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EXPERIMENTAL STUDY OF THE PHOSPHORUS CYCLE
IN FERTILIZED SALT WATER

BY

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ABSTRACT

The rates at which phosphorus is (1) assimilated by phytoplankton, (2) released into solution from dead cells, and (3) regenerated to inorganic state, were measured in outdoor concrete tanks containing sea water fertilized with inorganic phosphate and nitrate. Bottles wrapped in black cloth and bottles exposed to the light were filled with the tank water and suspended in the tanks; the three rates in the P cycle were calculated from the observed changes in the concentrations of inorganic and particulate P in the bottles. The maximum recorded rates were: assimilation = 0.36 µg-at. P/L of sea water in the tanks/day; solution = 0.38 µg-at./L/day; regeneration = 0.13 µg-at./L/day. In 18 series of measurements, during which phytoplankton increases predominated over decreases, the following relations were observed between the rates of phosphorus transformations and the size and absolute change of size of the phytoplankton populations: (1) The rate of phosphorus assimilation was significantly correlated with the size of the phytoplankton standing crop and showed an even stronger dependence upon the increment of growth. (2) The rate at which particulate organic phosphorus was released into solution was intimately related to the size of the standing crop and was independent of the change in population size (which in most cases was an increase). (3) The regeneration of dissolved inorganic phosphate from dissolved organic phosphorus depended upon the phytoplankton standing crop and showed no relation to the change in population size. If attached algae were allowed to grow on the sides and bottom of the tanks, in less than one month they removed three-fourths of the added phosphate from the waterphytoplankton system. Where attached algae were largely prevented from growing, four-fifths of the phosphorus originally present was detected in the water at the end of four weeks.

Our understanding of the dynamics of biological productivity is measured by our knowledge of certain rates of transfer of matter and energy in the environment (Clarke, 1946). The productivity of most aquatic environments depends heavily upon the growth and maintenance of the phytoplankton, and this in turn is frequently limited to the rates at which phosphorus is regenerated and assimilated (cf. Ketchum, 1947). The rates of these processes in the cycle of phosphorus thus assume the greatest theoretical interest. Furthermore, a knowledge of the rates of assimilation and regeneration of plant

1 Contribution number 497 from the Woods Hole Oceanographic Institution.
nutrients such as phosphorus is essential for the most intelligent direction of a program of fertilizing natural waters, salt or fresh. In spite of their cardinal importance from the practical as well as academic standpoint, the magnitude of such rates remains almost completely unknown. Our ignorance of these matters can probably be attributed largely to the difficulties of measurement.

Redfield, Smith and Ketchum (1937) have calculated the net or minimal rates of the phosphorus metabolism in a column of water in the Gulf of Maine on the basis of measurements made at three-month intervals throughout the year. Hutchinson (1941) has estimated the average minimal rates of regeneration and sedimentation of phosphorus in Linsley Pond over a period of several weeks in the summer. The rates arrived at in both of these researches are averages for relatively long periods of time and are minimal only.

The purpose of the present investigation was to make approximately accurate measurements of the absolute rates at which phosphorus passes through its cycle of assimilation, solution and regeneration, and to relate these rates to the standing crop and growth rate of the phytoplankton population. The studies were conducted in fertilized sea water in outdoor concrete tanks at the Woods Hole Oceanographic Institution. These experiments form part of a study of productivity in salt waters, initiated and guided by George L. Clarke. Some results of this project have been discussed by Edmondson and Edmondson (1947) and by Pratt (1949), while others are in preparation.

METHODS

Inorganic phosphate concentration, determined by the modified Deniges-Atkins method (Wattenberg, 1937), was measured in a Klett-Summerson photoelectric colorimeter. All determinations were made exactly five minutes after addition of the reagents, and correction for salt error was applied. Concentrations are expressed as microgram-atoms of phosphate phosphorus per liter (µg-at. PO₄P/L).

Particulate Organic Phosphorus. The method employed is that of Redfield, Smith and Ketchum (1937), except that the intensity of the blue color was measured in a Klett-Summerson photoelectric colorimeter exactly five minutes after addition of the molybdate and stannous chloride reagents. Concentrations are expressed as µg-at. PO₄P/L.

Total Phosphorus. In the hope of following changes in the concentration of the dissolved organic phosphorus fraction and of keeping a balance sheet of all phosphorus in the system, Harvey's (1948) procedure for estimating total phosphorus was tried. However, this method frequently gave readings of "total" phosphorus which were
less than the sum of the inorganic and particulate fractions. Moreover, in various preliminary tests Harvey's method did not yield altogether satisfactory results. Therefore, until the method's effectiveness in detecting all the organic phosphorus has been fully demonstrated, it seems wise not to place too much reliance in the absolute values of the measurements, although the close agreement between readings of identical samples warrants considerable confidence in the relative values obtained. Concentrations were determined in a Klett-Summerson photoelectric colorimeter and are expressed as µg-at. PO₄ P/L.

**Phytoplankton.** The standing crop of phytoplankton was measured by the concentration of chlorophyll. I am indebted to W. T. Edmondson for the chlorophyll measurements, which he made in connection with experiments being conducted simultaneously with those reported here. The method is that described by Edmondson and Edmondson (1947), with the exception that the Klett filter No. 66 was replaced by a Farrand filter of narrow transmission range centered on the peak of absorption for chlorophyll a. The approximate calibration factor is one unit on the Klett scale = 0.26 mg. chlorophyll a/m³, but since this may be slightly revised upon recalibration of the photocolorimeter, the chlorophyll concentrations are given in terms of Klett units.

In this investigation the importance of the phytoplankton standing crop and growth rate lies in the relations of these variables to the phosphorus cycle. The absorption of phosphate by the phytoplankton is a process intimately connected with photosynthesis. For this reason and because of the role of chlorophyll in photosynthesis, the use of chlorophyll concentration as an index of the phytoplankton population is particularly appropriate for our purpose.

**THE MEASUREMENT OF RATES IN THE PHOSPHORUS CYCLE**

**Procedure.** The concrete tanks, described by Edmondson and Edmondson (1947), had been filled with sea water for two summers previous to the present studies. Thus it seems likely that at least the principal exchanges of substances between water and concrete had already occurred before these investigations were begun. By the use of wooden partitions, the tanks were divided into four compartments, each being 2.74 meters long x 1.42 meters wide. On August 3, 1948, each of the four tanks was filled to a volume of 4.5 m³ with sea water (S₀/∞ 32.42) pumped from the harbor and passed through a No. 10 bolting silk net (mesh aperture diameter = 134 µ) to remove everything larger than small zooplankters. On the morning of August 6, Tanks 1 and 2 were fertilized with Reagent Na₂HPO₄.12H₂O in an
amount calculated to raise the inorganic phosphate phosphorus concentration by 5 µg-at./L; Tanks 3 and 4 received the same amount of phosphate plus Reagent NaNO₃ calculated to increase the nitrate nitrogen concentration by 80 µg-at./L. The ratio of added nitrogen to phosphorus was thus the average ratio in which they occur in marine plankton (Sverdrup, Johnson and Fleming, 1942). The observed increases in inorganic phosphate concentration resulting from the fertilization were, in Tanks 1–4 respectively, 4.87, 4.99, 4.61 and 5.11 µg-at./L. Nitrate concentration was not measured. On August 23 Tanks 1 and 2 were fertilized with Reagent NaNO₃ in an amount calculated to raise the nitrate nitrogen concentration by 80 µg-at./L.

Throughout the period in which experiments were conducted (August 6–September 1), the sides and bottom of Tank 4 were scraped with a stiff bristle brush every morning immediately after collecting the water samples. The other tanks were not thus treated, so that a thin mat of attached algae developed on their walls.

In the photosynthetic plankton, the most abundant forms were Prymnesium sp. (Chrysophyceae), Thalassiosira nana (Diatomaceae), and Nannochloris coccoïdes (Chlorophyceae). Also frequently observed were Gymnodinium punctatum, Glenodinium sp. (Dinophyceae), Platymonas sp. (Chlorophyceae) and Olisthodiscus luteus (Xanthophyceae). The flora of Tanks 1 and 2 were quite similar, with Prymnesium usually the dominant form. In Tanks 3 and 4, Nannochloris became the most abundant form during the latter half of August, and throughout the last week it constituted more than 90% of these populations. During the first two weeks, cell counts in Tanks 1 and 2 rarely exceeded 2 million cells/L, and in Tanks 3 and 4 they were generally less than 5 million cells/L. The densest populations occurred at the end of August, with the following approximate maxima: Tank 1, 7 million; Tank 2, 5 million; Tank 3, 250 million; and Tank 4, 400 million cells/L.

This study is concerned with the rates of the following three processes in the phosphorus cycle: step 1, assimilation—the rate at which dissolved inorganic phosphate is taken up by the phytoplankton; step 2, solution—the rate at which the particulate phosphorus of dead cells is released as dissolved organic phosphorus; and step 3, regeneration—the rate at which the dissolved organic fraction returns to the inorganic phase. The term regeneration is thus used in a somewhat restricted sense. It applies only to the conversion, in situ, of organic compounds in solution to inorganic state. It does not refer here to

2 Daily plankton identifications and counts, following centrifugation of fresh samples, were carried out by E. M. Hulburt and Albert Rosenberg.
the process of "solution" (step 2) nor to the influx of new supplies of phosphate through water movements.

The difficulty in measuring these three rates is that at least two of the processes occur simultaneously; the problem is to stop the cycle at one point so as to allow a measurable accumulation in one of the phosphorus fractions. Ketchum's (1939a) discovery that *Nitzschia* cultures would not assimilate phosphate in the dark (unless they were phosphorus deficient) apparently provides a means of arresting the cycle, with the accumulation of phosphorus in the dissolved inorganic fraction.

Under certain conditions this cessation of phosphate assimilation in the dark can be detected in natural populations that are adequately supplied with phosphorus. An earlier paper (Pratt, 1949) describes a marked diurnal fluctuation in inorganic phosphate values (high in the morning, low in the afternoon) observed in a salt pond after fertilization with superphosphate. This phosphate pulse is ascribed to a continuous (day and night) supply of phosphate from the fertilizer on the mud bottom, coupled with its discontinuous consumption by the phytoplankton during the hours of daylight only. These observations suggested the possibility of determining the regeneration rate by noting the increase in inorganic phosphate during the night in the open water of the tanks. Any detectable increase in the particulate fraction during the day would then represent the excess of assimilation over the solution of phosphorus from dead cells.

Accordingly, early morning and evening measurements were made of the dissolved inorganic and the particulate organic concentrations in the fertilized water of the tanks. In general, the inorganic fraction decreased by day, was restored by night, and these changes were mirrored in the concentrations of particulate phosphorus. The diurnal fluctuations were so slight, however, that their use for the reliable estimation of rates does not seem possible.

Under the conditions in the tanks it was necessary, therefore, to prolong the period of darkness artificially in order to permit a more accurately measurable accumulation of inorganic phosphate. This was done by the "dark and light bottle" technique widely used in the measurement of photosynthesis.

After thorough stirring of the tanks with an oar, clear glass-stoppered bottles of about 200 ml. capacity were filled by siphoning from a gallon sample collected approximately six inches below the surface. In siphoning, the water was passed through netting of such a mesh as to exclude any large clumps of detritus. Half of the bottles were then wrapped in several thicknesses of black cloth and, together with those exposed to the light, were suspended in the middle of the tank at
mid-depth. The concentrations of inorganic phosphate, particulate phosphorus and chlorophyll in the open water were determined immediately, using the remainder of the gallon sample. This sampling was always done between 9 and 9:30 A. M., with one exception (at 10:15). Forty-eight or 72 hours later the dark and light bottles were pulled up and their contents analyzed for dissolved inorganic and particulate organic phosphorus. At the same time the concentrations of these two phosphorus fractions and that of chlorophyll in the open water of the tanks were determined.

The rate of regeneration is measured by the increase in inorganic phosphate in the dark bottle during the two- or three-day period. The decrease of particulate organic phosphorus in the dark provides a measure of solution (step 2 in the cycle). The rate of assimilation can be measured in either of two ways. (1) It can be measured by changes in the particulate fraction. Since an increase in particulate phosphorus in the light bottle represents the assimilation of phosphate by the plankton in excess of solution, to that increase must be added the amount of solution, as measured in the dark bottle. (2) Similarly, the assimilation rate can be obtained from the decrease in inorganic phosphate in the light bottle, plus the amount of regeneration as observed in the dark bottle. This second method has been employed by Edmondson and Edmondson (1947).

If we introduce $\Delta p$ for the change per day in the concentration of particulate organic phosphorus, and $\Delta i$ for the change per day in the concentration of dissolved inorganic phosphorus, the scheme for measuring the rates can be expressed concisely as follows:

assimilation rate (step 1) = $\Delta p$ in light bottle - $\Delta p$ in dark bottle, or $\Delta i$ in dark bottle - $\Delta i$ in light bottle

solution rate (step 2) = - $\Delta p$ in dark bottle

regeneration rate (step 3) = $\Delta i$ in dark bottle

In the equations given for assimilation rate, $\Delta p$ in the dark bottle and $\Delta i$ in the light bottle must be preceded by minus signs because their values are ordinarily negative. The summation of terms in each of these equations thus gives the total rate.

Twenty-two series of measurements following this plan were conducted, according to the following schedule: Experiments in all four tanks August 6–9, 11–13, 20–23 and August 30–September 1; experiments in Tanks 1 and 2 alone during August 23–25, 25–27 and 27–30. The described method for measuring rates in the phosphorus cycle involves three assumptions.

1. There is no loss of phosphorus to the walls of the bottles. Apparently this assumption did not hold perfectly. Judging from
measurements made with Harvey's method, there was usually a slight decrease in total phosphorus in the bottles during the experiments, amounting to .05 µg-at./L. per day. The loss of inorganic phosphate from sea water stored in bottles is a familiar occurrence and probably can be attributed to a film of bacteria firmly attached to the walls of the vessel. Harvey (1948) has discussed this phenomenon and has given evidence suggesting that dissolved organic phosphorus may be adsorbed on a clean glass surface. It is not known what phosphorus fraction or fractions suffered the apparent losses in the present study. However, in 11 of the 22 experiments, the amount of detectable inorganic phosphate decreased in the dark bottle, which suggests that at least a part of the total phosphorus lost was inorganic. This is perhaps a serious defect in the bottle method, but it appears to be unavoidable. The result is that regeneration rates estimated from the change in inorganic phosphate in the dark bottle are minimal.

2. The phytoplankton does not assimilate phosphate in the dark. The basis for this assumption is the work of Ketchum (1939a) with laboratory cultures of *Nitzschia closterium*. Whether the populations of the tank water did or did not assimilate phosphorus in the dark cannot be stated with certainty. The loss of inorganic phosphate in dark bottles discussed above might be interpreted as evidence of phosphorus assimilation by the phytoplankton, but it seems likely that at least a considerable part of this loss can be ascribed to the bacterial film. The only unequivocal indication of phosphorus uptake in the absence of light that can be deduced from the existing data is an increase in particulate organic phosphorus in the dark bottle. In the course of 22 experiments this occurred only five times: During the first series of experiments (August 6–9) immediately after fertilizing, particulate phosphorus in the dark bottle increased in three of the tanks; in the second series (August 11–13) such an increase was again noted in one of the tanks; and immediately following the addition of nitrate to Tanks 1 and 2 on August 23, a similar increase was observed in Tank 1. The first four instances of phosphate assimilation in the dark occurred in populations which presumably had not yet restored an initial phosphorus deficiency (cf. Ketchum 1939a). The fifth case illustrates another phenomenon described by Ketchum (1939b). Within certain limits the rate of phosphorus uptake by Ketchum's *Nitzschia* cultures depended upon the concentration of nitrate. Apparently the nitrate fertilization of August 23 so accelerated the rate of phosphorus assimilation temporarily that in Tank 1 this process was allowed to go forward even in the dark.

That the phytoplankton did not usually assimilate phosphate in the dark cannot be proven, but every instance in which the assumption
demonstrably did not hold can be explained on the basis of the temporary effects of phosphorus deficiency or increased nitrate concentration.

3. The rates of solution and regeneration in the light bottle are the same as those in the dark bottle. In the absence of any evidence bearing on this assumption, its validity can be judged on theoretical grounds only. Quite possibly light affects phytoplankton and bacterial populations in various ways which influence phosphorus solution and regeneration. For example, Newcombe (1940) suggests that living phytoplankton cells release inorganic phosphate into solution by night, but this hypothesis lacks support from experimental evidence. The animal population may be important also in this connection. Under natural conditions in the sea, light intensity may influence the rate of feeding by the zooplankton and presumably the rate of excretion of phosphate. It is a significant fact, therefore, that the number of macroscopic animals in the tanks was negligible; indeed, no zooplankters visible to the unaided eye were observed during the period of study. Unidentified species of rotifers and nematodes occasionally appeared in small numbers. Aside from these, the plankton fauna consisted of flagellate and amoeboiid protozoa which rarely attained numerical importance. The effects of the light-influenced activities of this animal population on the phosphorus cycle cannot be assessed, but probably they were never significant. At least we can conclude that such effects resulting from the activities of macrozooplankton were absent in the tanks.

Degree of Agreement Between Independent Measures of Assimilation. It will be recalled that the changes in dissolved inorganic and particulate organic phosphorus in dark and light bottles yield two more or less independent measures of the rate of assimilation, viz. \( \Delta p \) in light - \( \Delta p \) in dark, and \( \Delta i \) in dark - \( \Delta i \) in light. Since one of these methods depends in part upon the solution rate (\( - \Delta p \) in dark) while the other involves the regeneration rate (\( \Delta i \) in dark), a comparison of the assimilation rates as measured by the two methods furnishes a critical test of the consistency of the dark and light bottle method. The relationship between the two measures is shown in Fig. 1, in which the least squares line has been fitted to the data from the 22 experiments. The correlation coefficient is .735, which is well above the 1% level of significance. In other words, in only one case in one hundred would 22 sets of data yield a correlation coefficient as high as .735, by chance alone. Of the two measures of assimilation rate, that derived from changes in the particulate fraction is regarded as the more trustworthy, since a decrease in inorganic phosphate is at best an indirect measure
of assimilation, and there is reason to believe that some inorganic phosphate is lost to bacteria on the walls of the bottles.

Comparison of Phosphorus Changes in the Light Bottle with Those in the Open Water. By the dark and light bottle method one can measure directly the rates of phosphorus conversions only as they occur within the experimental bottles. One must then meet the objection that, for marine phytoplankton, the interior of a small, tightly-stoppered bottle is a habitat somewhat less than likely. Rates of phosphorus transformations within the bottles are of little interest except in so far as they represent conditions in the larger environment—the open water of the tanks, in the present study. Ideally, the interrelations of phytoplankton and water in the light bottle should be identical to those in the open water. For example, the changes in concentrations of inorganic phosphate and of particulate organic phosphorus in the light bottle should be the same as those in the open water. A moment’s thought will show that the following relationship is also to be expected. Since an observed decrease in inorganic phosphate in the
open water represents the excess of assimilation over regeneration, the rate of that observed decrease should equal the rate of phosphorus assimilation in the light bottle less the regeneration rate. Thus we have the bases for three comparisons between the light bottle and the open water, and each of these provides a measure of the usefulness of the dark and light bottle method for estimating the rates of phosphorus transformations in the larger environment.

![Graph](https://via.placeholder.com/150)

Figure 2. Correlation between rate of change of particulate phosphorus concentration in light bottles and that rate in the open water, in µg-at./L/day.

The correlation coefficients for these relationships are as follows: (1) for Δp in open water vs. Δp in light bottle, r = .566, which is significant to the 1% level of probability; (2) for Δi in open water vs. Δi in light bottle, r = .596, significant to the 1% level; (3) for Δi in open water vs. Δp in light bottle − Δp in dark bottle − Δi in dark bottle, r = .461, significant to the 5% level. The less satisfactory agreement in the third correlation as compared to the first two can probably be laid to inaccuracies in the measurement of regeneration,
for, as we have seen above, there is reason to believe that changes in inorganic phosphate in the dark bottle give erroneously low values for the regeneration rate. The plots of these three correlations, with the least squares line fitted to each, are given in Figs. 2, 3 and 4.

The line of regression for phosphorus uptake as measured by changes in the particulate fraction (Fig. 2) shows nearly a 1 : 1 ratio between open water and light bottle, and it almost passes through the origin. This indicates that in so far as phosphorus assimilation is concerned, environmental conditions in the light bottle were statistically the same as the average of conditions throughout the tank.

A different sort of relationship between rates in open water and rates in bottles is evident in comparisons 2 and 3. It will be seen in Fig. 3 that the line of regression is notably displaced to the left of the origin, indicating that the rate of disappearance of inorganic phosphate from the open water is greater, by a nearly constant amount, than the rate of its decrease in the light bottle. An analogous relationship is evident in the third comparison (Fig. 4): The rate of decrease of inorganic phosphate in the open water is consistently higher than the rate
by which assimilation exceeds regeneration in the bottles. The asymmetric relationship in each of these comparisons is readily explained when one recalls that, with the exception of Tank 4, a layer of attached algae was allowed to accumulate on the tank walls. The demands which these plants made for dissolved phosphate account for the higher rate of phosphate disappearance in the open water than in the clean bottles (Fig. 3), and for the related anomaly in Fig. 4. In general, the data from Tank 4 do not show an excess loss of inorganic phosphate in the open water comparable to that in the other tanks.

We can conclude that, in spite of certain limitations, the dark and light bottle technique provides estimates of the rates of phosphorus assimilation, solution and regeneration which are highly consistent inter se. Moreover, such changes in phosphorus concentrations as could be measured in the surrounding water are significantly correlated with those in the bottles, indicating that the rates of phosphorus transformation measured within the bottles are representative of those prevailing in the larger environment.
RELATION BETWEEN PHOSPHORUS METABOLISM AND THE STANDING CROP AND GROWTH OF PHYTOPLANKTON

During the four weeks of measurement, the rates of the phosphorus transformations varied, as to be expected, with varying concentrations of phytoplankton and dissolved nutrients in the tank water. The highest recorded values for each of the three rates in the phosphorus cycle were as follows: assimilation (step 1) as measured by \( \Delta p \) in light \(-\Delta p \) in dark = 0.33 \( \mu \)g-at./L/day; assimilation (step 1) as measured by \( \Delta i \) in dark \(-\Delta i \) in light = 0.36 \( \mu \)g-at./L/day; solution (step 2) = 0.38 \( \mu \)g-at./L/day; regeneration (step 3) = 0.13 \( \mu \)g-at./L/day. Due to errors in measurement or limitations in the bottle technique, negative values for assimilation, solution and regeneration occasionally appear in the data. These are theoretically impossible, of course, and should not be interpreted literally.

During the period of phosphorus measurements, the mean and maximum of observed chlorophyll concentrations were approximately 10.4 and 25.2 mg. chlorophyll \( m^3 \), respectively. In order that these values may be compared with concentrations observed in natural salt waters, data published by Riley are listed in Table I. The Long Island Sound and Georges Bank measurements cover all seasons of the year; those for the Tortugas are midsummer values.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Mg. chlorophyll/( m^3 )</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long Island Sound</td>
<td>17.44</td>
<td>max. 62.00</td>
</tr>
<tr>
<td>Georges Bank</td>
<td>5.58</td>
<td>max. 27.20</td>
</tr>
<tr>
<td>Tortugas region</td>
<td>0.33</td>
<td>max. 0.47</td>
</tr>
</tbody>
</table>

In view of the importance of phosphorus in the growth and maintenance of the phytoplankton, the rates at which this element is taken up by living plant populations and released into solution by dead or dying cells, and perhaps even the rate of its regeneration to inorganic form, may be expected to show consistent relationships with the size and rate of increase or decrease of the phytoplankton population.

Correlation coefficients describing these relations are presented in Table II. The standing crop is measured as the arithmetic mean of the chlorophyll concentration in the open water at the beginning and at the end of the experiment, while the rate of population change is expressed as the increment (or decrement), per day, of the chlorophyll concentration during the experiment. Chlorophyll data are available for all
experiments with the exception of those running between August 11 and 13, giving a total of 18 complete sets of data. For this number, the values of the correlation coefficient for the 1%, 2% and 5% levels of significance are, respectively, .5897, .5425 and .4683.

**TABLE II.—CORRELATION COEFFICIENTS FOR RATES OF PHOSPHORUS ASSIMILATION, SOLUTION AND REGENERATION VERSUS MEAN CHLOROPHYLL CONCENTRATION AND RATE OF CHANGE OF CHLOROPHYLL CONCENTRATION**

<table>
<thead>
<tr>
<th>Assimilation rate ($\Delta p$ in light $-$ $\Delta p$ in dark)</th>
<th>Mean chlorophyll</th>
<th>Rate of change of chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assimilation rate ($\Delta i$ in dark $-$ $\Delta i$ in light)</td>
<td>.438</td>
<td>.598</td>
</tr>
<tr>
<td>Solution rate ($-\Delta p$ in dark)</td>
<td>.868</td>
<td>.038</td>
</tr>
<tr>
<td>Regeneration rate ($\Delta i$ in dark)</td>
<td>.551</td>
<td>.176</td>
</tr>
</tbody>
</table>

**Assimilation.** The correlation of rate of phosphate uptake (step 1 in the cycle) with the phytoplankton standing crop differs considerably, depending upon which index of assimilation is used. Employing the measurement believed to be the more reliable, namely the changes

![Figure 5](image-url)
in particulate phosphorus (Fig. 5), the correlation is significant to the 2% level. Since it is to be expected on theoretical grounds that, in general, the larger the population the greater will be its demands for phosphate, the rather high correlation probably indicates a causal connection.

![Graph](image)

**Figure 6.** Correlation between rate of phosphorus assimilation (as measured by change in particulate phosphorus) in µg-at./L/day, and change in concentration of chlorophyll a in Klett units/m²/day (see text).

The rate of phosphorus assimilation shows a still stronger correlation with the growth rate of the phytoplankton (Figs. 6 and 7). By either measure of assimilation the correlation is significant to the 1% level. This again can be interpreted on biological grounds. A certain rate of phosphorus uptake is required for mere maintenance of the phytoplankton at a constant population density; the demands of
a growing population are considerably greater. However, if we can attach biological significance to the fact that the assimilation rate is more strongly correlated with population growth than with population size, an interesting deduction emerges from this comparison of the correlations. Provided we can assume that most or all of the phos-

![Figure 7. Correlation between rate of phosphorus assimilation (as measured by changes in inorganic phosphate) in µg-at./L/day, and change in concentration of chlorophyll a in Klett units/m²/day (see text).](image)

phorus taken up is used in the synthesis of new organic matter through cell division (rather than in a metabolism of individual cells, absorbing and secreting phosphate, as postulated by Newcombe, 1940), the rate of phosphorus assimilation is a function of the rate of cell division. If this is true, the higher correlation of assimilation rate with population growth rather than with population size means that changes in population size were governed largely by changes in the rate of cell division rather than by changes in the death rate. That changes in
the division rate formed the principal mechanism of fluctuation in the standing crop is undoubtedly related to the fact that most of the population changes were increases. (Note that the single point in Figs. 6 and 7 representing a rapidly declining population quite visibly weakens the correlations.) However, there is no logical necessity relating the mechanism of change to the proponderance of increases; neither of these phenomena could have been predicted \textit{a priori} from the other. Theoretically a population can increase by lowering the death rate, the “birth rate” remaining constant.

While the rate of phosphorus uptake is determined by both the size and the rate of increase of the population, it might be reasonably expected to depend also upon the concentration of phosphate available for assimilation. Ketchum (1939b) has shown a direct relation between the phosphate content of culture media and the rate of phosphorus absorption by \textit{Nitzschia}. However, the correlation of assimilation rate with phosphate concentration in the tanks (not figured) falls a little short of the 5\% level of significance, even when such factors as the presence or absence of added nitrate and the abundance of phytoplankton are taken into account by excluding experiments in which added nitrate was lacking and by plotting assimilation rate as the rate per unit of chlorophyll. If the rate of phosphorus uptake was significantly influenced by the phosphate content of the tank water, this relationship was partially obscured by other factors that were not measured.

\textit{Solution.} The rate at which phosphorus is released into solution through the death and breakdown of cells (step 2 in the cycle) is closely correlated with the mean standing crop (Fig. 8), and there is every reason to believe that these variables are causally related.

The solution rate shows no significant relationship with the rate of population change. Here one might have expected to find a high negative correlation, but a moment’s reflection will show that such a correlation would derive its principal strength from rapidly declining populations, which were almost entirely lacking in these experiments. In other words, while one tends to associate rapid decrease of particulate phosphorus with rapid decline of phytoplankton, there are no grounds for supposing that slowly \textit{increasing} populations lose particulate phosphorus faster than rapidly increasing populations. There is, then, no reason to anticipate a strong negative correlation where population increases predominate, as in the present data.

\textit{Regeneration.} It is clear from Fig. 9 that the relation between the rate of regeneration (step 3) and mean chlorophyll concentration could be described more accurately by a nonlinear equation. How-
ever, even the somewhat inadequate straight line equation gives a correlation significant to the 2% level. In a general way, the larger the population the more rapidly is inorganic phosphate regenerated. Two factors appear to be operating here. As we have seen above, the rate of disintegration of particulate phosphorus is intimately related to the population size, and it may be that the dissolved organic products of that breakdown are almost instantaneously converted to inorganic form. Aside from this possible direct effect of population size upon regeneration rate, a secondary effect seems likely. The bacterial activity responsible for the regeneration of inorganic phosphate is doubtless a function of the surface area available to the bacteria, and this in turn is related to the abundance of phytoplankton (cf. ZoBell, 1946). It seems unlikely that all of the organic phosphorus released from the particulate phase reappears instantly as inorganic phosphate. Probably some or most of it is regenerated only after a lag of unknown duration in which the decomposing phosphorus compounds are present in dissolved organic form. In an attempt to detect such a lag, the regeneration rate as measured in each experiment was plotted against the mean chlorophyll value of the preceding two- or three-day period. However, simple inspection of the plot (not
figured) shows a correlation lower than that between regeneration rate and mean chlorophyll of the same experimental period. Evidently the peak of regeneration (if there is one at all) occurs either simultaneously with the decomposition of particulate phosphorus or more than two or three days later.

![Figure 9. Correlation between rate of phosphorus regeneration in µg-at./L/day and mean concentration of chlorophyll a in Klett units/m² (see text).](image)

The regeneration rate shows no significant relation to the rate of population growth or decline. Since regeneration is the process linking the two dissolved phosphorus fractions and is only remotely related to the particulate, it is to be expected that the rate of regeneration be more closely correlated with the concentration of dissolved organic phosphorus than with the particulate phase in the cycle. Unfortunately, dissolved organic phosphorus can be measured only as the difference between total phosphorus and the sum of the particulate and inorganic. As stated earlier, the measurement of total phosphorus frequently gave readings which were less than the sum of the inorganic and particulate fractions, resulting in “negative” values for the dissolved organic component.
A correlation of regeneration rate with the concentration of dissolved organic phosphorus as measured would thus be meaningless.

In summary, both the standing crop and the rate of change of the population determine its demands for phosphorus; whereas, in populations such as those studied, the rates of return of phosphorus to solution and to inorganic form depend upon the standing crop and are not affected by the rate of change of the population.

**PHOSPHORUS CHANGES IN THE OPEN TANK WATER**

One of the original objectives of this investigation was to draw up a balance sheet in which all of the changes in the various phosphorus fractions in the water-phytoplankton system could be accounted for.

![Figure 10](image-url)

Figure 10. Concentrations of inorganic, particulate and "total" phosphorus in the open water of the tanks, August 5–September 1. Black area = particulate organic phosphorus; white area beneath black area = inorganic phosphate; total area beneath dashed line = "total" phosphorus (see text).

How imperfectly this aim was realized, except in Tank 4, can be seen immediately in Fig. 10 and Table III, which show the changes in the particulate and organic fractions and in the "total" phosphorus of the tank water over the entire period of observation. The concentration of inorganic phosphate dropped from initial values of more than 5 µg-at./L on August 6 to less than 1 µg-at./L on September 1. In three of the tanks, the increase of particulate phosphorus during the same interval was less than 1 µg-at./L. Thus the rapid disappearance
of inorganic phosphate was not balanced by a commensurate increase in particulate phosphorus. Nor, apparently, can it be accounted for by increase in the dissolved organic fraction, for measurements of total phosphorus, in so far as they are reliable, indicate losses amounting to as much as two-thirds of the phosphorus that was present in the water immediately after fertilizing.

**TABLE III.—PHOSPHORUS CHANGES IN THE OPEN WATER OF THE TANKS, AUGUST 6—SEPTEMBER 1**

<table>
<thead>
<tr>
<th>Tank</th>
<th>Inorganic µg-at./L</th>
<th>Particulate µg-at./L</th>
<th>Loss of “total” phosphorus in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-4.57</td>
<td>+0.73</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>-5.14</td>
<td>+0.80</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>-5.22</td>
<td>+0.94</td>
<td>66</td>
</tr>
<tr>
<td>4</td>
<td>-5.22</td>
<td>+4.07</td>
<td>21</td>
</tr>
</tbody>
</table>

With respect to over-all changes in particulate and total phosphorus, the contrast between Tank 4 and the other tanks is conspicuous and instructive. Daily scraping of the sides and bottom of Tank 4 minimized the growth of attached algae, which in the remaining tanks produced a perceptible mat. The much greater loss of total phosphorus in the water of the first three tanks than in Tank 4 doubtless represents the demands of these fixed algae for dissolved phosphate. While the over-all decrease of inorganic phosphate was substantially the same in all tanks, by the end of the month a much greater proportion of this phosphorus was present in particulate form in Tank 4 than in the others. During the last two weeks of August, the water of Tank 4 supported a consistently denser phytoplankton population (by cell count) than did the other tanks, but it also contained considerably more detritus, as judged by gross inspection. The extent to which detritus contributed to the readings of particulate phosphorus cannot be estimated. It is quite clear, however, that scraping the tank walls profoundly affected the final distribution of phosphorus with the result that when observations were discontinued, four-fifths of the phosphorus originally present was accounted for by direct measurement. In contrast, where attached algae were allowed to grow undisturbed, they competed successfully with the plankton for the added phosphate, much of which was thus lost from the water.

Having added the benthic algae to our water-phytoplankton system, we can regard the momentary concentration of phosphorus locked up in this third component as resulting from the excess of uptake over release of phosphorus by these algae. Evidently these processes struck an equilibrium in Tank 3 on about August 20; thereafter the
return of phosphorus to the water just balanced its removal by the attached algae, and the concentration of total phosphorus in the water remained nearly constant at 2.2 µg-at./L. In Tank 4, where attached algae played a less important role, this equilibrium was poised at about 3.8 µg-at./L. (There is reason to believe that the apparent fluctuations about this value and the indicated increase in total phosphorus at the end of the period may have been due to varying degrees of thoroughness in scraping and stirring Tank 4, rather than to biological changes.) In the water of Tanks 1 and 2, total phosphorus did not reach a constant value and was still decreasing at the end of the investigation.

Finally, Fig. 10 illustrates the fact demonstrated by Ketchum (1939b) with Nitzschia cultures that the rate of phosphate assimilation depends upon the nitrate content of the medium. In Tanks 3 and 4, which were fertilized with nitrate as well as phosphate, the inorganic phosphate concentration dropped at a steady pace from August 6 to 18. Using the same interval for comparison with the tanks initially fertilized with phosphorus alone, we find the following rates of decrease in inorganic phosphate per day; Tank 1, 0.18; Tank 2, 0.15; Tank 3, 0.40; Tank 4, 0.38 µg-at./L. Following the addition of nitrate to Tanks 1 and 2 on August 20, the rate of phosphate depletion from the water in Tank 2 was exactly doubled. For unknown reasons, in Tank 1 the rate of phosphorus assimilation was not appreciably accelerated by the addition of nitrate.

**DISCUSSION**

In fresh or salt water ponds and in the sea, one may frequently observe dense phytoplankton blooms associated with extreme dilutions of inorganic phosphate. At first glance this may appear paradoxical, in view of the well established importance of phosphate to plant growth. However, consideration of the dynamic aspects of phytoplankton nutritution prevents one from falling into the error of concluding from this observation that phosphate is not an important nutrient. The essential factors are the rate of assimilation of phosphate and the rate of its replenishment.

Thus it becomes a matter of great theoretical interest to know the actual rates at which phosphorus passes from one form to the next in its cycle, and how these rates are related to the abundance of phytoplankton, its increase or decrease, and to the concentration of inorganic phosphate in the water. In the experiments described, it was shown that assimilation rate depends more upon the rate of population increase than upon its absolute size. Thus the phosphorus requirements of a large population which is not increasing its mass may be
satisfied by a relatively low rate of assimilation. It was also found that the quantity of phosphorus assimilated per day can be as great as the momentary concentration of inorganic phosphate in the water; in one experiment a dense population (approximately 19.6 mg. chlorophyll a/m³) removed .08 µg-at. P/L per day from water in which the phosphate concentration was .08 µg-at./L at the beginning and at the end of the experiment. This makes it appear probable that in natural situations where phytoplankton blooms are associated with low concentrations of inorganic phosphate, the time required for the complete replacement of that phosphate may be only a matter of hours.

The rate of regeneration as measured was consistently lower than the rates of assimilation and solution. Probably part of this difference is due to the incomplete measurement of regeneration, but in so far as the comparison is real it indicates that the rate of regeneration limits the velocity of the entire phosphorus cycle. This conclusion has an important bearing on the problem of organic production in the sea, for wherever phosphate is present in concentrations low enough to limit plant growth, the rate of its replenishment will determine the rate at which it can be assimilated. On the basis of phosphorus measurements in the surface waters of the Sargasso Sea, where the concentration of dissolved organic is rather high, the particulate low and the inorganic almost negligible, B. H. Ketchum (in litt.) has advanced the opinion that the rate of phosphorus regeneration may limit the rate of production of organic matter. His view is supported by the present data.

Since zooplankton organisms excrete inorganic phosphate (Gardiner, 1937) derived from the phytoplankton (and detritus) upon which they subsist, it is possible that they play an important role in the phosphorus cycle. However, until suitable measurements are made to investigate this problem, the effect of the zooplankton upon the circulation of phosphorus must remain a matter of conjecture.

A knowledge of phosphorus metabolism rates is essential to the most efficient management of a program of fertilizing natural waters. In published reports dealing with the fertilization of natural waters, one frequently finds the assumption that phosphorus assimilated by the phytoplankton can be equated with the disappearance of added phosphate from the water. The folly of this supposition can be illustrated from the present data. The concentration of inorganic phosphate immediately following fertilization was more than 5 µg-at./L. If attached algae were allowed to grow on the tank sides and bottom, by the end of the month only 23 to 32% of this initial phosphate endowment was present in the plankton; 73 to 82% had found its way into the attached flora. Moreover, in natural bodies of
water, even in the absence of any fixed vegetation competing for nutrients with the plankton, an important part of the added phosphate is apt to be adsorbed, at least temporarily, on colloidal bottom deposits (Ohle, 1937; Einsele, 1938; Hutchinson, 1941; Mortimer, 1941). In the well weathered concrete tanks this loss of phosphorus from the water-phytoplankton system was reduced to a minimum. Even so, the escape of phosphorus from the open water to attached vegetation was so rapid as to render impossible any estimate of phosphorus utilization by the plankton, from rates of phosphate depletion alone. Thus it has been shown that the actual assimilation by the phytoplankton can be determined only by direct measurement of the phosphorus taken into the plant cells.

**SUMMARY**

1. By the use of the "dark and light bottle" method in outdoor concrete tanks, measurements have been made of the rates at which phosphorus is regenerated, assimilated, and released into solution in fertilized salt water and its contained phytoplankton. The maximum recorded rates were: regeneration = 0.13 µg-at./L of sea water in the tanks/day; assimilation = 0.36 µg-at./L/day; solution = 0.38 µg-at./L/day. The highest observed assimilation rate was measured in water containing approximately 20 million cells/L and 25 mg. chlorophyll a/m³.

2. In 18 series of measurements, during which phytoplankton increases predominated over decreases, the following relations were observed between the rates of phosphorus transformations and the size and absolute change of size of the phytoplankton populations:

   a. The rate of phosphorus assimilation was significantly correlated with the size of the phytoplankton standing crop and showed an even stronger dependence upon the increment of growth.

   b. The rate at which particulate organic phosphorus was released into solution from dead plankton organisms was intimately related to the size of the standing crop and was independent of the change in population size (which in most cases was an increase).

   c. The regeneration of dissolved inorganic phosphate from dissolved organic phosphorus depended upon the phytoplankton standing crop and showed no relation to the change in population size.

3. If attached algae were allowed to grow on the sides and bottom of the tanks, in less than one month they removed three-fourths of the added phosphate from the water-phytoplankton system. Where attached algae were largely prevented from growing, four-fifths of the phosphorus originally present was detected in the water at the end of four weeks.
REFERENCES

Clarke, G. L.

Edmondson, W. T. and Y. H. Edmondson

Einsele, W.

Gardiner, A. C.

Harvey, H. W.

Hutchinson, G. E.

Ketchum, B. H.


Mortimer, C. H.

Newcombe, C. L.

Ohle, W.

Pratt, D. M.

Redfield, A. C., H. P. Smith and B. H. Ketchum

Riley, G. A.


SVERDRUP, H. U., M. W. JOHNSON AND R. H. FLEMING

WATTENBERG, H.

ZOBELL, C. E.