel. to lake water |
|---|---|---|---|
| Lake water | 689 | 24.8 | 1.0 |
| Lake water ¼ (c-Fe) | 9,952 | 358 | 14 |
| Lake water ½ (c-Fe) | 17,113 | 924 | 25 |
| Lake water 1 (c-Fe) | 53,419 | 1923 | 78 |
| Lake water ¼ c | 14,376 | 518 | 21 |
| Lake water ½ c | 31,608 | 974 | 40 |
| Lake water 1 c | 124,190 | 4470 | 180 |

Although iron, where not applied, evidently became the limiting factor at the increased rates of productivity, it is interesting to note that increased applications of fertilizers without iron still caused increased growth. These findings seem to indicate that the iron possibly was available as contaminant in the other fertilizers. The present materials do not allow analyzes in this regard, and several possibilities for its explanation exist. It might be suggested that some other element partly can replace iron in matebolic processes or that the increased fertilizer concentraction changes the redox potential enough to alter the availability of naturally existing iron. Also, selection of different communities of organisms having different iron response rates might likely have occurred.

Care should be used in interpreting results from the experiments to the situation in the lake. The enclosure of small volumes of lake water in containers creates a serious change in the plankton ecology. Significant is the increased ratio of surface to volume of water; also the effects from changes in light intensity, wind mixing, and air temperature are seriously altered, and together these factors could influence both the composition and the activities of the primary producers. It is also obvious that the 15 litre samples do not represent a complete miniature of the ecosystem of the lake, and the intereffect by organisms in the containers is more or less artificial. However, using the results obtained relative to those from non-treated containers, the mentioned effects are minimized or, in some cases, even eliminated. It is thus felt that the general conclusions drawn should be valid also to the lake itself under steady state conditions. It is interesting to note that the effect of full proposed fertilization with iron included was identical to the maximum effects recorded in the pilot experiment with the plastic silo. However, with respect to maximum effects, these should be considerably lower in the lake under natural conditions where dispersion and dilution would reduce the amount of fertilizers available to the primary producers.

The total rate of gross production of the lake is the sum of all the rates per unit volume at any place and depth, $P_{a,z}$. The latter is again related to the rate of gross production measured under standardized conditions ($P_i$) in the incubator the following way

$$P_{a,z} = P_i \cdot \frac{I_{a,z}}{I_i}$$

where $I_{a,z}$ = effective light at a,z and $I_i$ = effective light in the incubator. The relation is valid as long as light intensities are below saturation for the producing population. For the major part of the lake's annual gross production, the relation is assumed to be valid. Forming the relation between the rates of production in fertilized water ($P'_{z,a}$) and in non-fertilized water ($P_{a,z}$), we have

$$\frac{P'_{a,z}}{P_{a,z}} = \frac{P'_i}{P_i} \cdot \frac{I'_{a,z}}{I_{a,z}} = \frac{P'_i}{P_i} \cdot \frac{T'}{T}$$

where T and T′ are the light transmissions of the water before and after fertilization. The total effect of the fertilization on the *in situ* rates of production at any place and depth of the lake is thus equal to the relative change in the rate of production at standardized conditions of the same sample times the relative change in light transmission of the water.

## CONCLUSION

From the results presented, the following conclusions can be drawn.

Application to Fern Lake water of the proposed concentration of fertilizers, with and without iron, acts as a preliminary inhibitor upon the photosynthetic activity of the phytoplankton. For each of the two compositions of fertilizers used, the extent and duration of the inhibitions are nearly proportional to the amount of fertilizers introduced; addition of iron sulfate reduced the duration of the inhibition.

Following preliminary inhibition, a strong increase in the rate of production per unit volume and light occurs, increasing with increasing amounts of fertilizers. The trend of the results points out that the optimum effects will be reached with higher applications than proposed.

At the raised levels of photosynthetic activity, iron became a limiting factor if not applied. Application of iron raised the effect of fertilizers on the ultimate rate of gross production in the containers with an average factor of about 2. However, chemical reactions occurred in the water, forming colloidal materials which decreased the light transmission. The pilot experiments with Fern Lake water showed a 75% reduction in light transmission, a situation that seemed to persist over several months. Even with a twofold gain in the rate of gross production under standardized conditions relative to that of the iron-free parallels, the counteracting effect of the reduced transparency of the lake would cause a probable loss in the total gross production of the lake, as long as the colloidal materials persisted.

Since technical grade chemicals were planned to be used in the fertilization of the lake, it was anticipated that iron might be present as a contaminant, sufficiently not to become limiting.

The following advices for a successful improvement of the primary productivity can be given:

1. Fertilizers should be added to the lake in the full proposed concentration, exclusive of iron. By extrapolating the results, it seems evident that higher effects can be reached with higher applications.

2. Application of 100 ppb of iron ($Fe^{2+}$) increases the effect of fertilizers upon the rates of gross production under standardized light conditions. However, due to the effect of this amount upon transparency, it will reduce the integrated rate of gross production of the lake, and should, therefore, not be included.

3. After completed fertilization as above, bioassays with lake water should be repeated to investigate whether iron had become the limiting factor on the rate of production, and from similar experiments, possibly indicate quantities of eventual later additions.

4. The applications of fertilizers should be made sequentially, using one quart or less at a time, in order to minimize inhibition of the primary producers. For the same reason, rapid and thorough mixing with the whole water column seems important. The time intervals between applications should be three days or more to ensure rehabilitation in the plankton productivity between applications.

## SUMMARY

1. With the object of predicting effects of different amounts of fertilizers, upon the rate of primary production in Fern Lake, a bioassay was carried out in a series of 14 twenty liters glass carboys.
2. The experiment revealed that the rate of gross production increased nearly proportionally with the amounts of fertilizers added and a maximum increase of 180 times the orginal rate of gross production was obtained.
3. When iron was included with the fertilizers colloidal substances were formed acting upon the transparency of the water. When it was excluded it became limiting on the rates of primary production.
4. It is demonstrated that due to reduced transparency the inclusion of 100 ppm iron would not increase the total primary rate of production of the lake any further.
5. Suggestions for the fertilization of Fern Lake are given.

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# APPENDIX

Table I. Fern Lake water analysis together with the proposed addition of fertilizers. (Data from Olsen *et al.* (1967) and Olsen (1965).

| Macro-elements mg/l | Lake Surface | Water Bottom | Proposed added (c) | Fertilizers Sources and Amounts |
|---|---|---|---|---|
| Ca | 1.4 | 1.6 | 2.5 | $Ca(NO_3)_2$ 5.12 mg/l |
| $NO_3$ | | | 3.8 | |
| $NH_4$ | | | 0.1 | $CaCl_2$ 3.46 » |
| Cl | 2.8 | 3.1 | 2.2 | |
| Mg | 0.3 | 0.2 | 0.5 | $MgSO_4 \cdot 7H_2$ 05.0 » |
| $SO_4$ | | | 2.6 | |
| K | 0.2 | 0.1 | 0.5 | $K_2SO_4$ 1.42 » |
| Na | 1.5 | 0.5 | 1.0 | $NaHCO_3$ 3.66 » |
| $HCO_3$ | | | 2.7 | |
| $PO_4$-P | 0.004 | 0.009 | 0.2 | $NH_4H_2PO_4$ 0.74 » |
| **Micro-elements µg/l** | | | | |
| B | | | 10 | $H_3BO_3$ 57.1 µg/l |
| Co | 6.4 | 11 | 10 | $CoCl_2$ 22.0 » |
| I | | | 50 | KICl 65.4 » |
| Mn | | 24 | 50 | $MnCl_2$ 179.8 » |
| Mo | 0.2 | 0.2 | 10 | $H_2MoO_4 \cdot H_2O$ 18.8 » |
| Zn | 18.6 | 30.5 | 15 | $ZnSO_4 \cdot 7H_2O$ 65.9 » |
| Cu | 7.0 | 15 | 0 | |
| Ni | 5.4 | 2.5 | 0 | |
| Fe | 110 | 134 | 100 | $FeSO_4 . 7H_2O$ 500 » |

Table II. C-14 fixation in plankton from containers with untreated and with fertilized Fern Lake water.
Values in net CPM of C-14 assimilated from $2\frac{1}{2}$ $\mu$Ci $^{14}CO_2$ per 125 ml of sample. Parallel runs in four hours light-box incubations.
c = full proposed concentration of fertilizer in lake water. c-Fe = full proposed concentration of fertilizer less iron in lake water.

Time after fertilization

| Container # and contents | 9/VIII-4 hrs. CPM | 10/VIII-1 day CPM | 11/VIII-2 days CPM | 13/VIII-4 days CPM | 16/VIII-7 days CPM | 20/VIII-11 days CPM | 26/VIII-17 days CPM |
|---|---|---|---|---|---|---|---|
| No. 1: Lake Water | 968 | 1379 | 1119 | 760 | 987 | 992 | 683 |
| | | 1275 | | 761 | 1077 | 937 | 664 |
| 5: Lake Water | 1079 | 1143 | 1140 | 529 | 807 | 2062 (b) | 714 |
| | 1151 | 1312 | 1304 | 542 | 906 | | 694 |
| 2: $\frac{1}{4}$ (c-Fe) | 858 | 1273 | 1491 | 1528 | 6927 | 12804 | 12031 |
| | 806 | 1223 | 1397 | | 6007 | 11611 | 8992 |
| 9: $\frac{1}{4}$ (c-Fe) | 853 | 1423 | 1716 | 1297 | 4678 | 14269 | 8992 |
| | 927 | 1419 | 1725 | 1297 | 4064 | 13682 | 9692 |
| 3: $\frac{1}{2}$ (c-Fe) | 798 | 1124 | 1109 | | 5831 | 17840 | 66650 |
| | 760 | 1094 | 1205 | 1221 | 5831 | 18165 | 71411 |
| 10: $\frac{1}{2}$ (c-Fe) | 784 | 1106 | 1426 | 1276 | 4256 | 32241 | 18851 |
| | 688 | 1090 | 1323 | 1142 | 3673 | 32241 | 16376 |
| 4: 1 (c-Fe) | 603 | 807 | 988 | 1580 | 4933 | 31233 | 52615 |
| | 588 | 808 | 941 | 1115 | 4487 | 24983 | 49963 |
| 11: 1 (c-Fe) | 574 | 761 | 808 | 1352 | 4791 | 24376 | 55539 |
| | 591 | 798 | 888 | 1287 | 4678 | 25624 | 55539 |
| 6: $\frac{1}{4}\cdot$c | 897 | 1500 | 1943 | 1432 | 3593 | 15778 | 14476 |
| | 856 | 1545 | 1895 | 1704 | 3935 | 15856 | 14476 |
| 12: $\frac{1}{4}\cdot$c | 840 | 1349 | 1772 | 1424 | 9157 | 37020 (a) | 13872 |
| | 813 | 1423 | 1788 | 1510 | 5218 | 31233 (a) | 14689 |
| 7: $\frac{1}{2}\cdot$c | 762 | 1270 | 1646 | 1655 | 5033 | 45437 | 37020 |
| | 832 | 1351 | 1692 | 1818 | 6832 | 37020 | 43461 |
| 13: $\frac{1}{2}\cdot$c | 669 | 1016 | 1216 | 1588 | 3361 | 29395 | 23239 |
| | 687 | 1039 | 1241 | 1570 | 4312 | 31233 | 22710 |
| 8: 1$\cdot$c | 570 | 1091 | 1381 | 1397 | 4202 | 13141 | 142840 |
| | 698 | 1064 | 1342 | 1331 | 3756 | 11347 | 142840 |
| 14: 1$\cdot$c | 573 | 1349 | 1024 | 4098 (a) | 4064 | 27761 | 111094 |
| | 624 | 1423 | 1008 | 1678 | 1503 | 23793 | 99983 |

(a) 5 $\mu$Ci added. (b) Two samples on one filter.

Table III. $^{14}C$ fixation in dark bottles during four hours incubator experiments. Values are net cpm of $^{14}C$ fixed from $2\frac{1}{2}$ $\mu Ci$ $^{14}CO_2$ per 125 ml sample.
c = full proposed concentration of fertilizers.

| | Time after start of experiment | | | |
|---|---|---|---|---|
| | 2 days | 4 days | 11 days | 17 days |
| Lake Water | 94 | 90 | 110 | 103 |
| $\frac{1}{2}\cdot$c+ Lake Water | 104 | 107 | 200 | 300 |
| 1 ·c+ Lake Water | 113 | 93 | 219 | 269 |

Table IV. Physical-chemical properties in the experimental containers.

| | Total Alk. | t°C | pH | $CO_2$mg C/l |
|---|---|---|---|---|
| Lake water, before exp. | 8.20 | 22.5 | 7.2 | 2.26 |
| Lake water, at end of exp. | 7.92 | 23 | 6.65 | 2.87 |
| Lake water with $\frac{1}{4}$ c fert. at and of exp. | 9.08 | 23 | 9.30 | 2.00 |
| Lake water with $\frac{1}{2}$ c fert. at and of exp. | 9.60 | 23 | 9.80 | 2.11 |
| Lake water with 1 c fert. at end of exp. | 10.20 | 23 | 9.70 | 2.24 |
| Average total $CO_2$ as mg C/l = | | | | 2.30 |