BIOTIC INFLUENCES AFFECTING POPULATION GROWTH OF PLANKTONIC ALGAE

BY THEODORE R. RICE

FISHERY BULLETIN 87

UNITED STATES DEPARTMENT OF THE INTERIOR, Douglas McKay, *Secretary*
FISH AND WILDLIFE SERVICE, John L. Farley, *Director*

ABSTRACT

A culture medium in which both *Chlorella* and *Nitzschia* would grow well was devised so that the effect of each on the other could be tested when grown together in the same medium. An increase in inhibition of the growth rate of *Chlorella* populations of the same size was demonstrated with an increase in the number of *Nitzschia* cells used to start the culture. A similar increase in inhibition of growth rate of *Nitzschia* cells required a relatively larger increase in the number of *Chlorella* cells used to start the cultures.

If culture medium in which either *Chlorella* or *Nitzschia* had been growing was Berkefeld filtered, the pH adjusted, and nutrients added, it was found that the growth rates of both species were inhibited when they were again grown in this medium. However, if a portion of this medium was also washed with Norit A and autoclaved the growth rate of neither species was inhibited. It was therefore concluded that the antagonistic substances produced by the algae were either removed or destroyed by the latter treatment. The growth rates of both *Chlorella* and *Nitzschia* were also inhibited in culture medium prepared from pond water that had supported a bloom of *Pandorina* for a period of 2 weeks.

It is concluded that antagonistic substances arising from the metabolism of phytoplankton are important, at least in fresh-water ponds, in influencing the seasonal fluctuations in total phytoplankton numbers and in numbers of each species, and in inducing a definite succession of the phytoplankton.

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BIOTIC INFLUENCES AFFECTING POPULATION GROWTH OF PLANKTONIC ALGAE

By THEODORE R. RICE, *Fishery Research Biologist*

The enormous fluctuation in abundance of phytoplankton in oceans, lakes, and ponds has attracted the attention of oceanographers and limnologists for over half a century. Many hypotheses have been advanced to explain this phenomenon. The common belief has been that the abundance of each species, the size of the total population, and the succession of species during the season are controlled by changes in the physical factors or by a lack of the necessary nutrients, or by a combination of the two influences. Attention has also been called to the action of filter-feeding animals in reducing the numbers of the phytoplankton.

The further suggestion has been made that the intensive growth, or bloom,[1] of one species might affect the growth of another species, thus exerting an influence on the seasonal succession of species in the body of water concerned. Akehurst (1931) made a study of phytoplankton in freshwater ponds over a period of 4 years. All attempts to correlate fluctuations with chemical and physical factors failed. Therefore, he reasoned that other factors such as the complicated action of toxins were very important. These toxins were believed to originate from the phytoplankton and were defined as "excretion products which serve as accessory nutrients," inhibiting the growth of some species of phytoplankton and stimulating the growth of others. He further believed that the toxins of a particular species not only inhibited its own further growth after a certain length of time, but that the toxins of the "oil producing group" of algae inhibited the growth of some species and stimulated the growth of other species in this group while all members of the "starch group" were stimulated. The same general action of toxins of the "starch producing group" were also suggested. While Akehurst made very detailed studies in the field, it is believed by the author that his conclusions which treated all algae as either oil or starch producers are too general. A study of this complicated type of interaction should be made between species and preferably under controlled laboratory conditions.

More recently it has been shown in laboratory cultures of planktonic algae that metabolites, or other biological influences, of a species tend to inhibit the growth of that species. Pratt and Fong (1940) grew cultures of *Chlorella vulgaris* in inorganic medium for different periods of time and until different population sizes had been obtained. The cells were then removed by filtering the culture medium. This filtered culture medium, which shall be referred to as "conditioned medium," was used in different proportions with fresh medium after the pH had been adjusted to prepare new cultures of *Chlorella*. The growth of *Chlorella* in this medium was found to be slower than in culture medium to which no "conditioned medium" had been added. The conclusions Pratt drew from these experiments are as follows: (1) That the growth of *Chlorella* is inhibited by the presence of the "conditioned medium" in the culture medium; (2) that the depression of growth increases as the percentage of "conditioned medium" increases; and (3) that for a given concentration of "conditioned medium" in the culture medium, the depression of growth varies inversely with the size of *Chlorella* population in the "conditioned medium" prior to filtering.

NOTE.—This paper is a revision of a thesis that was submitted to Harvard University in 1949 in partial fulfillment of the requirements for the degree of doctor of philosophy. The author acknowledges his sincere appreciation to Dr. George L. Clarke, for stimulating criticism and kindly encouragement which contributed much to the completion of this work.

[1] The terms "bloom," "flowering," "outburst," and "pulse" refer to a rapid increase in the numbers of phytoplankton above the level of abundance previously existing in the area. No numerical value has been agreed upon which defines a bloom, but population densities above 1 million cells per liter have been commonly referred to as blooms. A bloom is often accompanied by a coloring of the water.

An apparent case of algal antagonism, similar to that observed by Pratt, was reported by J. Storey (Worthington 1943), who found that the water of Lake Windermere, when an *Asterionella* bloom is disappearing, is unsuitable for the preparation of culture medium for *Asterionella*. This suggests the possibility that *Asterionella* produces a substance similar to chlorellin produced by *Chlorella*. Experiments also showed that *Skeletonema* produces a substance which inhibits its further growth (Levring 1945).

Rodhe (1948) has conducted experiments on the effects of growing two species of planktonic algae in a common medium. He found that *Asterionella formosa* cultured in the presence of *Chlorella* had a lower rate of division than when grown alone, but a detailed study of the interaction was not made. Since completion of the present investigation the author has learned of the work of Lefevre, Nisbet, and Jakob (1949). These investigators reported inhibition in the growth of some species of algae by "algastatic" substances secreted into the medium by other species of algae. They tested the growth of several species of algae in culture medium prepared with filtered medium in which *Scenedesmus* had previously grown and in other culture medium prepared with medium in which *Pandorina* had previously grown. Most of those species tested in these media grew at a slower rate and later the cells of some shrank and died.

The purpose of the present study was to ascertain whether the biological products of a species could influence its own growth as well as the growth of another species under conditions which could be tested in laboratory cultures, and to consider whether these materials actually do exert an effect under natural conditions.

PREPARATION OF CULTURES

Two species of fresh-water algae, *Chlorella vulgaris*, class Chlorophyceae, and *Nitzschia frustulum*, class Bacillarieae, were used in these experiments. The author is grateful to Dr. W. T. Edmondson for a subculture of *Chlorella* and to Dr. Ruth Patrick for identifying the *Nitzschia*. Also the author appreciates the assistance of Dr. E. G. Pringsheim in isolating the *Nitzschia* into pure culture. *Nitzschia frustulum* was originally isolated by the author from a mixture of algae obtained from the Carolina Biological Supply Company. This is the first record that this species of *Nitzschia* has been isolated and cultured in the laboratory.

LIQUID CULTURE MEDIUM

These experiments required that both *Nitzschia* and *Chlorella* grow well in the same culture medium, thus increasing the difficulty of finding a suitable medium. Many of the better-known culture media were tried but were found to be unsatisfactory for the growth of one or the other of these algae. The author, therefore, devised a nutrient culture medium which proved to be satisfactory for the growth of both *Nitzschia* and *Chlorella*.

Only Pyrex glassware was used and it was cleaned with a mixture of sulfuric acid and potassium dichromate, followed by a thorough rinsing first in tap water and then in distilled water. The culture medium was always autoclaved at least 1 day prior to the time it was to be used, since many investigators have stated that the growth of algae is inhibited if autoclaved medium is used sooner. The formula for this medium which will be referred to as standard culture medium is as follows:

$Ca(NO_3)4H_2O$	0.04 gram
$MgSO_4 \cdot 7H_2O$	.02 gram
KCl	.04 gram
Na_2SiO_3	.025 gram
$FeCl_3$	.001 gram
KH_2PO_4	10 μgAP
"A-Z" solution	1 cubic centimeter
Double-distilled H_2O	1,000 cubic centimeters

An "A-Z" minor nutrient solution, containing small quantities of a number of elements thought to be necessary for plants, has been suggested by Hoagland and Snyder (1933). This solution, modified by omitting the $CuSO_4 \cdot 5\,H_2O$ and TiO_2, was added to the standard culture medium.

STERILITY

These experiments were conducted on bacteria-free cultures, as tested from time to time by streaks made on nutrient agar containing 2 percent glucose and ½ percent peptone. *Chlorella* was maintained in a pure state by culturing it on agar slants prepared with Detmer's (diluted to two-thirds its normal strength) and Bristol's solutions,

both containing 2 percent glucose and ½ percent peptone. Only Detmer's solution was used for culturing *Nitzschia*.

ILLUMINATION OF CULTURES

A set of four 40-watt daylight fluorescent bulbs was arranged on each of the four shelves in the culture cabinet. Approximately 5 inches above each set of lights was a platform made of glass which had been ground with carborundum to ensure an even dispersal of light and on which were placed the cultures of algae. In these experiments, daylight fluorescent lamps were used which produced an illumination on the culture flasks of 375-foot candles as measured by a Weston illumination meter. The cultures were illuminated daily from 11 a. m. to 1 a. m.

TEMPERATURE

The culture cabinet was placed in a dark constant-temperature room. In these investigations a temperature of 18°±1.5° C. was maintained. The temperature variation was checked with a maximum and minimum thermometer. Two circulators were used to blow a stream of air over the cultures, thus ensuring an even distribution of temperature.

INORGANIC-PHOSPHATE DETERMINATION

Phosphate concentrations were determined by the Atkins-Denigès molybdate method as modified by Wattenberg (1937). Algal cells were removed by filtering the culture medium through a sintered glass filter before making determinations. The concentration of phosphorus is expressed as microgram-atoms of phosphate phosphorus per liter as recommended by the International Association of Physical Oceanography (Sverdrup et al. 1942; table 42).

PREPARATION OF ALGAL CELLS FOR EXPERIMENTS

Ketchum (1939) and Pratt (1940) both found that the growth of algae when transferred to fresh medium was influenced by the age of the culture from which the cells were taken. Cells from older cultures had a longer lag period and a slower rate of growth. Therefore, in these experiments *Chlorella* cells were always taken from a 6- to 7-day-old culture, while *Nitzschia* cells were taken from a 4- to 5-day-old culture.

The cells were removed from the culture medium in which they were growing by centrifuging at 2,500 r. p. m. for approximately 5 minutes. The cells were resuspended in the same type of culture medium which was being used in the experiment. A cell count was made on this concentrated suspension of cells so that the proper dilutions could be made to give the desired concentration of cells for the experiment.

DETERMINATION OF POPULATION SIZE

After thoroughly mixing the culture medium, 0.5 cc. was removed aseptically. This medium was used for making two cell counts with a Levy haemocytometer. The mean of the numbers of cells counted in all like cultures in any one experiment was taken as the population size. A careful check showed that this method is extremely accurate for determining unialgal population sizes. At the lowest population sizes used in these experiments, this counting method showed a coefficient of variation of 7.1 percent, while the largest population sizes gave even more accurate results, with a coefficient of variation of 4.6 percent. The accuracy of this method as determined by the author agrees favorably with that found by other investigators (Pearsall and Loose 1937; Pratt 1940; and Winokur 1948).

GROWTH RATES AND INTERACTIONS OF *CHLORELLA* AND *NITZSCHIA*

GROWTH CURVE AND DIVISION RATE OF *CHLORELLA*

Since it has been observed that phosphorus occasionally reaches concentrations as high as 10 μgAP/L in natural waters (Chandler and Weeks 1945), the growth rate of *Chlorella* was determined in culture medium containing this concentration. Standard culture medium was prepared with *Chlorella* in a concentration of 70 million cells per liter and placed on the illuminated shelves. At the end of each day cell counts, the pH (as determined with a Beckman pH meter), and phosphorus determinations were made for one culture.

The *Chlorella* population (fig. 1) was still increasing on the seventh day even though all the measurable phosphorus had been used by the end of the fourth day. Ketchum (1939) had shown that *Chlorella pyrenoidosa* when grown in a nonnutrient medium divides until the phosphorus

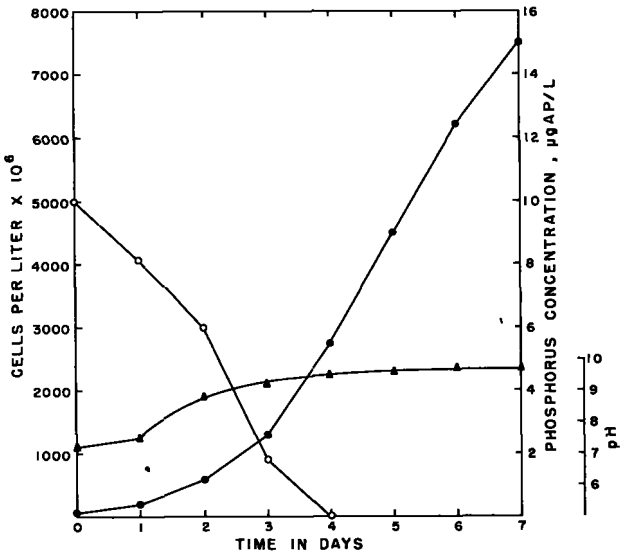

FIGURE 1.—Growth curve of *Chlorella* in standard culture medium. Dots represent growth curve of *Chlorella;* circles, phosphorus concentration: triangles, pH.

in the cells is reduced to one-half the normal content. Thus the cells in this experiment probably were able to continue dividing after all phosphorus disappeared from the medium by using intracellular phosphorus absorbed in excess of that needed during the first 4 days of the experiment. The division rate for *Chlorella* (fig. 2) was greater on the second than on the first day. The division rate on the third and fourth days was about the same as on the first day. From the fourth day through the seventh there was a steady decrease in the division rate.

Determinations of pH made at the end of each illumination period, when the culture medium was found to be most alkaline, are shown in figure 1. The pH rose from 7.2 on the first day to 8.3 on the second day, and continued to increase daily during the illumination period for the duration of the experiment. Determinations made at the end of each period of darkness showed that the pH had dropped each day to about 7.0 or 7.2.

GROWTH CURVE AND DIVISION RATE OF *NITZSCHIA*

The growth curve of *Nitzschia* under similar conditions of adequate nutrients was obtained by following the procedure described for *Chlorella* except that cultures were prepared with 10 million *Nitzschia* cells per liter. As shown in figure 3, the *Nitzschia* population reached a peak on the fifth day and was relatively constant for the remainder of the experiment. The division rate as shown in figure 4 varied from 1.5 to 1.2 on the first 3 days of the experiment and decreased rapidly on the fourth and fifth days. All measurable phosphate in the culture medium had been used by the end of the fifth day. Very little division occurred on the sixth day, and there was none on the seventh day. It appears that *Nitzschia* cells cannot continue to divide for as long a time as *Chlorella* cells after phosphorus has disappeared from the medium. It was found that *Chlorella* cells divide for 3 days after phosphorus disappears from the medium while *Nitzschia* cells divide for only 1 day. This may be due to *Chlorella* cells when grown in the presence of phosphorus absorbing much more than needed while *Nitzschia* cells grown in the presence of phosphorus may absorb only that needed for immediate use. The pH concentration, measured at the end of the light period, increased from day to day and reached a peak at the end of the third day. Beginning on the fifth day and continuing through the seventh day, the pH did not increase as much at the end of the light period as on previous days. Since the culture medium was not buffered, the pH increased as the size of the population increased. Thus the smaller increase in pH from the fifth day on was probably due to a reduced metabolic rate.

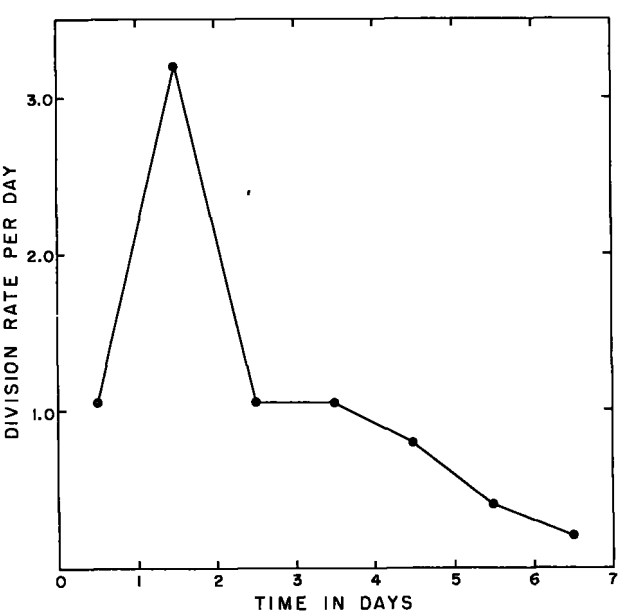

FIGURE 2.—Division rate of *Chlorella* in standard culture medium.

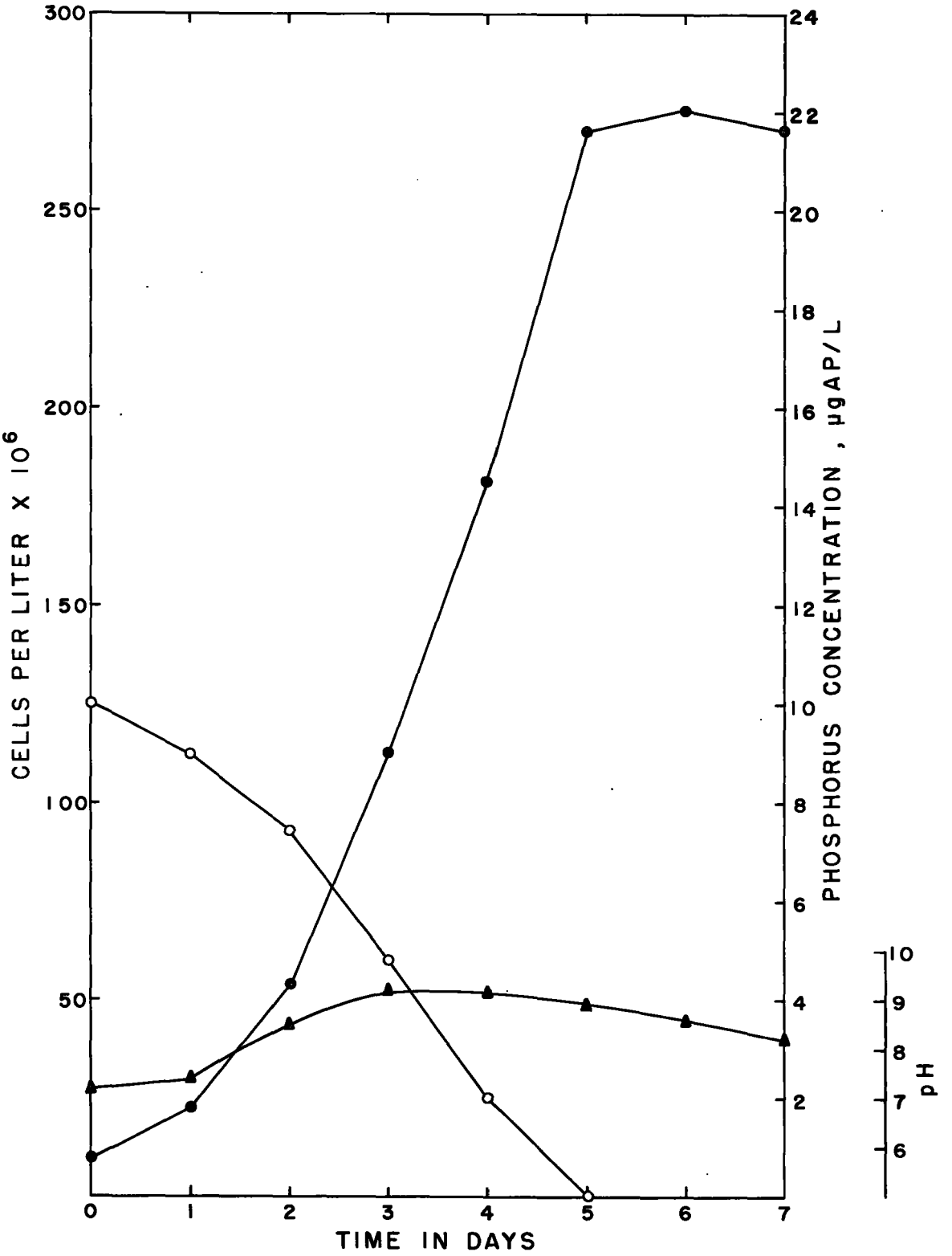

FIGURE 3.—Growth curve of *Nitzschia* in standard culture medium. Dots represent growth curve of *Nitzschia*; circles, phosphorus concentration; triangles, pH.

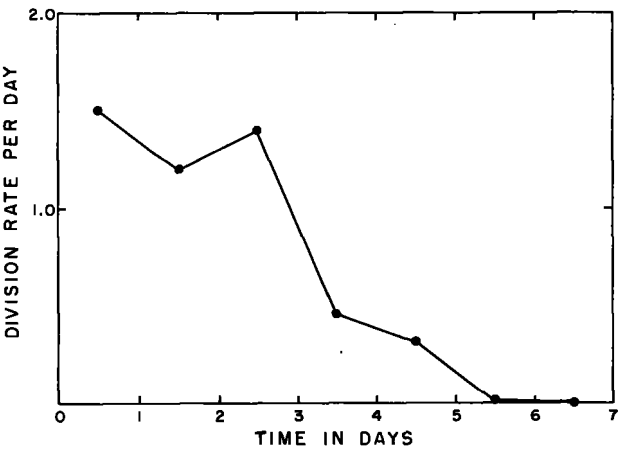

FIGURE 4.—Division rate of *Nitzschia* in standard culture medium.

INTERACTIONS OF *CHLORELLA* AND *NITZSCHIA*

To test whether or not one species of phytoplankton may inhibit the growth of another, the following experiment was run in which *Chlorella* and *Nitzschia* were grown together in mixed culture. The same procedure was followed in this experiment as described in the foregoing experiments, except that both *Chlorella* and *Nitzschia* were added initially to each flask.

Chlorella grown in mixed culture under the conditions of this experiment reached a population size only 60 percent of that attained when grown alone in the same type of medium (fig. 5); also the division rate was significantly less than when grown alone (fig. 6). There was considerable difference in the division rates on the second day and *Chlorella* grown in mixed culture continued to divide at a slower rate for the remainder of the experiment. *Nitzschia* in mixed culture reached a population size which was not significantly different from that obtained in the cultures in which *Nitzschia* was grown alone (fig. 7), and the division rates of *Nitzschia* under these conditions varied only slightly.

Even after taking into consideration the error of counting and the ordinary fluctuation occurring between two different cultures, it is evident that the results for *Chlorella* are significant. It was also found later that *Chlorella* can inhibit the growth of *Nitzschia* under certain conditions. Other investigators have shown that several algae inhibit their own growth, while in this experiment it has been demonstrated that a species of alga can also inhibit the growth of another species. Pratt and Fong (1940) reported that *Chlorella* produces and liberates into the culture medium an antibiotic substance which they named chlorellin. This author believes that *Nitzschia* also produces an antibiotic substance which inhibited the growth of *Chlorella* in the experiment described here. Further experiments supporting this conclusion are presented later.

Referring again to figure 5, it is seen that the phosphorus was depleted by the end of the fourth day in the mixed culture. However, this also occurred in the *Chlorella* culture (fig. 1). The pH increased a little more rapidly and was slightly higher in the mixed culture than in either the *Chlorella* or the *Nitzschia* cultures. Therefore the possibility must now be considered that the observed inhibition of *Chlorella* was due to either a depletion of nutrients or an unfavorable change in pH concentration instead of the production of an antibiotic substance by *Nitzschia*.

In initially enriched medium:

To determine whether a lack of nutrients had limited the growth of either or both *Chlorella* and *Nitzschia*, experiments similar to the preceding ones were run again with twice the initial concentration of nutrients in the culture medium. In the cultures in which *Chlorella* and *Nitzschia* were grown alone and in mixed cultures, the sizes of

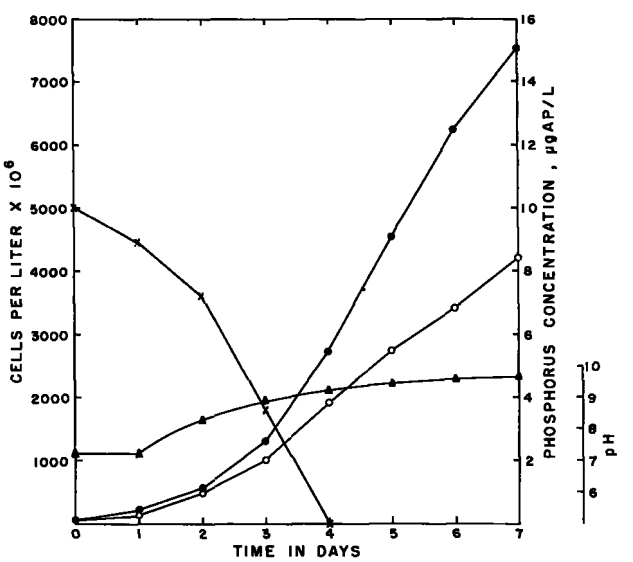

FIGURE 5.—Comparison of growth curves of *Chlorella* in *Chlorella* culture and in mixed culture prepared with standard culture medium. Dots represent growth curve in *Chlorella* culture; circles, growth curve in mixed culture; X's, phosphorus concentration; triangles, pH.

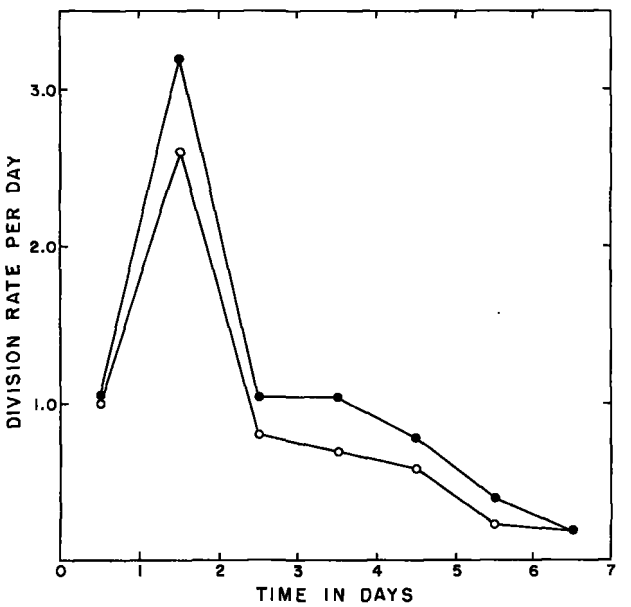

FIGURE 6.—Comparison of division rates of *Chlorella* in *Chlorella* culture and in mixed culture prepared with standard culture medium. Dots represent division rate in *Chlorella* culture; circles, division rate in mixed culture.

the populations reached on the seventh day in this experiment with twice the amount of nutrients was not significantly different from those obtained in the previous experiments. Also the division rates did not vary significantly.

In medium enriched daily:

Since nutrients may inhibit the growth of some algae if present in too high a concentration, the preceding experiment was repeated except that the extra amount of nutrients was added daily from the end of the second day through the sixth day. This method made possible tests on the effect of higher concentrations of nutrients without increasing the initial concentration. To the first group of flasks, an amount equal to 10 percent of the initial concentration of nutrients was added to each flask. To the second group an amount equal to 30 percent, and to the third group an amount equal to 50 percent of the initial concentrations of nutrients was added daily to each flask.

At the end of these experiments, the size of the populations reached in the *Chlorella*, the *Nitzschia*, and the mixed cultures were not significantly different from those obtained in the two previous experiments. Division rates obtained for *Chlorella* when grown in the presence of *Nitzschia* in mixed cultures were lower than division rates of *Chlorella* grown alone. As was found in previous experiments, division rates for *Nitzschia* were as high when grown in the presence of *Chlorella* as when grown alone. From the results of this and the previous experiment, it can be concluded that a lack of nutrients is not responsible for the observed inhibition of growth rate of *Chlorella*.

In buffered medium:

In the first experiment testing interactions between *Chlorella* and *Nitzschia* the hydrogen-ion concentration increased considerably, especially as the cultures aged. To test whether the growth of either *Chlorella* or *Nitzschia* had been limited by this change, experiments were run in which the hydrogen-ion concentration was prevented from fluctuating as widely. In this experiment the culture medium was buffered at pH 7.2 with .001 molar KH_2PO_4 and K_2HPO_4. The same

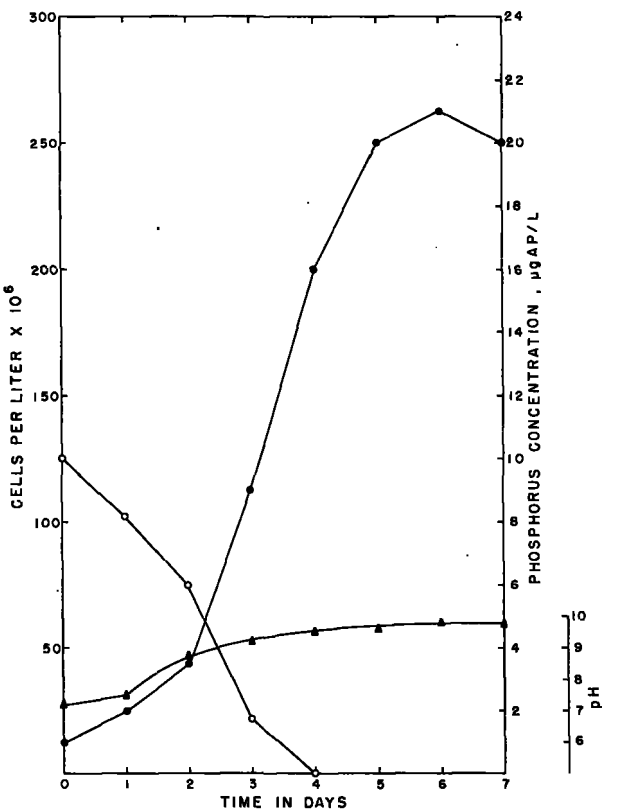

FIGURE 7.—Growth curve of *Nitzschia* in mixed culture prepared with standard culture medium. Dots represent growth curve of *Nitzschia*; circles, phosphorus concentration; triangles, pH.

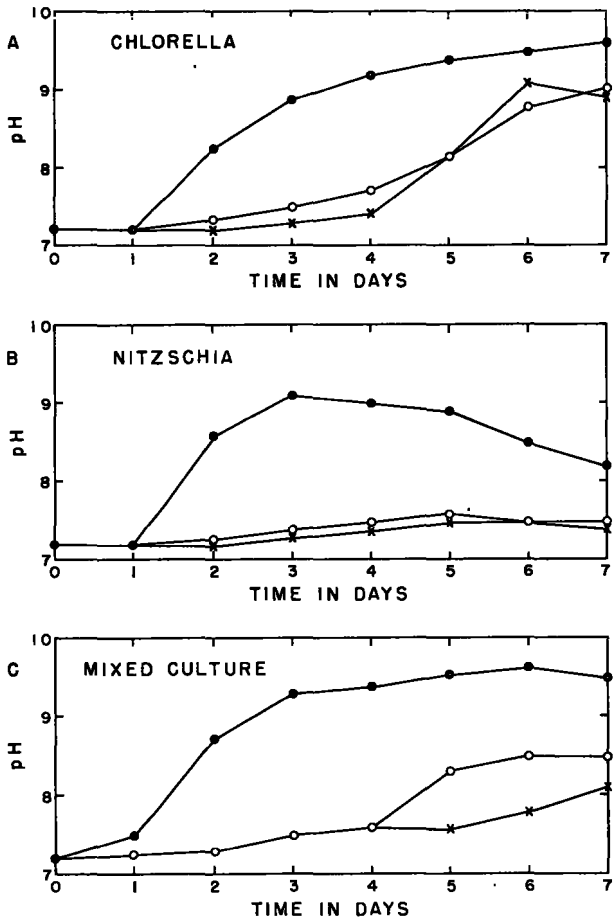

FIGURE 8.—Comparison of pH changes in unbuffered, buffered, and aerated culture media. Dots represent pH changes in unbuffered culture medium; circles, pH changes in medium buffered with .001 molar KH_2PO_4 and K_2HPO_4; X's, pH changes in aerated medium.

procedure used in previous experiments for the preparation of cultures was followed. At the end of each day for 7 days, cell counts and a pH determination were made.

The greatest pH concentration reached in any of the buffered and aerated cultures during the first 4 days of the experiment was 7.7 (fig. 8, A, B, and C) and it was not exceeded during the fifth, sixth, and seventh days in the *Nitzschia* culture (fig. 8, B). In the buffered flasks a pH of 9 was reached on the seventh day in the *Chlorella* culture, while in the mixed cultures the pH increased to only 8.5 on the sixth and seventh days. *Chlorella* cells growing alone had a higher rate of growth than the *Chlorella* cells in mixed culture. When compared with the results of the previous experiments, there was no significant difference in the total population sizes or the division rates for either *Chlorella* or *Nitzschia* when grown alone, and a similar inhibition of *Chlorella* occurred in the mixed cultures as in the previous experiments. Therefore pH can be eliminated as the factor responsible for the observed inhibition of growth of *Chlorella* in the mixed cultures.

In aerated medium:

Another experiment was run in which the pH was prevented from fluctuating as widely as in unbuffered medium by bubbling air through the culture medium during the period of illumination. In addition to preventing as great a fluctuation in pH, this method also furnished a larger supply of carbon dioxide for photosynthesis.

In this experiment, seven flasks were each fitted with a rolled cotton-wool plug through which an air inlet tube extended to the bottom of the flask so that air could be bubbled through the culture medium. A cotton filter, consisting of a calcium chloride drying tube filled with cotton-wool, was attached to the air inlet tube of each flask and the entire apparatus was autoclaved. *Chlorella* cultures were prepared in each flask with a concentration of 70 million cells per liter. The air from an air pump was run into a jar fitted with a rubber stopper containing seven outlet tubes arranged in a circle around the inlet tube. This apparatus was placed on the illuminated shelves and one flask of *Chlorella* was attached to each outlet tube. At the end of each day for 7 days one culture was removed. A flask containing 100 cc. of distilled water was used to replace the culture in order that the remaining cultures would continue to receive a constant supply of air. Cell counts and a pH determination were made each day for the culture removed from the apparatus. This experiment was repeated using *Nitzschia* cultures with an initial concentration of 10 million cells per liter. The experiment was run a third time using a mixed culture of *Chlorella* and *Nitzschia* in the same respective initial concentrations as before.

Chlorella grown alone under the conditions of this experiment reached a population size approximately twice that obtained in previous experiments. While the *Chlorella* population showed a

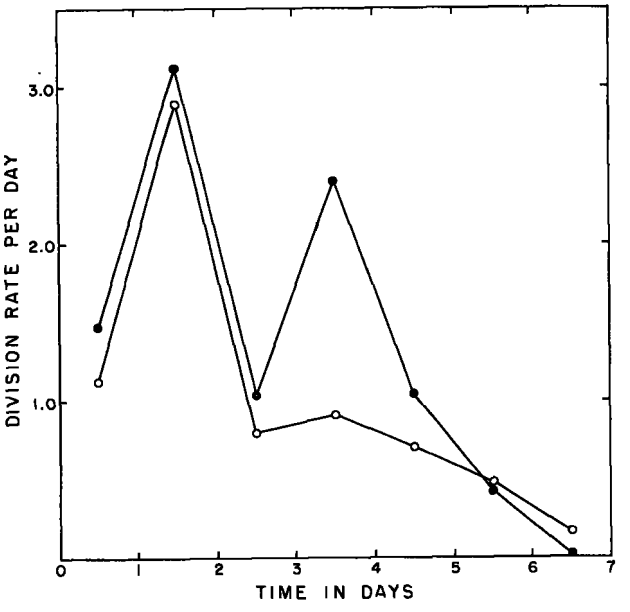

FIGURE 9.—Comparison of division rates of *Chlorella* grown in *Chlorella* culture and in mixed culture in aerated medium. Dots represent division rate in *Chlorella* culture; circles, division rate in mixed culture.

marked increase in size when grown in aerated mixed culture over that obtained when it was grown without aeration, it still reached only about one-half the population size of aerated cultures of *Chlorella* grown alone. The division rates in aerated cultures of *Nitzschia* were also larger than in unaerated cultures. The division rate of *Chlorella* when grown alone was greater than when grown in the mixed culture on every day except the sixth and seventh days when the division rates were about the same (fig. 9). It now appears beyond doubt that this inhibition of growth of *Chlorella* in mixed cultures was due not to a change in pH but to the presence of *Nitzschia*.

Nitzschia reached a total population size significantly larger when grown alone than when grown in the presence of *Chlorella*. The division rates for *Nitzschia* grown alone were generally higher than in mixed cultures (fig. 10), although the difference was perhaps not significant except on the fourth day. The relatively great depression of the division rate for *Nitzschia* in mixed culture on the fourth day may have been due to the large increase of *Chlorella* which occurred on the same day (fig. 9). The pH in the *Nitzschia* culture fluctuated only from 7.2 to 7.5 (fig. 8, B), and the growth of *Nitzschia* was significantly improved by aeration.

On solid agar:

Since solid medium has been used extensively for testing the antagonistic action of one organism on the growth of another, this experiment was designed to test the effect of chlorellin on *Nitzschia* when grown on agar. After much experimenting, it was found that *Chlorella* and *Nitzschia* grew well on agar medium prepared with Detmer's solution, diluted to two-thirds its normal concentration and containing 1 percent agar, 2 percent glucose, and ½ percent peptone. Since *Chlorella* is much less motile than *Nitzschia* when grown on agar, it was transferred to fresh agar slants which were then placed in a horizontal position 63 centimeters below a 50-watt Mazda lamp in a constant-temperature room.

After *Chlorella* had grown on the agar for 10 days, a small piece from an agar slant on which *Nitzschia* was growing was transferred to the agar slant on which *Chlorella* had been growing, being placed midway between the clump of *Chlorella* and the opposite end of the agar slant (fig. 11). It is a well-known fact that, compared with other organisms, algae grow very slowly on agar. Also algae require light to grow and the heat from the light shortens the length of time an agar slant can be used by speeding up dehydration of the agar.

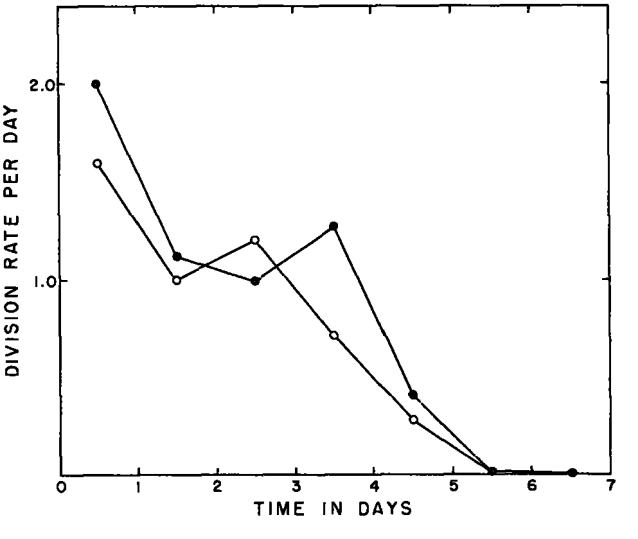

FIGURE 10.—Comparison of division rates of *Nitzschia* grown in *Nitzschia* culture and in mixed culture in aerated medium. Dots represent division rate in *Nitzschia* culture; circles, division rate in mixed culture.

This dehydration process can be partly overcome by keeping the agar slants in a cold room. However, to make this experiment feasible, it was necessary to accelerate the growth of *Nitzschia*. At this time it was discovered that by transferring a small piece of agar on which *Nitzschia* was growing (as described above) as much growth could be obtained in 1 week as was previously obtained in 4 to 6 weeks with transfers made by the conventional bacteriological streaking method.

In 3 to 5 days after *Nitzschia* was transferred to the agar slants containing *Chlorella*, it had grown as close to *Chlorella* as it would. It was quite evident that *Nitzschia* would not extend its growth so as to make contact with *Chlorella*. A small area remained between *Chlorella* and *Nitzschia* as shown at C in figure 11.

Another agar-slant experiment was run in which *Chlorella* was placed at the opposite end of the agar slant. Results were similar to those just discussed. *Nitzschia* grew most rapidly in the central portion of the slant. This was the area on which *Nitzschia* first reached a point beyond which it would grow no closer to *Chlorella*. Later *Nitzschia* covered the area to both sides of this point, leaving the same relative distance between it and *Chlorella*.

It might be thought that lack of nutrients prevented *Nitzschia* from growing until it came in contact with *Chlorella;* however, Detmer's medium diluted with distilled water to two-thirds its normal concentration still contains more of each nutrient than the liquid standard culture medium, and 16 times the total concentration of nutrients in that medium. It thus seems improbable that lack of nutrients could be responsible. Another possible cause was the use of test tubes instead of Petri dishes. The surface of the agar was not horizontal in the test tube, and this might have prevented *Nitzschia* from growing until contact was made with *Chlorella*. However, since *Nitzschia* is motile, and since it grows as rapidly upgrade as down, this factor can be disregarded. Further, as the agar was uniform in composition throughout, the sudden cessation of growth in the rapidly growing *Nitzschia* when it reached the proximity of *Chlorella* must have been due to some change in the agar so that *Nitzschia* would not grow on it. The failure of *Nitzschia* to grow on this area is believed to be due to the presence of chlorellin, the antibiotic substance shown by Pratt (1940) and in this report to be produced by *Chlorella*.

INHIBITION OF GROWTH RATE IN CONDITIONED MEDIA

INHIBITORY EFFECT ON *CHLORELLA* OF *NITZSCHIA*-CONDITIONED MEDIUM

It was found that the medium in *Nitzschia* cultures became conditioned by an accumulation of an antagonistic substance as the culture aged, so that greater inhibition in the growth rate of *Chlorella* occurred when that species was added to aged cultures than when it was added initially with *Nitzschia* to new medium. *Nitzschia* cultures with a concentration of 10 million cells per liter were prepared in six flasks with medium containing twice the concentration of nutrients used in the standard culture medium. At the end of the second day three cultures were removed from the illuminated shelves, and *Chlorella* was added in sufficient quantity to give a concentration of 70 million cells per liter; three cultures were left as controls. Also three *Chlorella* controls containing 70 million cells per liter were prepared with fresh culture medium.

Chlorella added to the *Nitzschia* cultures reached a population size about 45 percent of that obtained in the control cultures (fig. 12) and 65

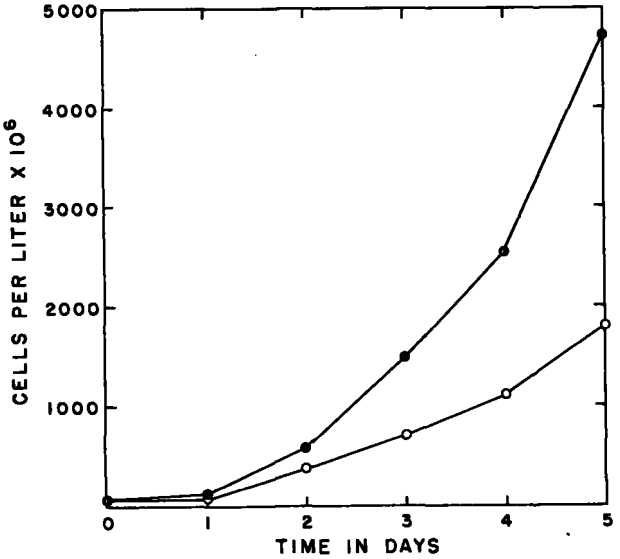

FIGURE 12.—Comparison of growth curves of *Chlorella* in control and in *Nitzschia*-conditioned medium. Dots represent growth curve in control cultures; circles, growth curve in *Nitzschia*-conditioned medium.

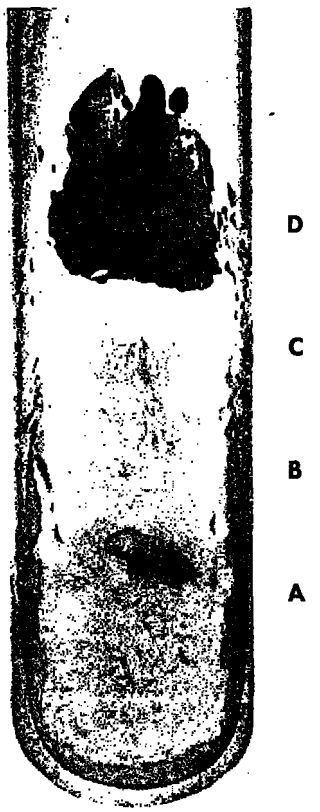

FIGURE 11.—The use of agar to demonstrate the inhibitory effect of *Chlorella* on *Nitzschia*. A. Original inoculation of *Nitzschia*. B. Area over which *Nitzschia* extended its growth. C. Area on which *Nitzschia* would not grow. D. *Chlorella*.

percent of that obtained in previous experiments when *Chlorella* was added initially with *Nitzschia* to new culture medium. The division rate of *Chlorella* added to the *Nitzschia* cultures was thus less than the division rate of *Chlorella* in the control cultures. The decreased division rate and subsequent smaller population obtained in this experiment can be attributed to *Nitzschia* having grown for 2 days in the medium before the addition of *Chlorella*. This gave sufficient time for the medium to become conditioned through the formation and accumulation of the antagonistic substance. The population size and division rate of *Nitzschia* in the cultures to which *Chlorella* was added were not significantly different from those obtained in the control cultures.

INHIBITORY EFFECT ON *NITZSCHIA* OF *CHLORELLA*-CONDITIONED MEDIUM

In the previous experiments when populations of *Chlorella* and *Nitzschia* were grown in mixed cultures, an inhibition always occurred in the growth rate of *Chlorella*, while there was no significant change in the growth rate of *Nitzschia*. Therefore, the following experiment was designed to ascertain whether an inhibition of *Nitzschia* would occur if the medium were conditioned by a longer and larger growth of *Chlorella*. The same procedure used to prepare the *Nitzschia*-conditioned medium was used to prepare the *Chlorella*-conditioned medium, except that *Chlorella* cultures were started with an initial concentration of 70 million cells per liter. At the end of the third day *Nitzschia* was added in sufficient quantity to give 10 million cells per liter to 3 of the *Chlorella* cultures, and 3 *Chlorella* cultures were left as controls. Three flasks with a concentration of 10 million *Nitzschia* cells per liter were prepared as controls.

The size of the *Nitzschia* population reached at the end of the experiment in the mixed cultures was only 70 percent of that attained in the controls (fig. 13). The division rate of the *Nitzschia* cells added to the *Chlorella* cultures were not as great as the division rate of *Nitzschia* in the control cultures. The population size and division rate of *Chlorella* in cultures to which *Nitzschia* was added were not significantly different from those obtained in the control cultures.

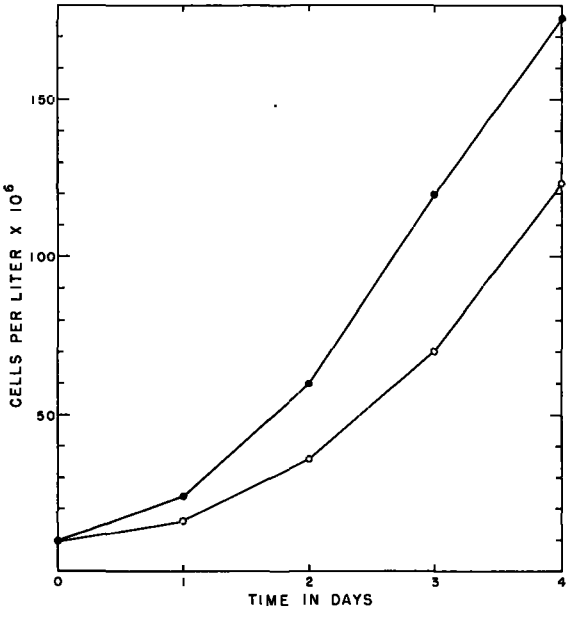

FIGURE 13.—Comparison of growth curves of *Nitzschia* in control and in *Chlorella*-conditioned cultures. Dots represent growth curve in control cultures; circles, growth curve in *Chlorella*-conditioned cultures.

INHIBITORY EFFECTS OF FILTRATE FROM CONDITIONED MEDIA

This experiment was designed to test the growth rates of both *Chlorella* and *Nitzschia* in culture media prepared from the filtrates of their own conditioned media as well as from filtrates of the conditioned medium of the other. *Nitzschia*-conditioned medium was prepared from 5-day-old cultures which had been started with an initial concentration of 10 million cells per liter in standard culture medium. At the end of the fifth day the cultures were centrifuged to remove the cells. The pH was adjusted to 7.0 with phosphoric acid. Phosphate determinations run at this point showed that approximately 5 μgAP/L had been added in the phosphoric acid. Next, 600 cc. of this conditioned medium was filtered through a Berkefeld filter. From this filtered medium, 100-cc. portions were transferred to each of three 250-cc. Erlenmeyer flasks. The remaining 300 cc. of the filtered, conditioned medium was washed with 1-percent Norit A (carbon) by bringing the medium to a boil. The medium was then paper filtered to remove the Norit A, divided into 100-cc. portions, placed in 250-cc. Erlenmeyer flasks, and autoclaved. The same concentration of nutrients used in the standard culture medium was added to

both the Berkefeld-filtered medium which had been Norit washed and the medium which was only Berkefeld filtered. Determinations of pH at this point showed that both the Norit-washed, Berkefeld-filtered and the Berkefeld-filtered media had a pH of 7.2±0.1. This was the pH concentration of culture media used in previous experiments. *Chlorella* cultures with an initial concentration of 70 million cells per liter were prepared with both the Berkefeld-filtered and Norit-washed media. Daily cell counts were made for each culture for a period of 7 days. More *Nitzschia*-conditioned medium was prepared but this time both the Norit-washed, Berkefeld-filtered and the Berkefeld-filtered, *Nitzschia*-conditioned media were used for the preparation of *Nitzschia* cultures with an initial concentration of 10 million cells per liter. These cultures were placed on the illuminated shelves and daily cell counts were made for all cultures for a period of 5 days.

Chlorella-conditioned medium was prepared in a way similar to that described for *Nitzschia* except that the *Chlorella* cultures were started with a concentration of 70 million cells per liter, and were grown for 7 days. Culture medium prepared with this *Chlorella*-conditioned medium was used for the preparation of *Nitzschia* cultures with a concentration of 10 million cells per liter, and cell counts were made daily for 5 days. More *Chlorella*-conditioned medium was prepared in the same way, and to the culture medium prepared with this conditioned medium, *Chlorella* cells were added in sufficient quantity to give a concentration of 70 million cells per liter. These cultures were placed on the illuminated shelves and counted each day for 7 days.

As previously stated, standard culture medium was used in the preparation of both *Nitzschia*-conditioned and *Chlorella*-conditioned media. Also, the same amounts of nutrients were again added to the conditioned culture media to be used in further growth studies. Thus, *Chlorella* and *Nitzschia* cells growing in *Chlorella*-conditioned and in *Nitzschia*-conditioned media were being supplied with concentrations of nutrients ranging between 1 and 2 times the concentration used in the standard culture medium. However, in experiments in which *Chlorella* and *Nitzschia* were grown in concentrations of nutrients 1 to 2 times the concentration contained in the standard culture medium, there was no significant difference in the total size of the population or in the division rate. Further experiments showed that the growth of neither *Chlorella* nor *Nitzschia* was changed significantly if distilled water which had been washed with Norit A by bringing the water to a boil was used in the preparation of culture media with the same concentration of nutrients. Therefore no substance which would change the division rate was added to the conditioned medium by washing it in Norit A.

The size of the *Chlorella* populations reached at the end of the seventh day in Berkefeld-filtered and Norit-washed *Nitzschia*- and *Chlorella*-conditioned medium was not significantly different from that obtained in cultures prepared with distilled water and the same concentration of nutrients. The *Chlorella* grown in culture medium prepared from *Nitzschia*- and *Chlorella*-conditioned medium at the end of 7 days had not reached population sizes as large as *Chlorella* grown in similar medium which in addition had been washed in Norit A and autoclaved (fig. 14). *Chlorella* grown in *Chlorella*-conditioned medium reached a population size larger than when grown in culture medium prepared from *Nitzschia*-conditioned medium. Pratt and Fong (1940) have shown in somewhat similar manner that the growth of *Chlorella* is inhibited when grown in culture medium prepared from *Chlorella*-conditioned medium

The division rate of *Chlorella* was not as high in culture medium prepared from Berkefeld-filtered *Nitzschia*- and *Chlorella*-conditioned medium as in the same type of medium which in addition had been washed in Norit A. The division rate of *Chlorella* in Norit-washed *Nitzschia*- and *Chlorella*-conditioned medium was not significantly different from growth obtained in standard culture medium. It can thus be concluded that either the heating of the medium or the washing with Norit A destroyed or absorbed the antagonistic substances formed by these algae. The division rate of *Chlorella* in culture medium prepared from *Nitzschia*-conditioned Berkefeld-filtered medium, which was not washed in Norit A, is less than that obtained in culture medium prepared from *Chlorella*-conditioned, Berkefeld-filtered medium and not washed in Norit A for

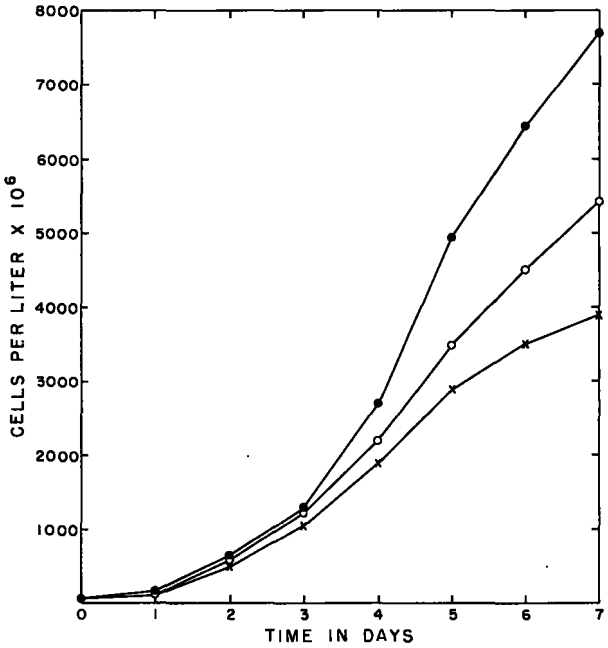

FIGURE 14.—Comparison of typical growth curves of *Chlorella* in culture medium prepared with Norit-washed and autoclaved *Chlorella*- and *Nitzschia*-conditioned medium with *Chlorella*-conditioned medium and with *Nitzschia*-conditioned medium. Dots represent growth curve in culture medium prepared with Norit-washed and autoclaved *Chlorella*- and *Nitzschia*-conditioned medium; circles, growth curve in culture medium prepared with *Chlorella*-conditioned medium; X's, growth curve in culture medium prepared with *Nitzschia*-conditioned medium.

the periods and the numbers of cells used in these experiments.

The size of the *Nitzschia* population reached at the end of the fifth day in Berkefeld-filtered and Norit-washed medium prepared from *Chlorella*- and *Nitzschia*-conditioned medium is not significantly different from that obtained in culture medium prepared with distilled water and the same concentration of nutrients. The *Nitzschia* grown in culture medium prepared from *Nitzschia*- and *Chlorella*-conditioned medium did not reach a population size as large as when grown in similar medium which in addition had been washed in Norit A (fig. 15). *Nitzschia* grown in culture medium prepared from *Nitzschia*-conditioned medium reached a population size larger than when grown in *Chlorella*-conditioned medium. The division rates of *Nitzschia* and of *Chlorella* in culture medium prepared from Berkefeld-filtered, *Nitzschia*- and *Chlorella*-conditioned medium is less than in similar medium which had been washed with Norit A. Thus it has been shown that both *Nitzschia* and *Chlorella* produce substances that remain in the medium after the cells have been filtered off which inhibit not only their own growth but also the growth of the other. Also, the antagonistic substances under the conditions in which they were tested inhibit the growth of the other species more than the species producing them. Finally, it has been determined that these substances can be removed from the medium by filtering and/or washing with Norit A and autoclaving. Lefevre et al. (1949) found that algastatic substances secreted into the medium by one species of alga which caused other species of alga to divide at slower rates could be destroyed by heat.

INHIBITORY EFFECT ON *CHLORELLA* AND *NITZSCHIA* OF *PANDORINA*-CONDITIONED POND WATER

During the time the experiments with conditioned media were being conducted in the laboratory, fluctuations in the phytoplankton populations of Belmont Hill Pond were being followed. This pond was fertilized during the last week of July 1949 and within a period of 3 days a bloom of *Pandorina* appeared with a population of 73 million cells per liter. After this alga had been growing in the pond for a period of 2 weeks and the population had dropped to 46 million cells per liter, a sample was collected and *Chlorella* and *Nitzschia* cultures were prepared as in the previous experiment testing conditioned medium.

Using *Pandorina*-conditioned pond water the population size reached by *Chlorella* after 7 days' growth in medium which had been Berkefeld filtered only was 81 percent of that obtained in similar medium which had also been washed with Norit A and autoclaved (table 1). Similarly, *Nitzschia*, after 5 days' growth in Berkefeld-filtered pond water, reached a population size only 70 percent of that obtained in culture medium which had also been washed with Norit A and autoclaved (table 1). From these observations it can be seen that a substance was present in the pond water which inhibited the growth of both *Chlorella* and *Nitzschia*. Also Lefevre et al. (1949) have grown several species of algae in culture medium prepared with filtered medium in which *Pandorina* had previously grown. Of the

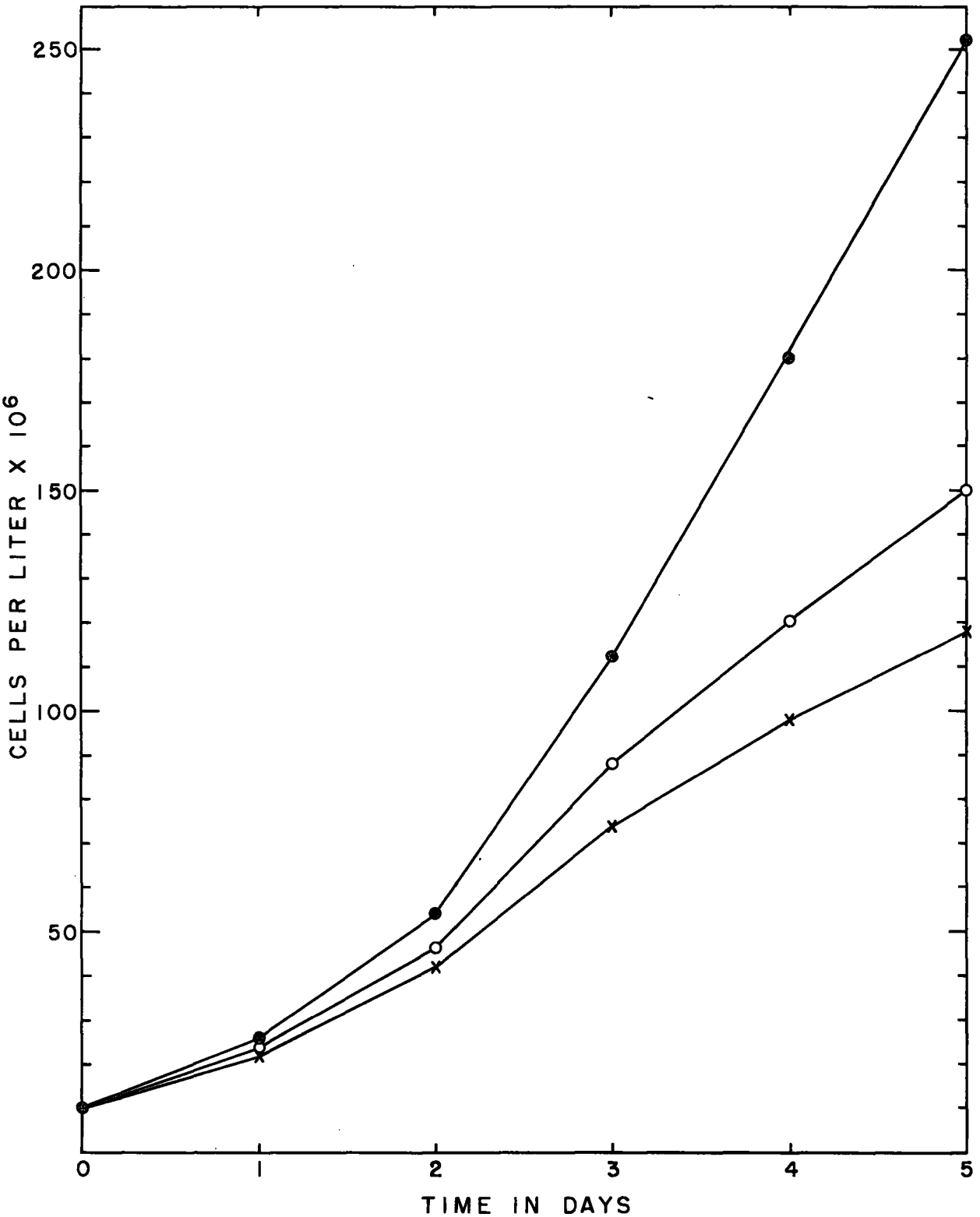

FIGURE 15.—Comparison of typical growth curves of *Nitzschia* in culture medium prepared with Norit-washed and autoclaved *Nitzschia*- and *Chlorella*-conditioned medium, with *Nitzschia*-conditioned medium, and with *Chlorella*-conditioned medium. Dots represent growth curve in culture medium prepared with Norit-washed and autoclaved *Nitzschia*- and *Chlorella*-conditioned medium; circles, growth curve in culture medium prepared with *Nitzschia*-conditioned medium; X's, growth curve in culture medium prepared with *Chlorella*-conditioned medium.

eight species those investigators tested, the majority divided poorly, and some later shrank in size and died.

TABLE 1.—*Populations of* Chlorella *and* Nitzschia *obtained in culture medium prepared from* Pandorina*-conditioned pond water*

[Data given in number of cells per liter]

Culture medium	*Chlorella*		*Nitzschia*	
	Initial population	Population after 7 days	Initial population	Population after 5 days
Pandorina-conditioned pond water:				
Berkefeld filtered	70 x 10^6	6,450 x 10^6	10 x 10^6	195 x 10^6.
Berkefeld-filtered, Norit-washed, and autoclaved.	70 x 10^6	7,930 x 10^6	10 x 10^6	280 x 10^6.
Control: Distilled water with standard culture nutrients added.	70 x 10^6	7,690 x 10^6	10 x 10^6	264 x 10^6.

EFFECT OF VARYING THE INITIAL CONCENTRATIONS OF *NITZSCHIA* AND *CHLORELLA*

INHIBITORY EFFECT ON *CHLORELLA* OF INCREASED CONCENTRATIONS OF *NITZSCHIA*

This experiment was designed to test the effect of different concentrations of *Nitzschia* on *Chlorella* populations as listed in table 2. Two cultures were prepared for each population in 50 cc. of standard culture medium buffered with .001 molar K_2HPO_4 and KH_2PO_4 at pH 7.2. The cultures were prepared in 120-cc. Erlenmeyer flasks and cell counts were made for each culture at the end of the first and second days.

Chlorella populations in cultures to which *Nitzschia* was added did not increase as much at the end of the first and second days as the *Chlorella* population in the cultures prepared as controls to which no *Nitzschia* was added (table 2). The larger the initial concentration of *Nitzschia* added to the *Chlorella* cultures the greater the inhibition of growth of *Chlorella*. *Chlorella* populations of 140 million cells per liter to which an initial concentration of 50 million *Nitzschia* cells per liter were added increased approximately threefold in 2 days, while the same size *Chlorella* population to which 150 million *Nitzschia* cells per liter had been added increased only 15 percent in 2 days. *Chlorella* populations of 560 million cells per liter to which an initial concentration of 50 million *Nitzschia* cells per liter were added reached a population size 50 percent of that in the cultures containing only *Chlorella*. Similar *Chlorella* populations to which 150 million *Nitzschia* cells were added were only about 50 percent of populations obtained in *Chlorella* cultures prepared with 50 million *Nitzschia* cells, and 25 percent of those obtained in cultures containing only *Chlorella*. *Chlorella* populations of 1,680 million cells per liter to which 50 million *Nitzschia* cells per liter were added reached 75 percent of the population in the *Chlorella* cultures.

There was no significant difference between the increased size of *Nitzschia* populations when grown in the presence of 140 million *Chlorella* cells per liter and when grown alone (table 2). *Nitzschia* added to *Chlorella* cultures of 560 million cells per liter reached population sizes slightly smaller than those obtained in the control cultures containing only *Nitzschia*, but the difference was not large enough to be significant. The *Nitzschia* added to *Chlorella* cultures of 1,680 mil-

TABLE 2.—*Effect of increased initial concentrations of* Nitzschia *on* Chlorella

[Data given in number of cells per liter]

Type of culture	Initial population of *Chlorella*	Population of *Nitzschia* during first 24 hours	Population of *Chlorella* after 24 hours	Population of *Nitzschia* during second 24 hours	Population of *Chlorella* after 48 hours
Mixed	140 x 10^6	50–140 x 10^6	215 x 10^6	140–245 x 10^6	410 x 10^6.
Do	140 x 10^6	100–250 x 10^6	160x 10^6	250–390 x 10^6	250 x 10^6.
Do	140 x 10^6	150–345 x 10^6	145 x 10^6	345–440 x 10^6	160 x 10^6.
Chlorella [1]	140 x 10^6		325 x 10^6		910 x 10^6.
Nitzschia [1]		50–135 x 10^6		135–260 x 10^6	
Do.[1]		100–280 x 10^6		280–415 x 10^6	
Do.[1]		150–330 x 10^6		330–445 x 10^6	
Mixed	560 x 10^6	50–125 x 10^6	940 x 10^6	125–230 x 10^6	1,310 x 10^6.
Do	560 x 10^6	100–260 x 10^6	825 x 10^6	260–410 x 10^6	910 x 10^6.
Do	560 x 10^6	150–335 x 10^6	680 x 10^6	325–425 x 10^6	745 x 10^6.
Chlorella [1]	560 x 10^6		1,180 x 10^6		3,065 x 10^6.
Mixed	1,680 x 10^6	50–115 x 10^6	2,420 x 10^6	115–195 x 10^6	3,050 x 10^6.
Do	1,680 x 10^6	100–230 x 10^6	2,140 x 10^6	230–360 x 10^6	2,580 x 10^6.
Do	1,680 x 10^6	150–315 x 10^6	1,965 x 10^6	310–405 x 10^6	2,340 x 10^6.
Chlorella [1]	1,680 x 10^6		2,900 x 10^6		4,630 x 10^6.

[1] Control.

lion cells per liter reached smaller populations than those in the control cultures. While this decrease in the *Nitzschia* population was not large enough to be important it occurred in all the mixed cultures. It is believed that the *Chlorella* population was approaching a concentration large enough to bring about an inhibition in the growth of these *Nitzschia* populations. Also the antagonistic substance produced by *Nitzschia* was divided among an increased number of *Chlorella* cells at the beginning of the experiment, thus producing less inhibition.

INHIBITORY EFFECT ON *NITZSCHIA* OF INCREASED CONCENTRATIONS OF *CHLORELLA*

In the previous experiment the effect of varying the initial population of *Nitzschia* on a population of *Chlorella* was tested. Since it has been shown that the concentrations of *Nitzschia* used in the mixed cultures in previous experiments inhibited the growth of *Chlorella*, it was now desirable to test the effect of large concentrations of *Chlorella* on a small concentration of *Nitzschia*. For this experiment, cultures were prepared as in previous experiments, except that different populations of *Chlorella*, as listed in table 3, were used.

The size of the *Nitzschia* population was smaller at the end of the second day in all cultures to which *Chlorella* had been added than in the control cultures containing only *Nitzschia*. The greatest inhibition in the division rate of *Nitzschia* occurred in cultures to which 5,000 million *Chlorella* cells per liter had been added. In these cultures, a 70-percent inhibition occurred in the division rate of *Nitzschia*. In the cultures which contained initially 20 million *Nitzschia* cells per liter and 400 million *Chlorella* cells per liter, a greater inhibition occurred in the division rate of *Chlorella* than of *Nitzschia*. However, in the cultures containing initially 20 million *Nitzschia* cells and 1,200 million *Chlorella* cells per liter considerable inhibition occurred in the division rate of both *Nitzschia* and *Chlorella*.

Since neither nutrients nor pH were limiting factors in these experiments, it can be concluded that the division rate of both *Chlorella* and *Nitzschia* are inhibited by varying the initial concentration of one or the other. The division rate of large concentrations of *Chlorella* is inhibited by relatively small concentrations of *Nitzschia*, while extremely large initial concentrations of *Chlorella* are required to inhibit the division rate of *Nitzschia*. It appears that *Chlorella* is more sensitive to the antagonistic substance produced by *Nitzschia* than *Nitzschia* is to chlorellin.

TABLE 3.—*Effect of increased initial concentrations of* Chlorella *on* Nitzschia

[Data given in number of cells per liter]

Type of culture	Initial population of *Nitzschia*	Population of *Chlorella* during first 24 hours	Population of *Nitzschia* after 24 hours	Population of *Chlorella* during second 24 hours	Population of *Nitzschia* after 48 hours
Mixed	20 x 10^6	400–650 x 10^6	50 x 10^6	650–1,490 x 10^6	135 x 10^6.
Do	20 x 10^6	1,200–2,170 x 10^6	47 x 10^6	2,160–4,970 x 10^6	100 x 10^6.
Do	20 x 10^6	5,000–5,940 x 10^6	30 x 10^6	5,940–9,685 x 10^6	45 x 10^6.
Nitzschia [1]	20 x 10^6		55 x 10^6		140 x 10^6.
Chlorella [1]		400–710 x 10^6		710–2,635 x 10^6	
Do.[1]		1,200–2,670 x 10^6		2,670–6,055 x 10^6	
Do.[1]		5,000–6,230 x 10^6		6,230–10,200 x 10^6	

[1] Control.

EFFECT OF ANTAGONISTIC SUBSTANCES ON PHYTOPLANKTON GROWTH

Up to the present time, our best understanding of the influence one plant exerts on another growing in association with it has been based on knowledge of the competition for some factor essential to growth, such as nutrients. Thus a thorough study was required to demonstrate conclusively the more complicated interactions between *Chlorella* and *Nitzschia* and the existence of antagonistic substances produced by them.

In the experiments reported in this paper, those chemical and physical factors which are known to have an effect upon the growth of algae were eliminated as responsible for the observed inhibition of growth by either maintaining a constant and adequate value for them or having them present in excess. Physical factors such as light and temperature were kept constant within small ranges. Nutrients were added in excess initially to the culture medium in some experiments, and daily to cultures in other experiments. Extreme

fluctuation in the hydrogen-ion concentration was prevented by using buffered and aerated medium. The experiment with aerated medium also eliminated lack of CO_2 as a factor responsible for inhibition of growth.

As a precautionary measure to guard against the physiological condition of the cells varying from one experiment to another, controls were run with every experiment. A comparison of the controls in different experiments revealed no significant difference in the physiological condition of the cells used. The control and the mixed cultures were always started with cells taken from the same culture.

The physical effect of one species upon the other in mixed culture cannot be considered as a factor in this inhibition, since experiments with culture medium prepared with conditioned medium from which all cells had been removed gave an inhibitory effect on the growth of cells subsequently cultured in the medium, even though the supply of nutrients and the pH were optimum.

A criticism raised against similar work on the growth of protozoa was that other organisms were used as a source of food for the animals under investigation and that the presence of the supplementary animals in the medium altered the results (Beers 1933; Johnson 1933). This criticism does not apply to the present investigation, since pure cultures of *Chlorella* and *Nitzschia* were used and the liquid media contained only inorganic nutrients. However, the solid medium used to test the effect of chlorellin on *Nitzschia* contained glucose and peptone in addition to the agar. Thus it can be concluded that the inhibition observed in these experiments was due to a substance originating from the algae. The mode of action of these antagonistic substances seemed to be only a retarding effect on the growth rate without any change in pigmentation of the cells or other observable deleterious effects.

Chlorella and *Nitzschia*, through the antagonistic substances produced by them, inhibited their own growth as well as the growth of the other, but each species inhibited the growth of the other more than its own. Rodhe (1948) working with *Scenedesmus* concluded that similar inhibitory conditions existed in his pure cultures.

The fact that a species of alga can inhibit its own further growth under any condition is of extreme importance. In the present laboratory experiments, population densities obtained in pure cultures were much higher than those ordinarily found in nature. Thus a shorter period of time was presumably required for the inhibition to show its effect and the degree of severity probably far exceeds that found under natural conditions. Therefore the results can be applied to natural situations only with caution.

The antagonistic substance produced by *Nitzschia* inhibited the growth rate of *Chlorella* in all experiments in which a concentration of 70 million *Chlorella* cells and 10 million *Nitzschia* cells per liter were used as initial populations. That the antagonistic substance accumulates in the medium of pure cultures as the cultures age was shown. *Nitzschia* cultures with 10 million cells per liter to which *Chlorella* was added on the second day gave a greater inhibition of growth rate for the *Chlorella* cells than when they were added initially with the *Nitzschia*. *Chlorella* cultures started with 70 million cells per liter inhibited the growth of *Nitzschia* when added in a concentration of 10 million cells per liter on the third day. Also when *Chlorella* was grown on agar, the antagonistic substance was absorbed by the agar creating an area on which the *Nitzschia* cells would not grow. An increase in the initial concentration of *Nitzschia* brought about a greater inhibition of the growth rate of *Chlorella*, and a similar increase in the inhibition of small populations of *Nitzschia* was brought about by increasing the initial concentration of *Chlorella*. However, a ratio of *Chlorella* to *Nitzschia* of 20 : 1 was required before the antagonistic substance from *Chlorella* would inhibit the growth rate of *Nitzschia*. When *Chlorella* and *Nitzschia* were grown together with a ratio of 7 : 1, the growth rate of *Chlorella* was always inhibited.

It is possible for antagonistic substances to accumulate in natural bodies of water supporting relatively small phytoplankton populations provided decomposition of these substances is slow. It will be important in the future to determine how long the substances remain effective. Similarly, large populations of phytoplankton may not suppress subsequent growth of certain species if antagonistic substances produced by them are

rapidly decomposed. Unfortunately, we have no information as yet on these important points.

In the experiment run with pond water which had supported a large growth of *Pandorina* for 2 weeks preceding the experiment, the growth rate of *Chlorella* was inhibited 19 percent and *Nitzschia* 30 percent in Berkefeld-filtered pond water when compared with growth rates in pond water, which in addition was washed with Norit A and autoclaved.

In natural bodies of water, one species of alga seldom if ever exists completely alone, but blooms are generally dominated by one species. However, during the blooms studied in the pond in these experiments, *Pandorina* became so dominant it is believed that the observed influence of the pond water on the growth rate of *Chlorella* and *Nitzschia* can be attributed to antagonistic substances produced by that dominant species. Another difficulty in the study of the effect of water from ponds, lakes, and the ocean on the growth of phytoplankton is that the water contains, in addition to the inorganic nutrients, organic materials derived not only from the phytoplankton but also from the soil, from animals, and from other plants.

From information obtained in this study and by other investigators, the author suggests that substances originating from phytoplankton may have one of the following effects upon the growth rate of some species of phytoplankton: (1) They may be necessary for any growth, (2) they may stimulate growth, or (3) they may inhibit growth. If these assumptions are correct, it can be seen that the seasonal fluctuations in total phytoplankton numbers and in the numbers of each species, as well as a definite succession of species, may in part be dependent upon the phytoplankton itself.

SUMMARY

1. The growth rates of both *Chlorella* and *Nitzschia* were less when the species were grown together in mixed cultures than when they were grown in pure culture, depending upon the size of the populations used.

2. It was demonstrated that an increase in the inhibition of growth rate of *Chlorella* populations of the same size occurred with an increase in the initial concentration of *Nitzschia*. Similarly, an increase in inhibition of the growth rate of small populations of *Nitzschia* was brought about by increasing the initial concentration of *Chlorella*.

3. If culture medium in which either *Chlorella* or *Nitzschia* had been growing was Berkefeld filtered to remove the cells and nutrients added to the medium and the pH adjusted, the growth rates of both *Chlorella* and *Nitzschia* were inhibited when the species were again grown in this medium. A portion of the same conditioned medium which in addition was washed with Norit A and autoclaved did not inhibit the growth rates of either *Chlorella* or *Nitzschia*. It was thus concluded that the antagonistic substance was either removed or destroyed by the latter treatment.

4. When *Chlorella* was grown on agar the antagonistic substance produced by it was absorbed by the agar and created an area on which the *Nitzschia* cells would not grow.

5. The growth rates of both *Chlorella* and *Nitzschia* were inhibited in culture medium prepared with pond water which had supported a large growth of *Pandorina* for a period of 2 weeks.

6. It is concluded that antagonistic substances arising from the metabolism of phytoplankton are important, at least in fresh-water ponds, in influencing the seasonal fluctuations in total phytoplankton numbers and in the numbers of each species, as well as in causing a definite succession of species.

7. It was found that by transferring a small piece of agar on which *Nitzschia* was growing to the fresh agar, as much growth resulted in 1 week as was previously obtained in from 4 to 6 weeks with transfers made by the conventional bacteriological streaking method.

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