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# EXPERIMENTS *on* POND FERTILIZATION

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# EXPERIMENTS *on* POND FERTILIZATION

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RESEARCH work on various phases of pond fertilization and management was begun by this Station in 1934.

Experiments with bluegills (*Lepomis macrochirus* Raf.) indicated a direct relationship between the average plankton content of pond water and the pounds of fish that these waters produced *(9)*. Further experiments were conducted to determine the types of inorganic fertilizer mixtures required for most economical plankton growth *(15)*. Results from tests with various commercial fertilizer ingredients, using mixed cultures of phytoplankton and distilled water in glass jars, indicated that the microscopic plants utilized nitrogen, phosphorus, and potassium at the approximate ratio of 4:1:1 (N-P-K). However, nitrogen was completely utilized at N-P ratios from 3:1 to 6:1. Since it was found that in pond waters colloidal clay and dissolved iron removed phosphorus from solution by adsorption and precipitation, the mixture used in pond fertilization experiments was a 4:2:1 (N-P-K) ratio and contained twice the phosphorus found necessary *in vitro*.

For commercial mixed fertilizers, the ratios are expressed as the percentages of N-$P_2O_5$-$K_2O$ in the mixture, instead of N-P-K. Using this system, it appeared that a 4-4.6-1.2, or approximately an 8-9-2 commercial fertilizer, would be the most desirable. Since in Alabama a commercial 6-8-4 was the nearest to the desired mixture, it was recommended that ponds be fertilized with 100 pounds 6-8-4 plus 10 pounds of nitrate of soda per acre per application (approximately a 7.6-8-4 ratio). This mixture has been used experimentally for the last 10 years. Its proper use was found to increase fish production 200 to 300 per cent. This mixture also has been used extensively by pond owners and hatcherymen. It is pointed out that, based on the results of the original experiments, commercial fertilizers varying from 6-9-2 to 12-9-2 should be satisfactory. It is also possible that under certain conditions a lower percentage of phosphorus might be equally effective, since the amount used in Alabama ponds is twice that required by the plankton *in vitro*. However, hundreds of analyses of water from ponds fertilized with 6-8-4 and nitrate of soda indicated that the nitrogen and phosphorus in this mixture were being completely utilized.

It was next found that the heavy plankton growth caused by inorganic fertilization kept water weeds under control by shading the pond bottom, and that submerged pond weeds, which had already become established, could be eradicated by winter and spring fertilization *(11, 12)*.

Additional work at this Station dealing with the use of cottonseed meal, soybean meal, peanut meal, and poultry laying mash alone and in combination with superphosphate was also reported *(10, 13)*. The use of these organic materials greatly increased fish production, but was less desirable than inorganic fertilizer because they produced large amounts of filamentous algae, which interfered with fishing and which increased the production of mosquitoes.

Experiments have been continued upon various other phases of pond fertilization to the present date. The progress made and some of the major unsolved problems encountered are presented in this bulletin.

## Experimental Methods

At the Alabama Agricultural Experiment Station, Auburn, a series of 30 concrete ponds (1/400 acre in size) and 102 excavated or earthen impoundment ponds (1/130 acre to 28 acres in size) are used for management studies. These ponds were stocked with definite numbers and weights of fish, and were given various predetermined fertilizer treatments. Appropriate chemical analyses were made and other pertinent records kept during the experiments. At the end of 1 or more years, the ponds were drained and the fish present counted and weighed.

Most of the results reported in this bulletin was gained as a direct result of planned experiments with fertilization. However, a considerable amount resulted indirectly from experiments with other pond management practices over the 12-year period.

## Variations in Plankton Growth in Response to Identical Fertilization

The theory that like causes produce like effects certainly does not appear to apply in the case of the production of plankton algae by fertilization. These differences have been especially striking in a series of 27 adjacent ¼-acre ponds at Auburn. These ponds have a common water supply, and are practically identical in length, width, and depth. Yet, when given identical fertilizer treatments, no two have the same appearance either to the naked eye or under the microscope. On any one day these pond waters appeared to be of various shades of green, brown, black, yellow, or red due to the different types of plankton dominant at that particular time. When these ponds were observed carefully, their appearance was found to change daily and even at various times during the same day.

Water samples from 30 to 50 ponds were examined at 2- to 3-week intervals under a microscope, and records were made over a 5-year period of the principal genera of algae present. Where the ponds were fertilized with commercial inorganic fertilizers, the principal algae present on any particular date in any one pond was a mixture of 4 to 6 or more genera, and different genera were dominant in different ponds receiving the same treatment. The dominant algae in practically all ponds varied at each examination.

The algae produced by inorganic fertilization were largely genera of the Chlorophyceae – *Scenedesmus, Ankistrodesmus, Chlorella, Staurastrum, Pandorina, Cosmarium, Chlamydomonas, Nannochloris, Pediastrum, Coelastrum,* and others. The Euglenophyceae – *Trachelomonas, Cryptoglena, Euglena,* and *Phacus* were also abundant and occasionally dominant. The Dinophyceae – *Glenodinium, Hemidinium,* and *Peridinium* were often present, but never in large numbers. Of the Chroococcaceae, or blue greens, *Coelosphaerium* and *Microcystis* occasionally became abundant for limited periods. Diatoms, which are so abundant in the marine plankton, were relatively unimportant in these fertilized fresh-water ponds.

Fertilization of ponds as was practiced, therefore, would appear to result in production of an unpredictable mixture of algae. It would not appear possible that all these forms of algae are equally desirable for the production of fish foods. Certainly, they are not equally desirable from the standpoint of growth habits, appearance, or odors. The present status of pond fertilization may be likened to that of a farmer who wished to increase his production of beef cattle but ignorant of desirable pasture plants applied fertilizer to a field of grasses and weeds without previous preparation or seeding to desirable species.

There is urgent need for a critical study of algal species to determine those that are most desirable for fish production, and to determine the conditions favoring production of these forms. At present it would not appear possible to raise cultures of a single species of alga in fish ponds. However, it should be possible to learn enough about the ecology of various species to seed the ponds and to maintain an abundance of the more desirable forms. It should also be possible to keep many undesirable species under control.

Variation in pond treatment has been observed to influence the dominance of certain genera of algae in the plankton at Auburn. In the alkaline waters of concrete pools, which are rich in calcium and magnesium, *Scenedesmus, Golenkinia,* and diatoms are especially abundant. In black swamp waters and in waters where woody plants are decaying, *Trachelomonas* often becomes very abundant following fertilization with inorganic fertilizers. Where organic matter rich in nitrogen is decaying, *Coelosphaerium, Microcystis,* and other colonial green and blue-green algae often become dominant. Under certain conditions, there would appear to be definite ecological successions of microscopic algae. Careful study should yield information of much practical as well as scientific interest. Many of the important advances in fertilization techniques will be made in this field.

## Factors Determining Production of Filamentous or Plankton Algae

While the relative values of filamentous algae and of plankton algae for production of fish food is at present unknown, there is no doubt as to their relative desirability in fish ponds or hatcheries.

Heavy growths of filamentous algae interfere with the removal of fish from the hatchery by seining or draining, and also with the catching of fish by casting, fly fishing, or by the use of live baits. Most fishermen consider them as obnoxious as a heavy growth of pond weeds.

In early experiments, the microscopic plankton algae were noted to result following fertilization with a 6-8-4 inorganic fertilizer, and filamentous algae to result from the use of cottonseed meal, hays, manures, and woody plants. Filamentous algae were also observed to grow upon the bottom of unfertilized ponds in early spring.

As a result, it was first thought that the growth of filamentous algae was correlated with a low nitrogen supply. Experiments were conducted in attempts to eliminate filamentous algae by the application of nitrate of soda to ponds. In many cases when a heavy application of nitrate of soda was sprinkled over the filamentous algae, the algae decayed and were replaced by the plankton forms. At other times it had no effect.

Further work with commercial 6-8-4 revealed that, when applied on clear water over underwater weed beds in January or February, this inorganic fertilizer caused a very heavy growth of filamentous algae, which shaded and killed the weeds *(12)*. If the water was clouded with plankton at the time of application, little or no filamentous algae were produced.

Also, it was found that 6-8-4 applied in cold weather to clear ponds often caused growth of filamentous algae. When applied in the spring, it caused growth of filamentous algae in some ponds and of plankton algae in others. If fertilization was continued into the summer, filamentous algae disappeared and all ponds became colored with plankton algae.

Cottonseed meal always caused growth of filamentous algae if first applied when the water was cold. If light applications were continued throughout the summer, the filamentous algae continued to grow. If the first application of meal was made during warm weather and followed by sufficiently heavy applications to give the water a dark color, plankton algae were produced instead of the filamentous forms.

Extremely heavy applications of manure, sufficient to stain the water a deep brown, produced plankton algae in either winter or summer, while light applications of manure produced the filamentous forms.

A study of these apparently contradictory results indicated that both the organic and inorganic fertilizers could produce either plankton algae or filamentous algae. Filamentous algae, however, could grow only if sunlight penetrated to the stratum upon which they rested – initially either on the pond bottom or on a submerged weed. If the plankton algae grew and reproduced rapidly, they shaded the bottom and filamentous algae could not grow. If filamentous algae got the head start, they gradually entrapped oxygen and floated to the surface, preventing the growth of the plankton forms.

Inorganic fertilizers, therefore, encouraged the growth of plankton algae because the plant nutrients present in the soluble form became distributed from top to bottom of the pond. This favored the growth of the free-floating algae, thus shading the bottom and preventing the growth of filamentous forms. When, however, the inorganic fertilizer was applied while the water was cold, it fell to the bottom and dissolved and diffused slowly, thus giving the filamentous forms of algae a better opportunity to become established.

Conversely, organic materials encouraged the growth of filamentous algae, because they fell to the pond bottom and the plant nutrients were released slowly upon decomposition. Extremely heavy applications of manure or cottonseed meal, however, colored the water brown, shaded the bottom, and released enough soluble plant nutrients to favor the growth of plankton algae.

Comprehension of these principles made the apparently conflicting results readily understandable, and also suggested a method for the control of filamentous algae in ponds during the spring.

It was found possible to kill filamentous algae in ponds when the water became warm in April and May by the following procedure: The floating filamentous masses were broken up with a fork or raked together and twisted into a ball and thrown into the deeper water. Commercial 6-8-4 fertilizer was then broadcast over the area. This caused some of the filamentous algae to sink, started part of it to decay, and encouraged the growth of the plankton algae by increasing the soluble nutrients throughout the water. One or 2 treatments were usually sufficient to eliminate all the filamentous algae and replace them with plankton forms. This method, however, was not successful in ponds shallower than 2 feet or where the pond contained large numbers of underwater weeds.

In experiments on weed control *(11, 12)* filamentous algae of the genera *Oedogonium, Spirogyra, Ulothrix,* and a few others were produced by winter fertilization with inorganic fertilizers. The species concerned were not determined, but they appeared to be winter species that normally died upon advent of hot weather. Unfortunately, the records are too incomplete to determine if hot weather or shading action caused by the increased growth of plankton was mainly responsible for the disappearance of these filamentous forms.

## Surface Scums and Dispersed Growth of Plankton Algae

Fertilization of ponds usually results in a growth of phytoplankton that is dispersed somewhat uniformly throughout the water. The following averages of analyses for plankton in a 1.3-acre fertilized pond at 3-week intervals for a 3-year period are typical of the depth distribution usually found:

| *Depth of sample* | *p.p.m. of plankton* |
|---|---|
| Surface | 4.9 |
| 2.5 feet | 5.8 |
| 5 feet | 7.0 |
| 7.5 feet | 7.8 |

Surface waters, it will be noted, had the lowest plankton concentration (expressed as p.p.m. of dry organic matter), with the concentration increasing at greater depths. The 5- and 7.5-foot depths contained living algae and also dead algae, which were gradually falling downward from the surface waters. Consequently, the oxygen content of the water during the daytime was not proportional to the plankton concentrations. The average oxygen concentrations at the surface and at 2.5 feet were approximately equal, but decreased rapidly at the 5- and 7.5-foot levels. Because of the higher concentration of live plankton, the 2.5-foot level often had a higher oxygen concentration than the surface waters. Typical of these results are the following analyses on the above pond made on July 26, 1940:

| | *p.p.m.* | |
|---|---|---|
| *Depth of sample* | *Plankton* | *Oxygen* |
| Surface | 3.8 | 4.1 |
| 2.5 feet | 5.2 | 5.3 |
| 5 feet | 6.4 | 1.6 |
| 7.5 feet | 6.2 | 1.0 |

Occasionally, however, the phytoplankton does not grow in a dispersed condition, but concentrates in a more or less thick film or scum at the surface. This shades the lower waters and causes the oxygen, resulting from photosynthesis, to be produced in the thin upper layer of water. Typical of these results are the following analyses on the same pond made on August 9, 1940:

| | *p.p.m.* | |
|---|---|---|
| *Depth of sample* | *Plankton* | *Oxygen* |
| Surface | 7.4 | 3.6 |
| 2.5 feet | 3.2 | 3.3 |
| 5.0 feet | 2.4 | 0.6 |
| 7.5 feet | 3.8 | 0.4 |

The surface concentration of plankton in this case materially reduced the oxygen in the lower waters.

The deleterious effect of surface scum formation by plankton was demonstrated more forcefully in a series of 1/130-acre ponds having a depth of 3 feet. These ponds were stocked with bluegills and fertilized with inorganic fertilizers at extremely high rates in attempts to get maximum plankton production.

On June 26, 1936, the plankton in one pond reached a concentration of 76.7 p.p.m., largely in the form of a thick surface scum. In another, it reached a concentration of 83.9 p.p.m., which remained uniformly dispersed throughout the water. In the former pond, the oxygen content of the water fell to 0.1 p.p.m. at 2 p.m., June 26, while in the latter, it was 6.5 p.p.m. All the adult bluegills died in the former pond. However, it is interesting to note that a recent hatch of bluegills in this pond did not die even at such a low concentration of oxygen. In the other pond no deaths occurred.

Heavy surface scums of plankton are also undesirable because they spoil the appearance of the water and often have an objectionable odor. However, it should be noted that for the production of goldfish and certain other minnows, much higher production results if the ponds are fertilized sufficiently heavy to produce a light scum of plankton over about one-third of the pond.

Regardless of the common undesirability of the concentration of phytoplankton in surface scums in fertilized ponds, little can be done about it at present because the factors causing this algal concentration are not sufficiently understood. Some of the factors involved appear to be the types of algae present, intensity of light, carbon dioxide content, and concentration of algae in the water.

The larger algal forms, such as *Euglena* and colonial species of greens and blue-greens appear most commonly in surface scums. These scums are affected greatly by the intensity of light. A scum of *Euglena* normally disappears during cloudy weather or at night, and it is most common in bright sunshine during the hottest weather. Some species form scums in cloudy weather, only to disperse when the sun shines, while others react the opposite.

The absence of sufficient amounts of carbonic acid or of bicarbonates in pond waters may prevent the growth of algae except at the pond surface, thus causing the formation of a very thin surface scum. Highly alkaline waters, such as occur in the West, are an example. The role of carbon dioxide in pond fertilization is discussed later in this bulletin.

In certain instances it is possible to fertilize a pond so heavily that the water becomes filled with plankton to the extent that a scum forms on the surface. The remedy here is relatively simple – a reduction in the amount of fertilizer applied. However, the factors causing the formation of surface scums, even under these conditions, are not understood.

It would appear that this problem is in need of careful investigation if pond fertilization is to be placed on as sound a basis as the fertilization of agricultural crops.

## Phytoplankton Pulses

In early experiments at this Station, ponds were fertilized with two or three heavy applications of fertilizer during the growing season. Shortly following fertilization, the plankton algae reached a high peak of abundance and then began to decay. The zooplankton shortly thereafter reached a peak, utilizing the food set free by the decomposing phytoplankton. Because of the dangers to the fish from these tremendous variations in phytoplankton abundance, further work was done, applying smaller amounts of fertilizers at more frequent intervals. This in effect smoothed out the curve of concentration of algae and has given high fish production at lower rates of fertilization. The following observations indicate that there is possible need for further investigations:

In concrete pools, it is possible to adjust the fertilizer applications so that one application will keep the pool continuously green with algae throughout the balance of the year. However, such a pool often gives lower fish production than one receiving heavier fertilizer applications where the algae grow in pulses.

A rock quarry pond showed similar reactions. One application of fertilizer kept a good algal growth continuously the rest of the year, but fish production was low.

Several excavated ponds in central Alabama, following fertilization for weed control for 2 years, maintained a relatively good growth of phytoplankton for 3 additional years with extremely light fertilization or no fertilization. Productivity however, did not remain at a high level.

It would appear in certain instances that, upon death of the algae, almost all the released nutrients may go into the production of other algae, while in other instances it goes into the production of animal organisms. The latter is the more desirable from the standpoint of fish production. A study of the relationships involved would yield much information of practical value to fish culture.

## Delayed Algal Growth Following Fertilization

When a pond is fertilized, the plankton algae normally become abundant enough to be readily noticed within 24 to 72 hours. In certain cases, however, even after repeated fertilization, the algae just do not grow. This condition may continue for 1 week, 2 weeks, or even 1 month; it is one of the most puzzling problems connected with pond fertilization. This condition sometimes occurred at the start of fertilization of a new pond. It also has happened in ponds that had been supporting a satisfactory plankton growth but where the plankton had precipitated on the pond bottom following a cold spell. One pond that supported a good plankton growth during the winter became muddy once each spring following very heavy rains. After the pond again became clear, the plankton did not resume growth for several weeks or longer each season.

In a series of 27 adjacent ponds that were fertilized similarly, there are each year one to three having delayed algal growth during the spring. A careful observer can even see a difference in the water in these ponds. The water lacks the sparkle or luster of that in adjacent ponds, and is called "dead" water by those working on the project. This condition rarely occurs during the summer or fall, and it seldom occurs in ponds that have been fertilized more than one year.

A considerable number of experiments have been conducted in an attempt to solve this problem. These are discussed under subsequent headings.

AERATION. Because of this lack of luster of the water, aeration was attempted in several small excavated pools (1/130 acre) with uniform depths of 3 feet. An outboard motor was clamped to a board and the water vigorously agitated for 1 hour on each of 2 successive days. This had no effect whatsoever upon the growth of plankton. Two weeks later both the untreated check pond and the aerated ponds became green with plankton almost overnight.

INOCULATION OF PONDS. It appeared possible that delayed growth might be due to the algae being in a resting stage at that particular time, or due to the pond water being practically sterile. A series of ponds, therefore, was inoculated with water from other adjacent ponds where various species of algae were growing vigorously. This was apparently without effect. When algae did begin to grow in the inoculated ponds, the dominant species were not those that were added.

MINOR FERTILIZER ELEMENTS. It also appeared possible that delayed growth might be due to a lack of essential elements other than those supplied in the 6-8-4 and nitrate of soda fertilizers. Of the so-called "minor essential elements," zinc, manganese, iron, iodine, boron, and copper were used in this experiment.

Water was carefully taken from a pond where the plankton would not grow, although both nitrogen and phosphorus were present at concentrations between 1 and 2 p.p.m. In one series the water was placed in 1-gallon battery jars of white glass and for the other series pyrex containers were used. The jars were placed upon the dam beside the pond from which the water was taken. In each series there were added in duplicate containers $ZnSO_4$, $MnSO_4$, $FeCl_3$, KI, and $HBO_3$ at rates of 0.1 p.p.m. and $CuSO_4$ at the rate of 0.05 p.p.m. These minor elements were used both singly and together. The water in the jars was then inoculated with centrifuged and washed algae and fertilized with 6-8-4 at rates sufficient to make 2 p.p.m. N in the water. While plankton made slight growth in all jars except those receiving copper, the growth was satisfactory in none. The boron and zinc treatments appeared to give slightly better growth than the others, although the difference was extremely small. Borax and zinc sulfate were then applied to a ¼-acre pond at rates of 10 pounds per acre; they, however, had no measurable effect.

The addition of all of the foregoing "minor elements" together with fertilization was also without effect.

VITAMINS. Since some work indicated that plants also needed vitamins, $B_1$ and a mixture of $B_1$ and $B_6$ were added to jars in the preceding experiment at rates of 0.1 p.p.m. This also did not increase plankton production.

Brewer's yeast added at the rate of 10 p.p.m. did increase the growth of algae, but cottonseed meal used at the same rate was equally effective. It was, therefore, concluded that the vitamins used were without effect.

BOTTOM ORGANIC MATTER. In certain instances delay in the appearance of phytoplankton growth following fertilization was found to be due to the presence of organic matter on the pond bottom. In such cases, the nitrogen and phosphorus were being completely utilized by bacteria in the decomposition of the organic deposit. Plant remains were found to decay at widely varying rates. Highly nitrogenous plants decayed rapidly in water, while those low in nitrogen and phosphorus decayed very slowly. The lignified stems of plants decayed the slowest of any part of the plant, often requiring more than 3 years in unfertilized ponds. The celluloses and hemicelluloses were capable of furnishing the necessary energy, but did not contain sufficient nitrogen, phosphorus, and possibly other nutrient elements for rapid bacterial growth. When these elements were supplied by the application of inorganic fertilizers to ponds, bacterial action was greatly stimulated and carbohydrate deposits were made available as food for zooplankton. Inorganic fertilization was in these cases producing zooplankton without the usual preliminary production of phytoplankton. This cause of delayed growth is temporary. It can be differentiated from other causes, because an analysis of the water will show that the nitrogen and phosphorus have been removed from solution and microscopic examination will show the presence of an abundance of zooplankton. The addition of more fertilizer than is required by the bacteria will result in a good growth of algae.

CARBON DIOXIDE. In certain cases where repeated applications of fertilizer have failed to produce plankton growth and water analyses have indicated the accumulation of considerable amounts of both soluble nitrogen and phosphorus, the cause has been found to be a lack of sufficient available carbon dioxide for algal growth. Since delayed algal growth and low fish production in fertilized ponds have both been found to be due to low availability of carbon dioxide in pond waters, the entire problem will be discussed in the next section. Here, it is sufficient to say that repeated fertilization of such waters will eventually result in phytoplankton growth due to absorption of carbon dioxide from the air, except in the case of waters so highly alkaline that no carbon dioxide can remain in solution in a form available to the algae.

It would appear that delayed algal growth is one of the most interesting and important problems connected with pond fertilization. The various problems involved could be solved most readily by a coordinated attack by algologists and physiological chemists, and their solution would be immensely valuable in placing water fertilization upon a more scientific basis.

## Carbon Dioxide as a Limiting Factor in Fish Production

That the available carbon is of great importance to the productivity of unfertilized lakes has been suggested by a number of workers *(8, 2)*. The work of Burr *(2)* and others has demonstrated that algae attain their highest efficiency at much higher tensions of carbon dioxide than are ever present in natural waters. The greater abundance of algae in hard-water lakes as compared to soft-water lakes in Wisconsin was considered by Birge and Juday to be directly traceable to the utilization in photosynthesis of dissolved bicarbonates abundantly present in the former *(8)*.

In most unfertilized ponds the carbon dioxide content of the water is not a limiting factor. The soluble nitrogen or phosphorus normally becomes a limiting factor in plankton and fish production long before the carbon dioxide is entirely utilized. It must be remembered that carbon dioxide is constantly being absorbed into the water from the air as long as the tension in the water is lower than that in the air. Nitrogen is added to a body of water in small amounts by rain water and in larger amounts by solution from the watershed. Phosphorus is tightly bound by the clay colloids of soils and enters the lake or pond in very small amounts by solution from the watershed. Consequently, the abundance of these latter two elements in the water and the speed of their replacement by solution from the watershed are normally the factors limiting the productivity of the body of water.

When waters are fertilized, however, the mineral elements can be supplied in abundance and the available $CO_2$ content of the water may be expected to limit plankton growth and fish production. Thus, it appears that it would be very desirable to increase the $CO_2$ tension of many waters.

$CO_2$ TENSION. Relatively little work in fisheries investigations has dealt with $CO_2$ tension of waters. Analyses for $CO_2$ are not normally expressed in tensions, but in p.p.m. $CO_2$, $HCO_3^-$, or $CO_3^{--}$. The reason for this is that the term "$CO_2$ tension" is not generally understood, and various methods for its determination are complicated and give conflicting results.

Assume a lake at sea-level contains pure water in which no plants are growing. The pressure of the air above the lake at sea-level equals 760 mm. of mercury. In the mixture of gases comprising the air, $CO_2$ equals 0.03 per cent of the total volume. It, therefore, exerts a "tension" or "partial pressure" equal to $760 \times .0003 = 0.228$ mm. of mercury upon the surface of the lake. As a result $CO_2$ will begin to be absorbed by the lake waters. This will continue until the pressure, or "tension," of the $CO_2$ within the lake equals that of the air above. In this case, the lake will absorb approximately 0.228 cc. of $CO_2$ per liter of water at 25° C., because at this temperature 1 liter of water dissolves approximately 1 cc. of $CO_2$ for each 1 millimeter of $CO_2$ tension in the air above the liquid *(4)*.

The $CO_2$ tension of a liquid is expressed as equal to the pressure, or "tension," of $CO_2$ in the air above after equilibrium has been reached. In the case of the example given, the $CO_2$ tension of the lake water would be 0.228 mm. of mercury. It might also be expressed as 0.0003 atmosphere, as $CO_2$ is present in the ratio of 3 cc. in 10,000 cc. of air.

If a solution of $NaHCO_3$ is placed under a bell jar and connected to a vacuum pump, and if the pressure of $CO_2$ above the solution is gradually lowered by removal of the air, $CO_2$ is released and the bicarbonate slowly changes into the carbonate:

$$2\ NaHCO_3 \rightarrow Na_2CO_3 + CO_2 + H_2O$$

Upon the completion of this reaction, further reduction in pressure releases practically no $CO_2$. It is apparent, therefore, that the carbonate in solution would exert practically no carbon dioxide tension, but that bicarbonates do exert a pressure in the solution equal to the tension or pressure in the air above, which is necessary to prevent their decomposition into the carbonate.

The action of phytoplankton during photosynthesis is equivalent to the action of the air pump. As the free $CO_2$ is used up, the tension is reduced and the bicarbonates release additional $CO_2$ and are transformed into carbonates. The plankton algae are apparently unable to utilize appreciable amounts of carbon in the carbonate form.

$CO_2$ tension, therefore, is not a measure of the total $CO_2$ present in the water but is a measure of its availability for photosynthetic purposes. The actual amount of $CO_2$ present as free $CO_2$ and as bicarbonate $CO_2$ at any given $CO_2$ tension varies depending upon the concentration of salts and kinds of salts in solution *(14)*, and the temperature of the solution.

The problem of whether the use of $CO_2$ tension is more valuable than the expression of active $CO_2$ as p.p.m. of $CO_2$ and $HCO_3^-$ to fisheries investigators has not yet been settled. However, the $CO_2$ tension is very useful and widely used in physiological chemical studies of both plants and animals. It must be understood to adequately understand the complex problems in relation to the role of $CO_2$ in the pond environment. It is especially important to understand the role of $CO_2$ tension in the respiration of fishes.

Carbon Dioxide Tension and Respiration of Fishes. The $CO_2$ tension of the fish's blood and that of the water in which it lives both exert a powerful influence upon respiration. Oxygen is absorbed into the blood from the water through the fine gill membranes and here combines chemically with the haemoglobin in the red blood cells to form oxyhaemoglobin. In this form it is carried to the various body tissues.

The presence of alkali increases both the speed with which haemoglobin combines with oxygen and the amount of oxygen that it will absorb at any given oxygen tension. Acids, including $CO_2$, have the opposite effect. A high $CO_2$ tension in the water would, therefore, have the effect of reducing both the speed of oxygen absorption and the amount carried by the haemoglobin. In experiments with sheep's blood, it was found that an increase in $CO_2$ tension from 5 mm. to 10 mm. reduced the oxygen-carrying capacity of the haemoglobin approximately 30 per cent *(5)*.

Upon reaching the tissues, some of the oxygen is released from the oxyhaemoglobin, partly because the oxygen tension is lower in the tissues than in the blood and partly because of the increase in acidity caused by rapid diffusion of carbon dioxide (a waste product of cell metabolism) from an area of high $CO_2$ tension (the body tissues) to an area of lower $CO_2$ tension (the blood).

In cold-blooded animals, the role of carbon dioxide in speeding the release of oxygen from the oxyhaemoglobin is especially important because of the low body temperatures. The carbon dioxide is carried by the blood from the cells to the gills where it diffuses into the surrounding water. The blood returning to the gills still contains a high percentage of its total oxygen, which acts as a temporary reserve.

It is evident, therefore, that there must be three levels of $CO_2$ tension involved: namely, high $CO_2$ tension in the body tissues, a lower one in the blood, and a still lower tension in the surrounding water. This is necessary to enable the transfer from body tissue to blood to water. If the $CO_2$ tension of the water should suddenly be increased above that of the fish's blood, then the fish would be unable to dispose of the carbon dioxide and the amount of oxygen carried by the haemoglobin would be greatly reduced. If the change was not too great, the increased $CO_2$ tension in the body tissues caused by inability of the blood to dispose of this waste product would release oxygen from the temporary reserve in the blood. However, a greater increase in $CO_2$ tension of the water would result eventually in death of the fish by asphyxiation. Attention was first called to this reason for fish mortality by Powers *(7)*.

It is evident, therefore, that in increasing the carbon dioxide tension of the water in order to increase algal production, great care must be taken to not raise the tension too rapidly or too high.

DETERMINATION OF $CO_2$ TENSION. There are at least two methods described for the determination of $CO_2$ tension of water. A method by Powers *(7)* and a revision of Powers' method *(6)* gave different results in tests at Auburn. Under such conditions there is need for a critical analysis of all available methods by a qualified authority and a simplification of the one adopted, so that it may be more generally used.

Both of these methods depend upon the accurate determination of the pH of the body of water being investigated. The experiences of the author in trying to accurately determine this value were not promising. At first, the water samples were collected from a series of ¼-acre fertilized ponds, taken to the laboratory, and analyzed for pH, $CO_2$ tension, and p.p.m. $CO_2$, $HCO_3^-$, $CO_3^{--}$, and Ca. In the laboratory, very accurate determinations of pH could be made of the water. Unfortunately the pH had changed between the time the sample was taken and when it was analyzed about 1 hour later. A portable glass electrode outfit with an immersion electrode was next used, and attempts were made to determine the pH at various depths in the pond between the hours of 7 to 9 a.m. If the sun was shining, there was a gradual drift of the indicator needle, making the reading at best only an approximation. Furthermore, at a uniform depth of 3 feet, three readings were taken approximately 4 feet apart. These individual readings varied normally by as much as 0.1 to 0.2 pH and occasionally by as much as 1.0 pH. In addition, of course, there were variations of 0.1 to 0.6 pH from top to bottom of the 6 feet of water, and from pH 7 to 9.5 in the same pond in the same position depending upon the time of day when the pH was taken, the amount of sunshine, and the phytoplankton concentration in the water. To minimize these difficulties, the pH reading and the water samples for analyses were taken at the same spot and at the same depth (3 feet). However, it would appear unwise to assume that the resulting figures closely represented conditions generally in the pond.

The Powers method was used for the determination of $CO_2$ tension. By this method, a water sample is aerated until in equilibrium with atmospheric air and the pH is then determined. Samples were first aerated 1 hour with compressed air, the pH determined, aerated for another hour, and again checked. Since the pH was changing, aeration was continued. Unfortunately, the pH was still changing after 24, 48, and even 72 hours. Since errors caused by slow oxidation of organic matter and inorganic salts in the sample may be expected from long continued aeration, it was decided arbitrarily to aerate all samples 12 hours for this determination.

The calcium was determined by precipitation as the oxalate and titration with permanganate by standard procedures. This was done because it was thought that the amount of soluble calcium would be an indirect measure of the amount of bicarbonates in solution. The $CO_2$, $HCO_3^-$, and $CO_3^{--}$ were determined by titration *(1)* of samples within 2 hours after they had been removed from the ponds.

RESULTS OF TESTS FOR $CO_2$ TENSION, pH, $CO_2$, AND Ca IN POND WATERS. The results of analyses for $CO_2$ tension, pH, $CO_2$, and Ca are given for a representative group of ponds in Table 1. Each figure given is the average of three analyses. If the $CO_2$ tension values as derived mean anything, these pond waters were

TABLE 1. THE pH, $CO_2$ TENSION, CARBON DIOXIDE, AND CALCIUM IN FERTILIZED POND WATERS

| Date Sampled | Pond No. | pH[1] | $CO_2$[2] Tension | $CO_2$[3] p.p.m. | $HCO_3^-$ p.p.m. | $CO_3^{--}$ p.p.m. | Ca p.p.m. | Water temp. °C. |
|---|---|---|---|---|---|---|---|---|
| Dec. 6, '43 | F-12 | 8.8 | 17.614 | 0.4 | 15.2 | 0 | 5.1 | 13 |
| " | 14 | 8.8 | 17.737 | 2.9 | 51.8 | 0 | 11.0 | 13 |
| " | 15 | 8.3 | 7.123 | 1.1 | 23.4 | 0 | 7.1 | 13 |
| " | 16 | 8.9 | 24.102 | 1.8 | 17.3 | 0 | 6.8 | 13 |
| " | 17 | 7.5 | 21.588 | 3.3 | 12.2 | 0 | 14.8 | 13 |
| Jan. 17, '44 | 12 | 8.8 | 13.387 | 2.8 | 16.8 | 0 | 6.4 | 5.5 |
| " | 14 | 9.0 | 9.160 | 3.3 | 21.4 | 0 | 12.8 | 5.5 |
| " | 15 | 8.7 | 11.575 | 2.2 | 24.4 | 0 | 7.6 | 5.5 |
| " | 16 | 9.0 | 12.022 | 2.2 | 10.7 | 0 | 7.2 | 5.5 |
| Feb. 7, '44 | 12 | 7.4 | 10.569 | 3.8 | 18.3 | 0 | ---- | 13.2 |
| " | 14 | 8.7 | 13.309 | 0.0 | 58.0 | 3.0 | 16.9 | 13.2 |
| " | 15 | 8.0 | 6.799 | 3.0 | 48.8 | 0.0 | 9.6 | 13.2 |
| " | 16 | 7.4 | 11.449 | 2.2 | 24.4 | 0.0 | 9.4 | 13.2 |

[1]The pH readings were made at a depth of 3 feet with an immersion glass electrode.

[2]Figures are the negative logarithms of the $CO_2$ tension in atmospheres. For reference, 3.5229 = the tension in air (containing 0.03% $CO_2$), 1.301 = the tension in the human lungs (containing 5% $CO_2$). High values indicate low $CO_2$ tension.

[3]Titrations for $CO_2$ were made upon samples taken at a depth of 3 feet. The samples were carried to the laboratory and analyzed within less than 2 hours, but within this short time the pH had changed to a lower value than that given in the column under pH.

practically devoid of $CO_2$ in a form available for use by algae in photosynthesis. However, titration for free $CO_2$ and bicarbonates gave results considerably at variance with the $CO_2$ tensions. It should be remembered that the samples for titration were kept in tightly stoppered flasks for up to 2 hours before analysis. The calcium content of the water showed agreement in a general way with the bicarbonates. The agreement was possibly as close as should be expected, in view of the fact that bicarbonates of Mg, Na, and K were also possibly present and that some of the calcium may have been combined with soluble phosphates. If all were present as $Ca(HCO_3)_2$, the p.p.m. $HCO_3^-$ should be approximately 3 times the p.p.m. Ca. The data roughly approximate this ratio.

It is evident that there is need for a careful reappraisal and standardization of all analytical methods dealing with $CO_2$ in pond waters. In addition to those mentioned here, there are many others in use that gave a variety of confusing results. It is quite probable that most methods in use are of extremely little actual value.

In addition to the standardization of methods, there is need for a standardization of the time of day when such chemical tests should be made. It appears probable that, in general, the values for pH, $CO_2$, and $CO_2$ tension for various bodies of water reported in the literature are worthless because the fisheries workers did not get up early enough. These values change rapidly during photosynthetic action. For example, the pH of a pond very productive of plankton may vary from 7.0 before daybreak to 9.5 or higher by the middle of the afternoon. An alkali pond may have a pH of 9.5 even before daybreak, indicating that it has practically no carbon dioxide in a form available for use in photosynthesis by the algae. It would appear that, if analyses of the above type are to be of value for comparative purposes, they should be made shortly before sunrise.

## Fertilizer Experiments with Organic and Inorganic Materials

Since $CO_2$ appeared to be a factor in delayed growth of phytoplankton and a limiting factor in fish production, experiments were conducted with inorganic fertilizers in combination with organic materials that would supply $CO_2$ upon decomposition.

The experiments were conducted in a series of ¼-acre excavated ponds. These had a maximum depth of 6 feet and were supplied by water from a small stream rising in the Piedmont Plateau area where the soils are low in calcium and in organic matter. The stream water as it entered the ponds was very low in bicarbonates (15 to 20 p.p.m. $HCO_3^-$). The ponds were stocked with bluegills and largemouth bass. Each series of experiments was run approximately 1 year. The ponds were then drained and the fish counted and weighed. Due to the unsatisfactory nature of chemical analytic methods, the results of the various treatments were measured only by the pounds of fish that they produced.

The experiments included tests of various inorganic fertilizer mixtures alone and in combination with organic materials as sources of $CO_2$. Tests were also run using hays, manures, and cottonseed meal for purposes of comparison.

Inorganic Fertilizer Alone. The 6.6-8-2, and the 6-8-4 plus nitrate of soda were used at rates giving approximately the same amounts of nitrogen and phosphorus per acre at each application. However, the ponds received 9 applications in 1945 and 12 in

TABLE 2. PRODUCTION OF FISH PER ACRE WITH VARIOUS FERTILIZER TREATMENTS[1], 1944

| Materials added, rate per acre | | | Fish produced per acre | | | |
|---|---|---|---|---|---|---|
| 6.6-8-2[2] | Lime-stone[3] | Other materials | Bass | Bluegills Large | Bluegills Small | Total |
| *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* |
| None | None | None | 7.5 | 55.0 | 37.5 | 100.0 |
| 1,440 | | | 38.0 | 145.2 | 106.4 | 289.6 |
| 1,440 | | | 28.0 | 180.4 | 79.6 | 288.0 |
| 1,440[4] | | | 30.0 | 94.0 | 156.0 | 280.0 |
| 5,760[5] | | | 15.2 | 181.0 | 76.0 | 276.2 |
| 1,440 | 400 | | 34.0 | 80.8 | 49.0 | 163.8 |
| 1,440 | 1,100[6] | 1,100—flour[6] | 13.0 | 122.0 | 60.0 | 195.0 |
| 1,440 | 120 | 120—flour[7] | 44.0 | 164.8 | 140.4 | 349.2 |
| 1,440 | | 4,000—manure | 32.0 | 182.0 | 196.4 | 410.4 |
| 1,440 | 800 | 4,000—manure[8] | 16.0 | 192.0 | 121.2 | 329.2 |
| 1,440 | 800 | 4,000—dry grasses[8] | 44.0 | 222.0 | 50.0 | 316.0 |
| 1,440 | 440 | 4,120—dry grasses | 42.0 | 134.0 | 105.0 | 281.0 |
| 1,440 | 800 | 4,000—hardwood leaves[8] | 52.0 | 104.4 | 44.4 | 200.8 |
| 1,440 | 800 | 4,000—dry pine needles[8] | 44.0 | 150.4 | 52.0 | 246.4 |
| 1,440 | 800 | 4,000—fresh pine sawdust[8] | 6.0 | 245.2 | 16.0 | 267.2 |
| 1,440 | | 84—$NaHCO_3$[9] | 37.0 | 109.6 | 114.0 | 260.6 |

[1]All ponds stocked with 1,500 bluegills per acre on 11/15/43 and 148 bass fry per acre 5/17/44. The ponds were drained November, 1944.
[2]Twelve applications of 120 pounds per acre of inorganic 6.6-8-2 fertilizer.
[3]Finely ground dolomitic limestone (agricultural lime).
[4]All nitrogen in this mixture derived from urea; $(NH_4)_2SO_4$ used in all others.
[5]Twelve applications of 480 pounds per acre.
[6]Four hundred pounds on 11/7/43, 300 pounds between 1/4 and 3/10/44, 400 pounds on 6/13/44.
[7]Ten pounds limestone and 10 pounds wheat flour mixed with each fertilizer application.
[8]Four hundred pounds limestone and 1 ton manure, leaves, grass or sawdust applied 11/29/43 and 4/8/44.
[9]Forty-two pounds applied 11/29/43 and an equal amount 3/16/44.

1946. In 1944 (Table 2) 1440 pounds of 6.6-8-2 per acre gave an average fish production of 286 pounds, while in 1945, (Table 3), 990 pounds of 6-8-4 and nitrate of soda gave an average production of 250 pounds of fish per acre. This production was so low it indicated that all the fertilizer added was not being efficiently utilized. Evidence that this was the case was given by another pond in the latter series, which received 5,760 pounds of 6.6-8-2 and gave a yield of only 276.2 pounds of fish. In another series of experiments (Table 4), a total of 1 ton of 6.6-8-2 per acre was applied to one pond at one time. The plankton production in this pond did not rise above that of an adjacent pond receiving only 120 pounds per acre per month until 5 months after the heavy application. This heavily fertilized pond did produce 341 pounds of fish per acre, as compared to 222 pounds produced by the one receiving 1,200 pounds of fertilizer in 10 equal applications. Such heavy fertilizer applications are dangerous, however, because, in ponds where sufficient $CO_2$ was available for heavy plankton growth, smaller applications produced such heavy algal growths that fish were killed.

UREA AS A SOURCE OF NITROGEN FOR FERTILIZERS. Since urea decomposes in water to form $CO_2$ and ammonia, it was hoped that the use of this material as a source of nitrogen in mixed fertilizers might be advantageous. Where all the nitrogen in a 6.6-8-2 was supplied by ammonium sulfate, 285.8 pounds of fish per acre was produced (Table 2). When urea was substituted as the source of nitrogen, the production was 280 pounds. Apparently, both sources of nitrogen were equally desirable, but the amount of $CO_2$ evolved from the urea was too small to make any difference in production.

ADDITION OF LIMESTONE TO FERTILIZED PONDS. In order to avoid any injurious effects of sudden changes in $CO_2$ tension upon the respiration of fishes, finely ground dolomitic limestone was added to ponds that were also to receive organic materials. It was hoped that the calcium carbonate would tie up as bicarbonates the carbon dioxide liberated by decay of the organic materials, thus acting as a storehouse for $CO_2$ and preventing injury to the fish from high $CO_2$ tensions. The addition of ground limestone reduced fish production in both the 1944 and 1945 experiments (Tables 2 and 3).

EFFECT OF THE ADDITION OF WHEAT FLOUR TO FERTILIZED PONDS. A low grade of white wheat flour was one of the organic materials used to increase the $CO_2$ content of ponds. In the first test, (Table 2), 1,100 pounds of flour and 1,100 pounds of ground limestone per acre were added to the pond, divided into 3 approximately equal applications, 2 in the winter and 1 in June. The pond was fertilized regularly with inorganic fertilizer. At the end of the experiment, the pond produced 195 pounds of fish. This was about 30 pounds more than the pond receiving inorganic fertilizer and limestone, but about 90 pounds less than where only inorganic fertilizer was used. It is believed that the large amount of limestone applied was the principal cause of low production, and also that too much flour was added at one time for efficient utilization of the $CO_2$.

In the next experiment, 10 pounds flour and 10 pounds ground

TABLE 3. PRODUCTION OF FISH PER ACRE WITH VARIOUS TREATMENTS[1], 1945

| Materials added, rate per acre | | | Fish produced per acre | | | |
|---|---|---|---|---|---|---|
| | | | | Bluegills | | |
| 6-8-4 | $NaNO_3$ | Other materials | Bass | Large | Small | Total |
| *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* |
| None | None | None | 35 | 65 | 25 | 125 |
| 900[2] | 90[2] | | 59 | 144 | 32 | 235 |
| 900 | 90 | | 32 | 148 | 66 | 246 |
| 900 | 90 | | 48 | 190 | 38 | 276 |
| 900 | 90 | 180—C. S. M.[3] | 40 | 140 | 72 | 252 |
| 900 | 90 | 180—C. S. M. | 74 | 147 | 106 | 296 |
| 900 | 90 | 180—Wheat flour | 20 | 176 | 106 | 302 |
| 1,800 | 180 | 180—Wheat flour | 30 | 124 | 186 | 340 |
| 900 | 90 | 800—Ground limestone | 54 | 132 | 16 | 202 |
| 900 | 90 | 800—Ground limestone | 54 | 136 | 60 | 250 |
| 900 | 90 | 800—Ground limestone, 180—Wheat flour | 47 | 196 | 62 | 297 |
| 900 | 90 | 800—Ground limestone, 180—Wheat flour | 36 | 164 | 26 | 234 |
| 900 | 90 | 900—Ground limestone, minor elements[4] | 32 | 154 | 33 | 219 |
| | | 900—C. S. M. + S. P. (3-1)[5] | 48 | 146 | 66 | 260 |
| | | 900—C. S. M. + S. P. (3-1)[5] | 32 | 128 | 80 | 240 |

[1]All ponds were stocked with 1,500 bluegills per acre 11/27/44 and with 120 bass fry per acre 4/13/45. The ponds were drained November, 1945.
[2]Nine equal applications during the experiment.
[3]C. S. M. = cottonseed meal.
[4]A commercial 6-8-4 used which contained minor elements Bo, Zn, Mn, Cu.
[5]Nine applications of 3-to-1 mixture of cottonseed meal and superphosphate.

limestone were mixed with each application of inorganic fertilizer. This treatment gave a yield of 349.2 pounds of fish, which was 63 pounds more than that produced by fertilization alone and 154 pounds more than was produced by fertilization plus limestone (Table 2).

In the 1945 experiments, (Table 3), the lime was omitted and the amount of flour was increased to 20 pounds per acre and mixed with each application of fertilizer. The production was 302 pounds of fish, or 50 pounds more than the average from fertilization alone in this series.

When 20 pounds limestone plus 20 pounds flour were mixed with each fertilizer application, the average fish production was 265.5 pounds (Table 3). This was an increase of 15 pounds over fertilization alone, but a decrease of 37 pounds due to the addition of limestone to the flour and fertilizer.

When twice the usual rate of fertilizer mixed with 20 pounds of flour was used, a production of 340 pounds of fish was obtained

(Table 3). This was an increase of 38 pounds due to doubling the amount of inorganic fertilizer.

It is apparent that mixing flour with each inorganic fertilizer application materially increased fish production. This would appear to be due to the carbon dioxide given off by the flour, as the increase in production is too great to be due to the food value of the flour alone.

It is also apparent that the addition of limestone reduced production. For want of a better explanation, it is assumed that the calcium and magnesium carbonates competed with the algae for the liberated carbon dioxide.

MINOR ELEMENTS. Since liming of soils occasionally causes deficiences in "minor elements" in the production of field crops, a commercial 6-8-4 containing these minor elements was used in a limed pond. The production was 219 pounds of fish, as compared with an average of 226 pounds for a similar treatment without the minor elements. Apparently, lack of "minor elements" was not the cause of the reduction of fish production caused by liming ponds.

EFFECT OF ADDITION OF GRASSES, LEAVES, AND SAWDUST TO FERTILIZED AND LIMED PONDS. Unfortunately, lime was added to all the ponds in these experiments and the application of inorganic fertilizer together with 2 tons of hardwood leaves, dry pine needles, or sawdust did not bring production up to that of the unlimed fertilized ponds. All of these treatments, however, did increase production materially above that of the inorganic fertilizer used alone in limed ponds (Table 2).

The addition of approximately 2 tons of dry grasses (mainly broomsage) to the limed and fertilized ponds gave an average production of 298 pounds of fish per acre, an increase of 12 pounds above inorganic fertilization alone and 134 pounds above that for fertilizer and ground limestone. The latter is a greater increase than could be accounted for by the food value of the grasses alone, since 4 tons of kudzu (legume) hay produced only 80 pounds of fish more than an unfertilized check, (Table 4). A considerable portion of the beneficial action must have been due to the carbon dioxide released.

EFFECT OF STABLE MANURE ALONE AND OF ITS ADDITION TO LIMED AND UNLIMED FERTILIZED PONDS. The application of 4 tons of stable manure (mule) in 4 equal applications (Table 4) yielded

272 pounds of fish. This was 50 pounds more than was obtained by inorganic fertilization alone where $CO_2$ appeared to be a severe limiting factor. Subtracting the production of the unfertilized check, each ton of manure produced approximately 43 pounds of fish.

The addition of 200 pounds of superphosphate to each ton of manure increased the production approximately 4.5 pounds above that of manure alone. This would not appear an economical procedure.

Four applications of 500 pounds of manure to a fertilized pond increased production only 16 pounds above inorganic fertilization alone (Table 4).

The addition of 2 applications of 1 ton of manure each to a fertilized pond increased production 125 pounds above that of inorganic fertilization alone (Table 2). Adding 800 pounds limestone and 2 tons of manure in 2 equal applications to a fertilized pond resulted in production of 44 pounds above that from in-

TABLE 4. EFFECT OF ORGANIC AND INORGANIC MATERIALS ON FISH PRODUCTION PER ACRE[1], 1943

| Inorganic fertilizers, rate per acre | Other materials, rate per acre | Fish produced per acre | | | |
|---|---|---|---|---|---|
| | | Bass | Bluegills Large | Bluegills Small | Total |
| | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* |
| 6.6-8-2, 2,240 lb.[2] | | 63.0 | 168.0 | 110.0 | 341.0 |
| 4-10-4, 700 lb.[3] | 300 C. S. M. + S. P. (3-1)[4] | 67.0 | 166.0 | 179.0 | 432.0 |
| 6.6-8-2, 1,200 lb.[5] | | 45.0 | 137.0 | 40.0 | 222.0 |
| 6.6-8-2, 1,200 lb.[5] | 2,000 manure[6] | 42.0 | 107.0 | 89.0 | 238.0 |
| | 8,000 manure[6] | 57.0 | 145.0 | 70.0 | 272.0 |
| | 8,000 manure + 800 S. P.[6] | 62.0 | 113.0 | 115.0 | 290.0 |
| | 8,000 kudzu hay[6] | 58.0 | 109.0 | 9.0 | 176.0 |
| | 8,000 kudzu hay + 800 S. P.[6] | 67.5 | 95.0 | 140.0 | 202.5 |
| None | None | 25.0 | 10.0 | 12.5 | 97.5 |

[1]All ponds stocked 10/22/42 with 1,500 bluegills per acre and on 5/10/43 with 150 bass fry per acre. The ponds were drained 10/29/ - 11/1/43.

[2]One hundred twenty lb. per acre 6.6-8-2 fertilizer added 11/14 and 12/5/42; 1 ton per acre added 3/29/43.

[3]Seven applications 100 lbs. per acre 4-10-4 added 11/14/42 to 5/14/43 and 3 applications of 100 lbs. per acre of a 3-to-1 mixture of cottonseed meal and superphosphate from 6/19 to 8/21/43.

[4]C.S.M. = cottonseed meal; S. P. = superphosphate.

[5]Divided into 10 equal applications from 11/14/42 to 10/22/43.

[6]Divided into 4 equal applications 11/14 and 12/5/42 and 5/29 and 8/21/43.

TABLE 5. PRODUCTION OF FISH PER ACRE WITH VARIOUS TREATMENTS[1], 1944

| Materials added, rate per acre | | | Fish produced per acre | | | | |
|---|---|---|---|---|---|---|---|
| | | | Bass | | Bluegill | | |
| 6-8-4 | $NaNO_3$ | Other materials | Large | Small | Large | Small | Total |
| *Pounds* | *Pounds* | | *Pounds* | *Pounds* | *Pounds* | *Pounds* | *Pounds* |
| 1,600[2] | 160[2] | | 44 | 0.0 | 210.0 | 102.0 | 356.0 |
| 1,600[2] | 160[2] | 6 T manure[3] + 6 T limestone[3] | 0.0[5] | 4.0 | 102.0[5] | 331.2 | 437.0 |
| 1,600[2] | 160[2] | 6 T manure[3] | 40 | 20.0 | 324.0 | 3.0 | 387.0 |
| 6,400[4] | 640[4] | | 70 | 4.8 | 257.2 | 60.0 | 392.0 |

[1]Ponds were stocked 8/26/43 with 1,500 bluegills (9.0 lb.) and 100 bass fingerlings (2.0 lb.) per acre. On 5/17/44, an additional 150 bass fry were added. The ponds were drained 10/26/44.
[2]Sixteen equal applications.
[3]Applied 2 tons per acre on 8/30/43, 1/1/44, and 4/10/44.
[4]Sixteen equal applications.
[5]On 7/18/44, 70 large bass and 844 large bluegills died presumably due to low oxygen. Total weight of dead fish = 207.0 lb.

organic fertilization alone, but 81 pounds less than that obtained without the limestone.

When 6 tons of manure per acre in 3 equal applications were added to a fertilized pond, the production was 387 pounds, or only 31 pounds more than was obtained by inorganic fertilization alone (Table 5). This experiment was run 14 months, or 2 months longer than that discussed in the preceding paragraph. Fish production was increased 5 pounds for each ton of manure used to supplement inorganic fertilization in the former case (Table 5) and 62 pounds per ton of manure in the latter (Table 2). Apparently, the addition during one season of 6 tons per acre of manure together with inorganic fertilization is excessive or 2 tons of manure was too large an amount to add at one time.

When 6 tons of manure and 6 tons limestone in 3 equal applications were added to a pond receiving inorganic fertilization, a very heavy plankton growth resulted and the fish grew at an excellent rate until July 18 when large numbers died (Table 5). Presumably this was due to oxygen depletion or possibly high $CO_2$ tension or both. Unfortunately, no analyses were made during the period when the fish were dying. A total of 207 pounds of dead bass and bream was removed from the pond. The fish that died were mainly large fish. Some of the large bream and all of the small bream lived; no further difficulties were experienced. When the pond was drained in the fall, 437 pounds of fish were recovered. This was 81 pounds more than was produced by inorganic fertilization alone and 50 pounds more than was produced without the limestone. If the 207 pounds that died previously in the pond is added to the total recovered in the fall, the yield would amount to 644 pounds of fish per acre during the 14-month period. However, this would not be a fair basis for comparison with the other treatments.

Two points should be noted. In this case, when heavy amounts of organic matter were applied, limestone increased fish production. At the same time, contrary to preconceived ideas, fish survival was greatest in the pond that did not receive limestone. Since the cause of the death of the fish is unknown, this does not in any way invalidate these theories, but merely indicates a need for further study.

EFFECT OF THE USE OF COTTONSEED MEAL ON FERTILIZED PONDS. Applications of 900 pounds of a mixture of 3 parts cottonseed meal to 1 part superphosphate in 9 equal treatments produced an average of 250 pounds of fish per acre; this is about the same average as was produced by inorganic fertilization alone (Table 3).

The addition of 20 pounds cottonseed meal to each application of inorganic fertilizer increased production 22 pounds above the average from fertilization alone, as compared with a 50-pound increase from an equal weight of wheat flour (Table 3).

Since it appeared that a combination of organic and inorganic fertilization would be an ideal solution, a pond was fertilized with an inorganic 4-10-4 fertilizer in the winter and early spring months and with a cottonseed meal-superphosphate mixture in the summer and fall. The inorganic fertilizer was used in the colder parts of the year to prevent the formation of filamentous algae. The treatment produced 432 pounds of fish per acre from 700 pounds of 4-10-4 and 300 pounds of a 3-to-1 mixture of cottonseed meal and superphosphate (Table 4). This was 210 pounds more than was produced by a 1,200-pound application of a 6.6-8-2 inorganic fertilizer, and 182 pounds more than was produced by 900 pounds of the cottonseed meal-superphosphate mixture alone. Apparently this combination of the two fertilizers was mutually beneficial.

EFFECTS OF LIMESTONE. All types of organic materials added to inorganically fertilized ponds increased fish production except where limestone also was used. The addition of limestone reduced production, except where it was combined with extremely large amounts of organic materials.

A study of these results indicated that the addition of limestone was unnecessary where moderate amounts of organic materials were applied, because the plankton algae were capable of absorbing all the carbon dioxide released. The addition of limestone decreased production, because it competed with the algae for the available carbon dioxide. After part of this gas had been tied up as the bicarbonate, the algae had to do work at reduced carbon dioxide tensions to recover it for use in photosynthesis. At reduced $CO_2$ tensions, the algae are much less efficient *(2)*.

EFFECT OF ADDITION OF SODIUM BICARBONATE TO A FERTILIZED POND. The addition of 84 pounds of sodium bicarbonate per acre in two equal applications to a fertilized pond gave a production of 25 pounds less than inorganic fertilization alone (Table 2). This difference is not considered significant.

HAY AS FERTILIZER. Application of 4 tons of kudzu hay per acre in 4 equal applications gave a total production of 176 pounds of fish (Table 4). This was 78 pounds more than was produced by the unfertilized check. Each ton of hay, therefore, produced approximately 19 pounds of fish.

The addition of 200 pounds of superphosphate to each ton of hay increased the production of fish by 6 pounds.

Other experiments with Johnson grass hay gave similar results. Apparently, the use of hays for fish production is uneconomical, either with or without the addition of superphosphate.

ABSORPTION OF CARBON DIOXIDE FROM AIR. Although these studies have demonstrated that organic materials may be added to inorganic fertilizers to increase fish production, the added cost makes this generally uneconomical. Carbon dioxide can be obtained by fertilizing and waiting. It will gradually accumulate in the pond by absorption from the air. For example, ponds receiving inorganic fertilization were supporting an average of 285 pounds of fish per acre at the end of 12 months (Table 2), while a similar pond at the end of 14 months was supporting 356 pounds of fish (Table 5). It has been generally noted in experimental ponds that phytoplankton grows more readily and in greater abundance after the first year if the water is not displaced. On the other hand, in ponds where the water is displaced by winter rains and the accumulated carbon dioxide is lost, considerable trouble is experienced every year in obtaining a good algal growth during the spring and early summer.

RELEASE OF CARBON DIOXIDE FROM CARBONATES. When pond waters contained a considerable supply of carbonates, it appeared that the bound $CO_2$ could be made available for algal growth by the use of acid-forming fertilizers. Commercial fertilizer mixtures, such as the 6-8-4, have sufficient limestone added to neutralize the acid released after their addition to the soil. In alkaline waters especially, acid-forming fertilizers would appear beneficial.

When all the nitrogen for pond fertilization was derived from ammonium sulfate, sulfuric acid was released into the water as the algae utilized the ammonia nitrogen in photosynthesis. In soft-water ponds sufficient acid was released to give the water a pH 5.0.

When Ammophos (a commercial ammonium phosphate containing 11 per cent nitrogen and 48 per cent $P_2O_5$) was used for pond fertilization, the algae utilized all the ammonia and part of the phosphorus. The remaining phosphoric acid gave experimental ponds an acidity ranging between pH 4.5 to 6.5 during the year. When equal amounts of calcium sulfate were applied with the Ammophos, the pH varied from 4.5 to 5.8.

It is apparent, therefore, that fertilizer mixtures can be devised that will release appreciable amounts of acid as the algae utilize the mineral nutrients.

In order to test whether the released acid would be beneficial in the presence of carbonates, two ponds were fertilized with 1,900 pounds per acre of Ammophos, divided into 3 applications. To one of the ponds, 2 tons of limestone was added. The ponds were stocked with bluegills only and were drained after 8 months. Plankton analyses at 2-week intervals indicated that the limed pond produced a 20 per cent greater crop of plankton than the unlimed pond. The former produced 355 pounds and the latter 292 pounds of fish per acre. This is an increase of 63 pounds due to the addition of limestone. However, the pH of the limed pond varied from 6.1 to 6.9 compared with 4.5 to 6.5 in the unlimed pond at equivalent times. It is possible, therefore, that part of the increased production was due to a more favorable reaction in the former rather than to an increase in available $CO_2$.

The problem of the use of acid-forming fertilizers in water containing carbonates is in need of intensive study. It would appear to offer possibilities as a method for increasing fish production, especially in highly alkaline waters.

## Clearance of Muddy Waters

It has been observed that muddy waters clear rapidly above beds of underwater weeds, around the roots of floating hyacinths, and around decaying piles of vegetation. It appeared probable that the release of $CO_2$ caused the precipitation of the suspended soil colloids. New ponds were found to muddy readily from wading or by wave action, but, after a heavy crop of plankton had been produced by fertilization, the stirred up soil colloids precipitated rapidly.

Muddy ponds did not clear following applications to the water of calcium carbonate, basic slag, or superphosphate. The addition of considerable amounts of sodium nitrate to muddy waters resulted in the pond remaining muddy for much longer periods of time. The sodium united with the colloidal soil particles to form a sodium clay, which remained in suspension almost indefinitely.

Application of inorganic commercial fertilizers cleared muddy waters only if the waters were transparent to a depth of 6 inches or more. Under these conditions the fertilizer often caused the growth of phytoplankton and the presence of these algae caused the precipitation of the colloidal soil particles.

All organic fertilizers used aided in clearing muddy ponds. Similar results were obtained by Irvin in Oklahoma *(3)*. In experiments at Auburn repeated applications of a 3-to-1 mixture of cottonseed meal and superphosphate at the rate of 100 pounds per acre and of manure at the rate of 1 ton per acre were very effective. Where ponds become muddy only at rare intervals, these materials should be used until the water clears. Subsequently, inorganic fertilizers may be used.

## Summary

Fertilization of pond waters results in the growth of an unpredictable succession of mixed species of algae. The relative desirability of various species and methods for the production of these species have not yet been determined.

The concentration of phytoplankton in surface scums normally is less desirable than a dispersed type of growth in which the algal forms are somewhat uniformly distributed throughout the water. The size of the algae, the species, the concentration of algae, light, and $CO_2$ tension of the water are some of the factors

involved in surface scum formation. A detailed study of this problem is needed.

Organic fertilizers tend to cause the growth of filamentous algae because they decay slowly on the pond bottom. Inorganic fertilizers dissolve readily and are distributed throughout the water. Therefore, they encourage the growth of the floating plankton algae. Inorganic fertilizers applied in cold water are more slowly soluble, and under such conditions they may cause the growth of filamentous forms. Heavy growths of filamentous algae were controlled in the spring by breaking up the floating mats and applying inorganic fertilizer. This caused the growth of plankton algae, which shaded and killed the filamentous forms.

In certain cases the lack of pulsation in phytoplankton growth seemed to be undesirable, because it appeared to indicate that the algae were not being adequately utilized by aquatic animals. Under these conditions a greater portion of the available nutritive elements remain in a phytoplankton-to-phytoplankton cycle.

Delayed growth of phytoplankton following inorganic fertilization was a puzzling problem. Aeration of the water, inoculation with actively growing algae, addition of essential "minor elements," and addition of vitamins $B_{1+6}$ did not correct this condition. One cause was found to be a lack of sufficient available carbon dioxide for plankton growth. There are probably a number of other factors or combinations of factors involved, and this problem needs intensive study.

Carbon dioxide was found to be a limiting factor in fish production in ponds fertilized with inorganic materials. Analytical methods for $CO_2$ and $CO_2$ tension (Powers method) did not give comparable results. Research is needed to critically compare various methods for the determination of $CO_2$ tension and to simplify the procedure and calculations so that the method can be used by fisheries workers.

The addition of ground limestone to ponds receiving inorganic fertilizer decreased fish production. The added calcium and magnesium apparently competed with the algae for the available carbon dioxide. After the carbon dioxide was bound as the bicarbonate, it was released for photosynthesis only under decreased $CO_2$ tension. Under these conditions the algae were less efficient.

The addition of various organic materials to ponds receiving inorganic fertilizer increased fish production. This apparently was due in part to the carbon dioxide supplied for photosynthesis by the organic materials. White wheat flour, manure, grasses, and cottonseed meal were effective for this purpose. However, use of these materials adds considerably to the cost of fertilization and their use is not recommended pending further experimentation.

Fertilized ponds slowly accumulated carbon dioxide from the air and gave better phytoplankton growth and higher fish production after the first year.

Four tons of stable manure per acre in 4 equal applications, used alone as a fertilizer, increased fish production 43 pounds for each ton. The addition of 200 pounds of superphosphate to each application increased production 4.5 pounds above that for manure alone.

Kudzu hay used in the same manner and at the same rate increased fish production 19 pounds for each ton. The addition of the superphosphate gave an additional increase of 6 pounds of fish.

Muddy ponds could not be cleared by the addition to the water of limestone, basic slag or superphosphate. Plankton algae, underwater pond weeds, and decaying vegetation precipitated the suspended soil colloids. Repeated applications of 1 ton of manure per acre or 100 pounds of a 3-to-1 mixture of cottonseed meal and superphosphate was effective in clearing muddy water.

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