# THE ECOLOGY OF ALGAE

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University of Pittsburgh

The Pymatuning Symposia in Ecology

# THE ECOLOGY OF ALGAE

A Symposium Held at the Pymatuning
Laboratory of Field Biology on
June 18 and 19, 1959

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# INTRODUCTION

The papers which make up this volume were presented at a conference held June 18 and 19, 1959 at the Pymatuning Laboratory. The focal point of the conference and of this publication is encompassed in the title Ecology of Algae. The emphasis in the papers is on fresh-water algae but several of them present data from marine situations and implications can be extended to this related field.

Stimulation for many of the recent developments in our understanding of algae has come from outside the traditional boundaries of botanical sciences. Much has been contributed from those areas of ecology concerned with the hydrosphere, limnology and oceanography. The emphasis on the role of algae as the primary producers in aquatic ecosystems has resulted in a great deal of work on the physiology of algae. Certain problems of engineers concerned with sewage disposal, mass culture, and space travel have also been a great stimulus to algal studies.

The ecology of algae is here considered from the standpoint of ecological phycology i.e., we are interested in algae and their environmental relationships. These algal-environment interactions may be viewed from the traditional descriptive approach or they may be dealt with experimentally, either in terms of changes occurring in the environment due to the algae or in terms of changes that occur in the algae as a response to the environment.

The fact that algae are able to alter decisively the biotic factors of their environment, is brought out in the papers by Dr. Prescott and Dr. Hartman. Dr. Blum and Dr. Whitford present a picture of the occurrence and composition of algal communities in various ecological situations. Dr. Bartsch dwells on the functional changes or processes that occur in a unique algal community. Dr. Ryther continues this approach by presenting the results of algal activities in terms of productivity, which is, to many ecologists, the philosopher's stone that will provide understanding of the ecosystem. Dr. Provasoli shows what may be necessary in an environment in order for growth and development of algal communities to occur.

It is obvious that time did not permit a full exploration of the ecology of algae, even to the limited extent that the present development of the field would permit.

# ECOLOGICAL DISTRIBUTION OF FRESH-WATER ALGAE

L. A. Whitford
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Ecology is one of the younger divisions of biology, and yet the desire and search for ecological data is as old as man's culture of plants and animals. Needham and Lloyd (1916) say the science of limnology began with the musings of the contemplative fisherman. We can likewise say the science of ecology began when the cave man first began to wonder why his patches of half-wild peas and cereals grew better at certain times and places than others, and that the study of algal ecology began when the Chinese began to try to manage fish ponds, probably a thousand years ago.

Just as the ecology of land plants began with, and was stimulated by, such works as Kerner's Plant Life of the Danube Basin, and by Humbolt's studies of world floras, algal ecology also had its beginnings in florlstic studies. Many early students of algal floras were good observers and worked at such a leisurely pace that they took many notes. Furthermore, page space for publication was not at such a premium then as now. Many papers of a few decades ago have valuable ecological data among the more voluminous floristic and taxonomic material. Among those who have published material of this type are the Wests (W. and G. S.), Chodat, Fritsch, and Pascher. More recently in this country Smith, Taylor, Transeau, and Tiffany have published floristic and taxonomic papers from which ecological data can be gleaned. Some like Fritsch, Taylor, and Tiffany have also later written ecological papers.

As early as 1904 West pointed out that regions with drainage from pre-cambrian rocks have an algal flora richer in species than regions of more recent rocks. Later, a flora few in species but rich in numbers of individuals was recognized. These became known as the Caledonian and the Baltic floras. By 1920 this first classification of fresh-water algal floras was established in the literature. While the original theory as to the reason for the two types is now held to be invalid, the names can still be used to designate the types of flora.

The rapidly developing science of limnology, of course, has contributed most to algal ecology. Welch (1952) has given an excellent summary of the history of ecology from which an idea of its influence on algal ecology can be gained. We have taken over, however, a number of limnological terms and concepts without, perhaps, too critical an evaluation of their use and value. Thienemann and Naumann's oligotrophic, eutrophic, and dystrophic lake types are a case in point. Welch (1952, p. 344) says these terms have "drifted slowly into a certain limited, uncrystallized acceptance in American limnology, although no serious attempts have yet been made to reduce the diagnostic characters to positive specifications valid for American inland waters." The terms suggest a type of lake or type of habitat and they have also been defined in a number of different ways. If used in phycology they should designate a habitat and not a type of flora.

During the past sixty years phytoplankton studies have been by far the most numerous. Many limnological papers including phytoplankton and papers on algae alone have been published both in Europe and in this country. In the United States, the Wisconsin lakes, the New York lakes, and the Great Lakes have all received attention. Until recently the south and the west have been relatively neglected.

The algae of streams have also been investigated in the course of limnological studies. The Russians are reported to have worked especially on the large rivers. The algae of other European streams have received considerable attention and series of papers have been published by Budde, Butcher, and Fritsch. In this country stream algae have received relatively little attention, especially non-planktonic species. Published material is found mostly in limnological surveys. There are plankton papers by Allen (1920), Chandler (1937), Eddy (1932), Purdy (1916), Reinhard (1931) and Roach (1932). Recently there have been papers on non-plankton algae by Blum (1954, 1956, 1957) and Whitford (1956).

During the nineteen hundred forties a system using organisms to indicate limnological conditions was developed by Liebmann (1951). It seems to have some merit but it has not been widely tested by investigators. This is the saprobien system mentioned by Dr. Blum. Symoens also in 1951 outlined a classification of fresh-water algal communities. An acceptable system of ecological classification should be based on experimental data, but before it will be generally adopted, it must be widely tested under experimental conditions.

Algal ecology will someday have its Warming or its Forel, who will write a definitive treatise as Warming did for land plant ecology and Forel dld for limnology. We probably do not yet have a sufficient body of data for such a paper on fresh-water ecology.

In a consideration of the ecological distribution of algae it is obvious that the effects of light, temperature, and water quality should be

considered. A fourth factor, the effect of current, seems of considerable importance in streams and on wave-swept shores.

Since algae are photosynthetic plants, no one can deny that light is a very important habitat factor. Algae are thus restricted to the photic zone. The depth of this zone varies not only locally with the turbidity or color of the water, with the cloud cover, and in small streams, as pointed out by Blum (1956), with the leaf canopy; but also with season and latitude. Rodhe (1955) has recently postulated, however, that some species may grow heterotrophically in arctic winter darkness. Welch (1952) cites two marine observations indicating that the effective day length at some depth (10 to 40 m) is markedly less than near the surface. This effect is probably of less importance in fresh waters except at high latitudes or in winter.

As is true of other habitat factors, we do not have nearly enough data on the effect of light on algal distribution. It has not been easy to make under-water light measurements. Until recently there was no standard or commercially made meter for under-water measurements. Light and dark bottle experiments have been most common. Total diurnal incident light measurements are still difficult to make. Most fresh-water algae are probably "shade plants." Exceptions are algae growing on soils, in seeps, or epiphytically on land. Certain species of Zygnemataceae, Mesotaeniaceae, and Desmidiaceae growing in seeps seem to need full sunlight. Tilden (1935) proposed a theory on the relation between algal pigments, especially the accessory pigments, and algal evolution and distribution. This theory has never gained general acceptance except in one part not original with Tilden. It seems to have been adequately proved that the accessory pigments in some red algae render the light penetrating to some depth, more effective in photosynthesis. This seems to be true of fresh-water Rhodophyceae as well as marine species. Batrachospermum, Hildenbrandtia, Chantransia, and Thorea have been found growing at from ten to thirty meters in depth. The fresh-water red algae are said to be red only when growing in deep water. I have collected Thorea ramosissima, deep red in color, growing in Vaucheria mats at ten meters depth in Silver Springs, Florida, (Whitford 1956). We had thought that Batrachospermum is a cool water genus, but we have good evidence that low light is an equally important factor. Batrachospermum is found at cool seasons and persists longest in cool shady streams, but it is probable that low light is a more important factor than low temperature. Plants are found later in the spring under shady banks and in the shade of stones than elsewhere, although the water temperature here is identical with that in sunny reaches. We were surprised to find Batrachospermum much less widespread in the higher North Carolina mountains in summer than water temperatures would lead one to believe. On the other hand, at least one species, B. macrosporum, is abundant in the deep brown waters of Coastal Plain streams throughout the summer. It is most abundant in shady places. Water temperature may exceed 27°C where it is growing.[1]

We have made another observation which seems of some interest. In North Carolina spring and autumn are fairly long. In autumn we notice a reversal of the spring flora as regards species and time of abundance. Late spring species come in earliest in autumn and early spring species later. We note, however, that abundance of individuals in autumn is usually less than in spring although the period of the same water temperatures are approximately equal. In autumn some species seem to be caught between a fairly low temperature requirement and a high light requirement. In spring light intensity and duration are increasing as water temperatures become favorable but in autumn total incident light is decreasing as temperatures become favorable for certain species. We definitely note that if bright clear weather prevails in autumn we get a more nearly exact reversal of the spring flora than when it is cloudy. Oedogonium kurzii, an important stream species with us, most species of Draparnaldia, as well as some plankton algae such as Asterionella and Dinobryon have a low temperature, high light requirement.

It seems apparent that many green algae are high-light species and red algae are low-light species. Diatoms and Chrysophyceae seem more indifferent in light requirement. Perhaps they will respond to fairly high light intensities if other conditions are ideal. The high diatom populations in the cool Florida springs in summer seems to be an indication that this is true (Whitford 1956). Blue-greens probably respond more to high summer temperatures than to high light intensities. This would be an interesting laboratory problem as will be pointed out later.

Oberdorfer (1928) published detailed data on the distribution of algae in relation to the light factor in Lake Constance. His work and that of others has been summarized by Fritsch (1931).

Schiller (1930) showed by means of culture experiments that cold water (11-12°C) is frequently more productive than warm (23-25°C). The productivity of the northern fishing banks is well known and apparently diatoms are more abundant in northern lakes than in those at lower latitudes. The diatoms and Chrysophyceae are in general microthermal (oligothermal). Some species of

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[1] Schumacher and Whitford, unpublished data.

Peridinium (P. limbatum, P. willei) and apparently some blue-greens are also. In some blue-greens, however, such as Chamaesiphon low light and current may be more important than low temperature. We have recently found Chamaesiphon abundant in mid-Peruvian streams at summer temperatures.[1] The Chlorophyceae seem more eurythermal. Many green algae have good vegetative growth at fairly low temperatures, if light is adequate, and they continue growth at summer temperatures. The larger species of Spirogyra and Oedogonium and many of the Chlorococcales seem mesothermal (eurythermal). Among the diatoms Melosira granulata and Eunotia pectinalis are much more eurythermal than most species. In brown-water streams in North Carolina Eunotia pectinalis replaces the genus Fragilaria of northern waters in late winter and spring, and it is abundant after the water temperature well exceeds 20°. West (1909) cites Rhizosolenia as a diatom growing at warmer temperatures than most. We agree with this and also suggest that Terpsinoe may be another. Many species of fresh-water algae which are most abundant in spring and autumn may be mesothermal but data is lacking which will clearly separate light from temperature effects.

There are probably few megathermal algae in spite of the fact that certain blue-greens especially grow in hot springs. Some workers report that most thermal species grow as well at ordinary room temperatures as at high temperatures. Other reports differ, however. Synechococcus eximus is said to make maximum growth at 79°C and will not survive at a temperature below 70°. Oscillatora filiformis is said to tolerate a temperature above 85°C and it will survive at a temperature no lower than 59°C.

Recently we (Schumacher and Whitford) have been collecting algae in winter in North Carolina. We find that 15° seems to be the critical temperature for many microthermal species. A species of Chrysophyceae, Phaeosphaera perforata is perhaps the most remarkable. It has been under observation for more than ten years. It grows only at temperatures between 4 and 10°C, and disappears rapidly as the temperature approaches 15°. It is very abundant in Coastal Plain streams in February and March following cold winters, and fairly common in bogs and brooks somewhat later at Raleigh, 150 miles inland. We do not have conclusive evidence, but apparently it is rare near the coast southward because water temperatures do not drop below 10° for a long enough period for it to become abundant.

Another species in the Xanthophyceae, Chlorosaccus fluidus Luther grows in somewhat similar habitats at about the same temperatures. The diatom, Meridion circulare seems another microthermal species. It is found growing with us only in winter and early spring except in the mountains. Single living but depauperate cells can occasionally be collected in the ooze of stream bottoms at any season but the typical half-wheel colonies occur only at temperatures below 15°. Actinella punctata Lewis, a supposedly rare diatom, has been collected in ten counties in the Coastal Plain. It is an abundant epiphyte on algae and stems during the cold months but, like Meridion, is present only as an occasional single cell in the ooze during warm seasons. Chaetophora incrassata is common in brooks in late winter but disappears rapidly when the water temperature exceeds 15°. Fragillaria is very rare at lower elevations except in late winter but is common in streams at high elevations, where temperatures remain low, in summer.

The factor of water quality is the most complex and diverse especially if one includes such things as water color, turbidity, and pH, in addition to mineral content, and dissolved gases. Nevertheless they are all closely related and interacting and have to be considered together. There is a voluminous literature on water quality and it is useless to try to summarize it in a paper of this length. There have been numerous attempts to generalize by using types of flora, or groups of organisms as indicators of over all conditions. Frequently single supposedly important factors have been considered so important that such terms as calciphilic and halophytic have been coined. Much investigation has been done on phosphorus and iron as limiting factors. Undoubtedly these as well as nitrate are often limiting. There is no doubt that lack of available silicon limits diatom growth, for instance. In communities of microorganisms, however, conditions can change so rapidly that stenoecious species (that is those with a narrow range of tolerance) may become abundant or disappear with amazing rapidity. As Hutchinson said (1944) it is the interaction of a complex of both physical and chemical factors which produces both seasonal fluctuations and sporadic blooms.

The terms eutrophic and oligotrophic have been most used to indicate general productivity, but we cannot yet reduce them to positive specifications. We can only say that a eutrophic habitat is one with a high pH where available organic matter is rapidly reduced to liberate an abundance of the vital mineral elements. The oligotrophic habitat has a low pH and the mineral nutrients are low. Thus pH seems the best single indicator of the type of algal flora. At least several American phycologists agree with this statement. The pH results, of course, from the interaction of a complex of factors.

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[1] Whitford, unpublished data.

As mentioned above, I am not qualified to judge whether the saprobien system which uses indicator organisms will prove to be useful and workable. Dominant species and indicator organisms have been widely used in other branches of ecology.

Separate studies of individual species might go a long way toward solving some of the problems of the effects of water quality.

One habitat factor which is of considerable importance in stream ecology seems to be misunderstood by many limnologists and phycologists. This is the effect of current. For a long time it has been known that certain species grow only in a current of water or grow better there. This fact was at first explained by assuming that running water has a higher content of dissolved gases and a lower temperature than still water. There is now much evidence that these assumptions are incorrect, but there is evidence that some rapids species have a higher respiratory rate than corresponding lenitic species. Many investigators recognize this "inherent current demand" of such species but offer no explanation. Ruttner (1926) seems to have offered the first partial explanation. His statement as translated by Frey and Fry (Ruttner, 1953) is as follows: "In quiet or in weakly agitated water the organisms are surrounded by a closely adhering film of liquid, which speedily forms around the animal or plant, a cloak impoverished of substances important for life. In a rapid current, however, the formation of such exchange-hindering investitures is prevented, and the absorbing surfaces are continually brought into contact with new portions of water as yet unutilized." Moving water, he continues, "is not absolutely but rather physiologically richer in oxygen and nutrients."

The reason for this "physiological richness" can be explained, I believe, by the laws of cohesion and diffusion which are familiar to most limnologists and phycologists. It is a simple matter of the diffusion gradient being steeper around plants in rapid water. If all other factors are equal, diffusion will occur twice as fast at half the distance. There is formed around a cell in still or slowly moving water a gradient of concentration of diffusing materials. For a material diffusing inward the concentration in the medium is least at the cell wall and the material increases in concentration outward a certain distance until it reaches 100% of that in the surrounding water. For the smaller inorganic molecules this distance seems to be about 1/4 mm. Even in a current cohesion holds a film of relatively still water against the surface. In a rapid current at least part of this film is swept away bringing the region of high solute concentration closer to the surface--in other words making a steeper diffusion gradient and therefore increasing the rate of diffusion. Ferrell, Beatty, and Richardson, (1955) have shown that the speed of current necessary to displace this film is of the order of one-half foot (15 cm.) per second.

In case of oxygen and carbon dioxide, which are more soluble in cold water, low temperature may reduce or eliminate the "current demand." Cedergren (1938) reports that certain algae which grow in still water during the cooler seasons are found only in rapids in summer. I discovered this to be true for Stigeoclonium and Draparnaldia in North Carolina, many years before seeing a reference to Cedergren's work. One species of Stigeoclonium grows in summer in very swift water at a temperature averaging close to 25°C.

We[1] have data which indicate that some fifteen or more species of algae require a rapid current at least at temperatures above 15°C. These include species of Stigeoclonium, Chaetophora, Gongosira, Oedogonium, and Spirogyra as well as most of the species of red algae we have collected. A few crust-forming species of blue-green algae almost certainly belong in this group also.

One of our most important rapids species is Oedogonium kurzii. It is a perennial in rocky rapids especially in the Piedmont of the southeast. It seems to occupy the same place in soft water streams as does Cladophora in hard-water regions. In late spring it forms great masses and skeins over a meter in length, but by late summer it is reduced to short tufts attached to rock in the swiftest water. During long sunny autumns it may again become fairly abundant and it never completely disappears all winter.

The problem of communities has received the attention of numerous limnologists and phycologists. Everyone who does ecological work must consider it. Since there has been more work done on the plankton than on other communities there have been more attempts to classify the phytoplankton than other communities. Several hundred papers deal at least in part with community relationships. Perhaps the best summaries and lists of literature are those of Strøm (1924), Fritsch (1931), Symoens (1951), Tiffany (1951) and more recently Prescott (1956), and Blum (1956). In his summary of the ecology of fresh-water algae, Tiffany says, "...the ecological factors are identical with those affecting the larger land plants, but the degree of intensity, the availability, and distribution of such factors are different;...attention must be directed more and more to the microenvironments of algae. Algal communities, though quite distinct in many habitats, are more difficult to define and delimit than associations of many terrestrial seed plants. Successional phenomena in the algae are often matters of seasonal

[1] Schumacher and Whitford, unpublished data.

periodicity, determined by the occurrence and length of the vegetational span of the species involved. ...Climax associations are approached in some instances, but they are scarcely to be considered as the counterparts of such aggregations among the higher plants of terrestrial habitats."

He says also that it is almost impossible to make a classification of algal communities that will entirely separate one community from another or that has ecological significance.

Each ecological investigator should deal with the problem in his own way. He should use the classification which seems to fit his data best, or make his own. It is only in this way that a good, basic, and generally acceptable system of community classification will sometime be achieved. I cannot refrain from again quoting from Tiffany because I recently found a statement of his which clearly and succinctly expresses my own views, "...we are as yet not in a position to formulate very definite principles regarding algal associations /and/ successions, or even direct relationships between algal productivity and special causal environmental factors."

The last part of the above quotation is a good way to introduce the problem of seasonal distribution. We have a relatively large amount of data on the specific organisms abundant at the different seasons, and on many of the associated habitat factors. This is especially true of the phytoplankton. Can we, however, even occasionally be sure just what the chief causal factors are for seasonal abundance, or more particularly for the sporadic blooms of phytoplankton? This is even more true for the littoral and lotic communities where we have much less data. There is general agreement that temperature and light are important in seasonal distribution, but these factors act on a whole spectrum of autotrophic, heterotrophic, and parasitic organisms. As pointed out above, we cannot sometimes easily separate the effect of temperature from that of light. Can we ever be quite sure, on the basis of present data, that phosphorus or nitrogen is really a limiting factor in a natural community, except perhaps for a very short period of time? Some of you have read numerous papers on phosphorus as a limiting factor, but the problem does not seem to be settled yet. Here, it seems to me, is where combination laboratory and field studies of species could give us some exact answers. Laboratory culture work should always be checked against a study of the same species in natural communities. Only such studies give promise of solving the problem of the sporadic, objectional bloom as well as that of seasonal fluctuations.

It is amazing to those who have had an interest in fresh-water algae for many years, how frequently a species first described from one locality will turn up next in a locality very remote from the first one. William Bailey (1851) wrote an excellent paper on fresh-water algae collected along the south Atlantic coast. In this paper he described a new desmid genus, Triploceras. His two species were next collected in Scandinavia, I believe. Triploceras is now known from all five continents. Bailey's Ceratium carolinianum was also next found in Scandinavia. The genus Oedocladium was first collected in Germany, then Virginia, Puerto Rico, India, and Australia. Oedocladium operculatum described from Puerto Rico, is common in India and is known also from Mississippi. These few illustrations give the pattern, or rather the lack of a pattern, in the known world distribution of fresh-water algae. Sometimes one thinks there is a pattern of distribution for certain species. In 1936 Tiffany described a genus (Cloniophora) from Puerto Rico which somewhat resembles both Stigeoclonium and Draparnaldia. Recently when I found the plant common in Peruvian rivers in the Amazon watershed, I thought, "Here is a tropical species." When I came to examine the literature, however, I found that the species has been collected widely in the northern hemisphere but reported under the name Draparnaldia mutabilis.

In a review of the geographic distribution of one of the best known families of green algae, the Oedogoniaceae, Tiffany (1957) concludes that some species (of Oedogonium and Bulbochaete) are cosmopolitan, the largest aggregation of species occurs in the temperate zone (where most collecting has been done), a few species are arctic, many species are both temperate and tropical, and a few large species are almost exclusively tropical. These conclusions are anything but concise and definite but they indicate the state of our present knowledge. Some of the supposedly rare species have an interesting history. Cyclonexis annularis Stokes, Chrysophyceae, was described from New Jersey in 1886. It was next found in Germany about 1910, then in Ohio in 1933 and in Massachusetts in 1939. A closely related species was described from Russia a few years ago, and last winter we found Cyclonexis annularis again, in North Carolina. We have three second records of species described in Europe, 30, 40, and 50 years respectively after the original collection. The diatom, Actinella punctata, until two years ago was known from only two or three collections in the United States. Since then we have found it at 12 localities in 10 counties in the Coastal Plain of North Carolina. As many as 50 cells were seen in one clump this past winter. Is it possible that if we knew when and where to look there would be no really rare species of fresh-water algae? I believe this is probably true. Most genera have been in existence for a very long time and individuals have become widely distributed into suitable habitats throughout the world. Just the other day I found this statement in Strøm's 1924 paper, "They all

have in common that they do not possess any sharp geographical distribution. They occur where their claims upon the habitat are fulfilled".

Some species are more stenoecious in habitat requirements than others; that is, they will multiply and become abundant only within a narrow range of habitat conditions. These are the so-called rare species which are sometimes very abundant when found. Sometimes they are thought to be indigenous to a particular locality because they have been found a few times in one locality only. Is it not possible that most of them are really widespread? Suppose a species is just barely able to survive during unfavorable seasons or conditions and becomes abundant only when the habitat is exactly favorable. It would long be regarded as a rare species. If this theory is true how do species survive during unfavorable periods. Fritsch (1931) has discussed this problem. He postulates that they may survive as spores or other "resting cells," but also suggests that they survive in the bottom ooze of the littoral zone, in case of plankton species. We have considerable evidence that this is true of both lotic and lenitic species. Synura uvella can always be found during the warmest weather in massive net collections from North Carolina lakes. The colonies are very rare and contain only a few cells, but they are there. We have found occasional living but depauperate cells of Actinella in the bottom ooze of streams where the water temperature reaches 27°C. Batrachospermum seems to survive the summer as very tiny rhizoidal, microscopic colonies on stones in streams. I could give many other such instances.

Many species of algae are more euryecious in habitat requirements. They are abundant enough under fairly favorable conditions to be collected widely or at all seasons of the year. These are the so-called cosmopolitan species. I suppose Micrasteria radiata is likely to turn up in any collection in the world where desmids are found at all. Perhaps this species is just somewhat more tolerant of unfavorable conditions than some, but like them only becomes abundant when conditions are exactly right.

I propose a theory of micro-habitats in both time and space for species of fresh-water algae. It is suggested that stenoecious species survive unfavorable periods or in unfavorable places as resting cells or as vegetative cells in very small, uncollectable numbers, that more euryecious species survive in the same way but in numbers great enough to be more often collected; but that all species become abundant only under precisely suitable conditions. This theory would explain the sudden blooming of plankton species, the marked seasonal pulses of others, and also the apparent rarity of certain species and the general and cosmopolitan distribution of others.

If an ecological approach were made on this basis, that is on the basis that each species or perhaps small group of species occupies a micro-habitat, I believe it would help solve many of our ecological problems. It would help in the matter of an ecological classification, because the smallest group of species occupying a particular micro-habitat would be our smallest community. A sufficient body of data regarding these habitats would enable us to group them logically into larger units. It would help solve the problem of seasonal pulses and of sporadic blooms. When a narrow band of blooming Microcystis occurs across one of our larger lakes, the habitat factors in this area must be just slightly different from those on each side. If we knew the exact habitat of one of the rare species we could predict where in the world it could likely be found. We already have a fairly large body of ecological data some of which might be interpreted on this basis.

In conclusion, I should like to suggest to phycologists; first, that students of flora try to record and publish more than bare date and locality records, at least on apparently interesting species; and second that there be more studies of species ecology. Only from a backlog of accurate and detailed data on species can a really good ecology of fresh-water algae ever be derived.

The ecology of fresh-water algae will, for many years, be very productive both of important data and of new ideas. I strongly recommend it to students with an interest in the algae.

## Summary

The contribution of floristic studies and of limnology to algal ecology has been pointed out and also that certain concepts and terms have been borrowed from these disciplines.

The importance of such habitat factors as light, temperature, water quality, and speed of current have been discussed together with some of the problems encountered in obtaining accurate data.

The fact that there is no entirely satisfactory system of community classification was emphasized.

The problems of seasonal distribution and of world distribution of fresh-water algae were briefly discussed. It was indicated that data are lacking for good correlation of seasonal distribution with habitat factors, and likewise that we cannot yet hypothesize accurately as to the character of world floras.

A theory was presented that fresh-water algae occupy micro-habitats and species frequently are not abundant enough to be collected except when growing under precisely suitable conditions.

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# ALGAL POPULATIONS IN FLOWING WATERS

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The problems encountered in dealing with organisms that inhabit a current are different in many ways from those met in the study of communities of standing water. The fickle, unstable nature of both the surrounding medium and the solid substratum; the insecurity of being unattached in a current; the enhanced chances of survival bestowed by a protected nook wherein to live and grow; the physiological and mechanical stress which continued pulling, twisting, and abrasion provide; the linear alternation of the strikingly different environments of riffle and pool; the continual supply of fresh nutrients, along with silt and other debris from upstream area--all these factors so influence river algae as to render the problems inherent in sampling, counting, and even in determining them, different in certain ways, and often more difficult, than those involved in working with algae from still water.

## QUANTITATIVE METHODS OF SAMPLING STREAM ALGAE

Most of the special methods of sampling river algae which have been devised are intended for studies of the bottom vegetation. Of course, this vegetation can simply be pulled up or scraped off with a knife. Other methods better suited to give quantitative data on the vegetation have been devised by many workers. Probably the best known method is that of Butcher (1932) who used a submerged photographic frame of the type used for printing postcards. The frame, holding five microscope slides, is fastened horizontally in the river and held by chains which are stretched tightly on iron stakes driven into the river bed.

This method was used throughout a long series of government studies of British streams, in which Butcher took part. These studies provided the foundation for the most extensive information we have about stream vegetation of any given region of the world. It permits quantitative appraisal of the early stages of algal growth. The method has not been adapted to long periods of observation, and no proof has apparently been given that algae colonize the slides in quite the same way that they colonize the river bottom. There is some evidence that numerical results from slide counts are not, in fact, comparable to those from the river bottom, but that the species present are generally similar (Reese, 1935). However, a few species of algae, which are almost universally present on such slides, are little known from natural substrata. Large algae may easily be torn off smooth surfaces, and some algae may colonize such a glass surface very slowly or simply show slow growth (Lund and Talling 1957). This method of Butcher is hence somewhat inadequate, and particularly so, in recording changes in the mass or volume of the vegetation or its components. This is a serious drawback, for such changes can be spectacular, as well as inexplicable.

Among measures designed to measure epiphytic growth may be mentioned the method of Thurman and Kuehne (1952). They used this method for epiphytes of Cladophora glomerata (L.) Kütz., but it could be easily applied to those of many other filamentous algae. A cylinder of algae 1 cm. thick is prepared, and pieces 1 cm. in length are cut from this. The individual 1 cm. X 1 cm. cylinder pieces are placed in preservative, shaken, allowed to stand for a day; and counts of the epiphytic algae are made from the sediment contained in the liquid.

Algae, such as Oscillatoria and various other blue-greens which live unattached on sediment, are difficult to collect by means which permit quantitative comparison between different collections. Soft deposits may be sampled with a suction-tube sampler, using a hand- or foot-operated pump and sucking the surface deposit through a funnel which is passed over the deposit in the manner of a vacuum cleaner. The method does not permit the collection of comparable samples from different types of substrate, nor give the same ratio of mud to water from any one area on each sampling (Lund and Talling 1957).

Margalef (1949a) has devised a method for stripping the algae from surfaces of rocks from the stream bed. After fixing, staining, and dehydrating the algal layer in situ, a collodion solution is poured over it and, when dry, is removed from the surface of the rock. The method, similar to the one commonly used for fossil material, is claimed by Margalef to permit accurate counts of the populations present.

As a means of estimating algal growth on the bottoms of shallow rocky streams, I employed a modification of the transect method. A rope was marked off into decimeter and meter units and stretched across the stream at a riffle, just above the water surface. Presence was recorded in alternate decimeters of all visible algal species, an individual or colony of which, is crossed by the rope. The importance of the species was thus determined by the relative number of decimeters within which that species was present. Frequency was determined for each visible species by estimating the relative percentage of total decimeters in which it

was found in relation to the total number of decimeters whose composition was recorded. All transects were located with the intention of providing a typical cross-section of the riffle and were oriented at approximately right angles to the direction of water movement (Blum, 1957).

This method also has many drawbacks. It is not applicable to pools and is workable only with difficulty on riffles when the water is 20 cm. or more in depth and turbidity is high--conditions which are likely to be synchronous in a small stream.

Douglas (1958) has devised several useful instruments for working with epilithic algae. One of these consists of a short, tuft-like brush of nylon bristles, provided with a handle of steel rod. After removing a specimen rock from the water, a 50 ml. polythene bottle with bottom sawn off is inverted over the surface to be sampled. The brush is inserted into the bottle and the rock surface delimited by the neck of the bottle is scrubbed clean. Washing and brushing are carried out over an enamel sorting tray so that none of the sample is lost. From the area of the polythene bottle neck and the number of stones used in sampling, it is possible to calculate the total area of stone surface thus cleaned.

Another, more complex tool was devised for the sampling of algae from stones under water. Here the brush used is similar but has a hollow, tubular handle. The upper end of this handle connects by means of a length of rubber tubing to a specimen tube. The area brushed is delimited by a steel casing which shields the sampling area from the current and prevents the algae from washing away. A sponge rubber ring at the base of the casing tube provides a tight seal against the rock surface. During sampling with this equipment, algal material brushed from the rock surface is sucked up into the specimen tube through the hole in the brush. From the area covered by the innermost part of the casing and the number of such areas cleaned, the total area sampled can be calculated.

A third device, employed for the sampling of epiphytes on aquatic bryophytes, was developed by Douglas. This consists essentially of a hardened steel tube or borer sharpened at one end and a wire plunger which can be pushed through it. The borer is forced through the moss layer against the underlying rock, thus cutting out a small cylinder of vegetation which is subsequently removed by the plunger. An elaborate cleaning process follows. As in the other two methods, counts of the sample are made microscopically after delivering portions of the sample to a counting cell (Douglas, 1958).

Among the algae, which the stream collector records by these, or other methods, are forms which are characteristic of standing waters, as well as others which are indifferent; still others which are practically limited to running water. Some of these latter forms have been recorded from so many streams, particularly of Europe, that it becomes possible to correlate the data from many surveys and to a degree, to characterize the usual habitat of each alga. Much of the following information on algal species has been taken from the recent paper of Hornung on the Echaz (1959).

## ECOLOGICAL CHARACTERIZATION OF RHEOPHILIC ALGAE

Achanthes lanceolata Brèb. This is a diatom common and widespread in alkaline streams, and particularly common in springs and small brooks. It exhibits a degree of resistance to certain poisons and to sewage pollution (Schroeder, 1939). It exhibits a spring growth maximum, according to Hornung (1959), and a maximum in spring and fall in the results reported by Schroeder. Schroeder states further that in polluted water, its growth maximum is attained in winter.

Cocconeis placentula Ehr., frequently an epiphyte, grows best and is most common in running water, in mildly alkaline streams. It is somewhat sensitive to pollutants and seems to exhibit a growth maximum in autumn.

Diatoma vulgare Bory is common and widespread, especially in running water, and prefers somewhat alkaline water. According to Kolkwitz (1950) and Hustedt (1944), it is characteristic of the $\beta$-mesosaprobic zone or the region of advanced oxidation of pollutants in a polluted stream. But, I have found its best growth in the oligosaprobic portion of a polluted stream, and various authors have credited it to every one of the principal zones of the saprobic system. In the streams of southern Michigan, which I studied, it was the dominant form, covering all available rocks in the current during the fall months, and again, in spring, (Blum, 1957). A related species, D. hiemale (Lyngb.) Heiberg is characteristic of mountain brooks.

Gomphonema angustatum (Kütz.) Rabh. is typical of small brooks and is adapted to alkaline waters. Kolkwitz (1950) considers it to be an oligosaprobic form, but Hornung reports it from highly polluted localities.

The similar Gomphonema olivaceum (Lyngb.) Kütz. is not confined to running water but forms thick, gelatinous mats in favorable stream situations. In the Michigan streams investigated, it replaced Diatoma vulgare in winter and became a strong competitor for favorable sites with Diatoma in the following spring. In European streams this form seems to be associated with polluted waters, occupying the mesosaprobic zone, according to Kolkwitz (1950), Fjerdingstad (1950), and Liebmann (1951). Melosira varians C.A.Ag. is found in both

standing and flowing eutrophic waters. Both Kolkwitz and Liebmann consider it to be characteristic of the β-mesosaprobic zone. Growth maxima of this species have been found both in spring and in summer. Nitzschia linearis W. Smith is a true rheobiont, characteristic of springs and flowing water. The related species, Nitzschia palea (Kütz.) W. Smith is perhaps the most resistant and most tolerant of all diatoms, a eurytopic, euryhaline eurythermic form which grows conspicuously in polluted water where it forms a rich brown surface layer on rocks of rapids, as well as in quiet water on shallow silt banks. Both Kolkwitz and Liebmann place it in the α-mesosaprobic zone of incipient oxidation in a stream, and it is often used as an indicator of polluted water although its ubiquitous tendencies permit it to grow throughout the entire gamut of water purity.

Hildenbrandia rivularis (Liebm.) J. Ag. is a rheobiont, a crust-forming red alga which is found in many hard waters of Europe. It seems to be absent, however, from streams with extremely high calcium content. In a paper reviewing its occurrence, Luther (1954) has concluded that it is limited to shaded situations. Thus, it is often found in rather deep clear water, being at the extreme outpost of macroscopic vegetation due to light limitation. In sunny situations and in clear water it may be found only on the under side of rocks. Whereas, if the water is turbid, it is more likely to be found on the upper side. In the waters of Lough Neagh in Ireland it was not found on basalt or other dark-colored rocks but was found on the under side of quartz rocks where it apparently received most of its light directly through the rock. It seems to have a wide amplitude of tolerance for temperature, as well as for organic pollution. Its absence from otherwise suitable waters has been interpreted as brought about, in some cases, by competition with other forms; in other cases, by deposition of silt around rocks inhabited by young plants (Luther, 1954).

Ulothrix zonata Kütz. is a rheobiont inhabiting cool, well-oxygenated streams. It shows a vegetative growth maximum in late spring and early summer. Fjerdingstad (1950), Kolkwitz (1950), and Liebmann (1951) characterize it as oligosaprobic in habit, but there is some indication that its growth is enhanced in polluted waters.

Cladophora glomerata L.) Kütz. is one of the largest and most widespread of rheobiontic algae. It is known to be highly sensitive to iron and relatively tolerant of high pH values. It seems to require alkaline and calcareous water. It is tolerant of relatively large amounts of sewage in the water and of weak salinity. The length of its summer growth period varies from a few days to several months. Usually it is inconspicuous in winter although its full development has been recorded in February and March, as well as in October; and Jaag (1938) has observed it throughout the winter. It can apparently produce swarmers throughout the entire year but is probably sensitive to temperatures higher than about 25° C. After periods of turbidity, the plants of Cladophora often become heavily loaded with silt and epiphytes, a factor which may be significant in the suppression of growth or in the mechanical detachment of the thallus.

Stigeoclonium tenue Kütz. is a large, common stream alga which thrives in polluted waters of the α- and β-mesosaprobic zones and is seldom found growing luxuriantly elsewhere. Budde (1930) concluded that it favored cool waters, but I have found it attaining its best growth in early summer and midsummer. Like Cladophora glomerata it is likely to be found in the most rapid water of a brook, occupying the crest of a shallows. Its preference for polluted water is not limited to organically enriched water, for it can frequently be found downstream from industrial outfalls (which, however, frequently contain sewage wastes) whereas it is apparently absent upstream from the outfall.

Hydrurus foetidus (Vill.) Trev., a filamentous member of the Chrysophyceae, is a rheobiont adapted to cold water (approximately 0°-16° C.). Like Hildenbrandia it appears to favor shaded situations. In Europe, where it has been found much more frequently than in North America, its growth maximum is apparently attained in spring and in autumn.

Phormidium autumnale (Ag.) Gom. is common in hard-water streams and is frequently associated in polluted water with Sphaerotilus and with Stigeoclonium tenue. With the sheaths which surround its filaments, it forms mats of medium thickness and rubbery consistency which adhere to the surfaces of rocks in the stream bed.

The species I have taken up here are only a few of many common and widespread stream forms, and I have tried to give merely general information on the ecological preferences of most of them. Much more precise data on the diatom species as concerns their halobion spectrum, the pH spectrum, and the current spectrum are available in the recent work of Foged (1947-48, 1954).

## ECOLOGICAL CLASSIFICATION OF RHEOPHILIC ALGAE

The Naming of Communities.--In streams it is the exception, rather than the rule, to find extensive areas inhabited by recognizable groups of algal species which maintain a definite order of dominance or inferiority with respect to each other throughout all the seasons, although this relationship is more commonly found if higher plants are considered. Many algal populations appear to be

relatively haphazard minglings of two to many unattached species. These groupings may exhibit no constant composition from place to place within the stream. Frequently, too, their composition changes rapidly from week to week. In the composition of any community of aquatic algae, chance probably plays a major role throughout the formative period, which may last for many years, perhaps centuries. Thus, it is only in large, old, aquatic situations that one may hope to find associations so stable that the absence of one form or another would be of real biological significance (Symoens, 1951). The availability of numerous diatom and other species, any one or any combination of which is able to take over a site and quickly achieve local dominance, makes it particularly difficult and unrewarding to apply formal names to such groupings. Only when there is (1) a permanent, distinctive community structure which can be found repeated again and again in a series of streams with essentially the same species composition of algal dominants throughout the series, or (2) a consistent reappearance of a specific algal vegetation in a given season of the year, does there seem to be sufficient grounds for applying a formal ecological name to the vegetation. It seems preferable to deny formal status to the less frequently observed combinations. Just as in land vegetation, the application of formal names to all types of variant transition combinations found along ecotones is liable to create confusion without serving any redeeming practical purpose. It is futile to name, aquatic communities without evidence that they are of more than purely local occurrence. Panknin (1945) points out that phytoplankton can, in this way, be divided up into an endless series of associations. In the benthos, extensive stands of one or more species of dominant algae are relatively more common than among land communities. Often these stands are unialgal, or nearly so, to the exclusion of other green plants below them. Often they grow with great rapidity, carpeting the stream floor in ten days or less when they experience favorable environmental conditions. Many small species are epiphytes (or endophytes) on larger algae which support, and to a certain extent protect, them. The ephemeral nature of attached algae is, furthermore, one of the unique features of the stream communities. With the detachment of a large thallus, it, and its entire collection of epiphytes, is immediately taken downstream. All these organisms with the nutrients they contain are effectively lost to the local ecocosm.

These are rather deep-seated differences from the conditions on land and from the conditions in standing water. It has been suggested that we may be basically in error in attempting to apply to the hydrosere terms such as "climate" and "climax" which were first used for land vegetation and which may, upon scrutiny, carry with them too many implications of no validity in hydrobiology. For one thing, a plant community which takes over a site and reproduces there, maintaining dominance and successfully resisting invasion so long as environmental conditions remain substantially unmodified, has fulfilled the conditions for a climax community. But, with reference to river algae, this community may not have been preceded by any other green plants whatever--colonization and dominance are thus synchronous. With no previous community occupying this site, succession has not occurred and use of the term "climax" seems inappropriate (Blum, 1956a).

Eddy has concluded that permanent freshwater communities exist, reach maturity and show aspects comparable to terrestrial communities, and he points out that the maintenance of given climatic conditions necessary for the establishment of a "climatic" climax is not confined to land communities, but can also be found in permanent streams (Eddy, 1934). The generation and the life span of dominant algae are so much shorter than those of most dominant vascular plants that "permanent" climatic conditions can be achieved in a relatively shorter time and more rapid succession is to be expected. For extremely short-lived microphytes, a single growing season in temperate regions may be sufficiently long to represent a "permanent" climate (Blum, 1956a).

Panknin (1941) has discussed the classification of seasonal communities which assume temporary dominance upon a given site and has concluded that such algae should not be regarded as constituting seasonal associations but rather as making up seasonal aspects of the entire association. He considers that community names based on only two diagnostic species are inadequate if only one season has been studied, and the aspect of the vegetation in other seasons is unknown. The "true" algal community recognized by Panknin includes no higher plants and occurs only on the seacoast, in deep mountain lakes, in the phytoplankton, and in streams. All other algal communities are "dependent," the algae being merely an undergrowth in an assemblage of higher plants. (Panknin, 1945). This refusal to recognize many common algal communities separate from those of higher plants around them led, as Symoens (1951) has pointed out, to lumping them with plants mostly larger than themselves and to designating such heterogeneous communities as the Micrasterieto-Sparganietum. Various communities have been named exclusively on the basis of desmids present or on diatoms present while, at the other extreme, Margalef (1947, 1949) understandably introduces even Protozoa, Cladocera, Copepods, and insect larvae into associations made up partly of algae (Symoens, 1951).

The Classification of Stream Algae According to Structural Adaptation.--Among schemes of

classification which have been devised for the forms of plant body possessed by rheobiontic algae, that of Cedergren, sketched in brief form in 1938, is worth attention. Cedergren points out that benthic algae, which inhabit a current, exhibit adaptations of four principal types: (1) Richly branched filaments. These have strong anchorage and water may pass freely between adjacent filaments. The presence of a slippery gelatinous external coat is noteworthy in many of these such as Batrachospermum, Draparnaldia, and Stigeoclonium. (2) Long flexible cylinders. These align themselves perfectly with the current and thus offer little resistance to the water. (Encyonema spp., Tetraspora cylindrica (Wahlenb.) Ag. or Spirogyra fluviatilis Hilse) The presence of a firm attachment and a slippery exterior is remarkable in this group. (3) Spheric, wart-like or cushion-like colonies. These show numerous contained filaments as in Chaetophora or Nostoc which, because of the smooth external surface of the colony, offer little resistance to water movement. (4) Reduced, simplified, plate-like forms. These grow in a thin, appressed sheet. Although diverse in internal structure, externally such sheets are similar in usually following the contours of the rock or other substrate and in exposing relatively little surface as a colony to the water in relation to the enormous surface of the individual filaments or other units. Hildenbrandia and Phormidium are examples.

These four types can actually be further reduced. Groups 1 and 2 are those with flexible thalli which permit water to run through them and thus expose a large surface to the water. Groups 3 and 4 have inflexible bodies and a greatly reduced surface. A number of low-growing green and blue-green algae do not seem to fit into this system very well. Nevertheless, a great majority of stream algae would fall under Cedergren's groups 1 and 4.

Named Benthic Communities of Algae.--A number of authors have catalogued stream communities which have come under their observation, but relatively few have made a synthesis of observations from other regions with their own. Among recent contributors to algal sociology, Symoens, who has done important work on streams in Belgium, has presented an outline of algal communities based on (1) floristics, (2) aspect (physionomie), (3) syngenetic relationships, and (4) synecology. The system includes eighteen alliances which he lists "timidly and provisionally." These include three alliances of stream algae, as follows: (1) epilithic algae and crustose lichens, (2) benthic diatoms and (3) filamentous green and red algae (Symoens 1951). Mention was made of certain associations represented by each heading, but without extensive discussion or description. These associations were amplified in a subsequent study of streams in the Ardennes region (Symoens, 1957).

In England, Butcher (1946) has recorded what he considers to be two distinctive communities. The first of these, Achnanthes microcephala--Chaetopeltis, with associated Diatoma hiemale and and Eunotia, is the characteristic oligotrophic community in the streams studied. With increasing eutrophy in downstream areas, it may be replaced there by the Cocconeis-Ulvella-Chamaesiphon community. This is a eutrophic association which shows no seasonal periodicity and which is composed principally of Cocconeis placentula Ehr., Achnanthes minutissima Kütz., Ulvella frequens, Chamaesiphon incrustans, Grun. and Chamaesiphon irregularis.

Butcher's group observed the dominance of the first of these communities in the upper reaches of the Tees. This was replaced by the second wherever sewage drains flowed into the river in quantity. The first, or Achnanthes-Chaetopeltis, reappeared wherever the dilution was sufficient and decomposition rapid. Margalef (1948) has referred under a different name (Asociation Hydrococcetum rivularis) to what appears to be a directly comparable community living in streams of the Pyrenees.

Another community characteristic of the upper reaches of hard water streams is a crustose community dominated by Phormidium, Schizothrix, and Audouinella, and which is known also as the Chantransieto-Phormidietum incrustans (Symoens, 1957). It has been identified in Austria, Belgium, England, and the United States of America. It is probably widespread in hard water streams, perhaps throughout the world. The principal blue-green components of this crust, Schizothrix fasciculata, S. pulvinata and S. lacustris grow on submerged rocks, forming dense tufts of radiating filaments which may be only 0.5 mm. in height. As the Schizothrix grows, two other species frequently invade the tufts: Phormidium incrustatum, whose filaments mingle with the tufts and grow, with either horizontal or vertical orientation, twisted in and out between the Schizothrix filaments; and Audouinella sp., which grows radially like the Schizothrix out to the limits of the tufts, where it branches while its upward growth keeps pace with, or slightly surpasses, that of the Schizothrix and Phormidium species. These four or five organisms constitute a single layer, and apparently all of them may secrete calcium carbonate abundantly, thus converting the entire tuft, or stratum of continuous tufts, into a crust. This crust becomes especially stony and resistant in the presence of a dense admixture of Phormidium incrustatum. After growth for a year or more, the tufted layer may eventually attain a thickness of 4-5 mm. Frequently, at seasons when other benthic algae are abundant within the stream, it becomes an inferior layer, shaded and more or less enveloped by temporary dominants such as Cladophora glomerata (Blum, 1956).

Among the many benthic diatom communities which have been named, I shall mention only a few. The Diatoma vulgare-Melosira varians community, characterized by a substantial development of species of alkaline and eutrophic waters. Under typical conditions, at least in the Belgian streams studied by Symoens (1957), few non-diatomaceous algae are included. With time, Ulothrix, Cladophora, Vaucheria and Oscillatoria make their appearance. The latter is associated especially with organically enriched waters. When these algae become dominant, the physiognomy of the vegetation changes completely, and its formal classification under the alliances listed by Symoens would shift from group 2 (benthic diatoms) to group 3 (green and red filamentous algae) (Symoens, 1957).

Another diatom community is the Diameto-Meridionetum of Margalef (1948) and others. It is represented in various forms in different streams, with many species included, but the diagnostic ones are Diatoma hiemale and Meridion circulare Ag. In the Ardennes, Symoens (1957) considers this community to be associated with considerable elevation (150-2500 m.) and Margalef states that it is confined to waters of temperature between 10° and 15° C.

Under the associations of green and red filamentous algae Symoens has listed, among others a Cladophora glomerata association, known from Belgium, France, Western Germany, Catalonia, Switzerland, Balearic Isles, the U.S.A., and, with associated Podostemaceae, from central Africa. The diatoms associated in the Ardennes region are those of the Diatoma vulgaris-Melosira association. Cocconeis pediculus is a widespread, epiphyte on the Cladophora.

Another filamentous association listed is that of lime-rich waters dominated by Vaucheria spp., especially (in the Ardennes) V. debaryana Woronin, and characterized by much the same diatoms that are associated with the Cladophora vegetation.

An additional Vaucheria Association is listed. However, this is Vaucheria of soft waters and is accompanied by diatoms of the Diatoma hiemale-Meridion circulare community.

It is clear from these brief examples taken from the literature that a beginning has been made in the cataloguing and classification of stream communities. Work in future years will, no doubt, increase our knowledge of the validity and floristic affinities of the associations named thus far and give us more than a pioneer explorer's view of the vegetation of streams of the world.

## DISTRIBUTION OF ALGAL POPULATIONS IN TIME

Colonization and Succession.--From his work on the Hull (England), Butcher has concluded that colonization there reaches a maximum in May. Individual dominant species have periods of most rapid reproduction in various months from March through October, and the period of least colonization is in winter. In summer, colonization appears to be complete in about 20 days, but in cold months when growth is slower, 30 to 40 days are required. It might be supposed that vigorous current would impede algal colonization on so smooth a surface as a glass slide, but more algae are produced on the slides in regions of rapid flow than elsewhere.

In southern Michigan, colonization of rock surfaces by winter-dominant diatoms is very rapid, and macroscopically visible colonies can form in as little as ten days. The period within which Gomphonema olivaceum colonized bare rock surfaces extended from late November to early April, and colonization appeared to be possible at any time within this period. No evidence of succession was found prior to the establishment of this community, and the same may be said for the community of Diatoma vulgare characteristic of late fall--both these forms were at one and the same time colonists and seasonal dominants (Blum, 1956).

In the work of Butcher there is likewise little evidence of succession in the algal communities he has investigated. In the Cocconeis--Chamaesiphon community, Chamaesiphon arrives in the ensemble later than the other forms, but there is no true succession. Certain of these communities are regarded as representing a true climax, but their development is apparently accomplished with no steps separating invasion from climax conditions (Butcher, 1946).

Periodicity of Algal Populations.--The plankton algae of a great many streams have now been investigated for sustained periods. Most of these streams are in Europe, but some of those in the United States have been extensively studied also, and a few tropical streams are represented. Many streams exhibited considerable consistancy, during the period and to the extent of the observations made, as regards the time of development of the greatest densities of their plankton or of their benthic vegetation, whereas other studies have pointed out sharp differences in the year-to-year developmental pattern. A mere year's work upon a single stream may prove inadequate in that it reveals conditions essentially unlike the preceding and following years. In nearly all streams investigated, the principal phytoplankton pulse, if there is one, is to be expected at some time during the warm season, but individual plankters, e.g., Crucigenia rectangularis, Pediastrum boryanum, Fragilaria capucina, Meridion circulare and Synedra ulna, frequently exhibit population maxima in winter. Many streams are characterized by two separate periods of maximum plankton abundance. Some species of algae exhibit great variability in production, remaining uncommon in one year and

becoming a dominant in the next, exhibiting a pulse at widely variable times throughout the year, or a single pulse in one year and a bimodal one in another. Certain diatoms tend to exhibit a pulse in spring and/or in autumn rather than in midsummer.

Occasionally a river may develop a "bloom", although this phenomenon is more frequently seen in ponds. Organisms which have been found responsible for such blooms include Thalassiosira fluviatilis (Weser, Germany), Synedra delicatissima (Potomac, U.S.A.), Microcystis flos-aquae (Bug, U.S.S.R.), Anabaena spiroides (Mayenne, France), Aphanizomenon flosaquae (Don, U.S.S.R.) and Pandorina morum (Cumberland, U.S.A., and Kentucky, U.S.A.) (Blum, 1956). Lackey lists no fewer than 25 separate blooms for the Clinch River and adjacent waters in 1956 with a great variety of organisms represented (Lackey, 1958).

The best correlations which have been obtained by plankton work and chemical analyses over a period of years point to a time relationship between abundant nutrients and abundant phytoplankton, the plankton pulse usually following the period of highest concentration of the nutrients, as nitrates and nitrites, in such a way that the decrease in nutrients precedes by a few days to a few weeks the maximum development of phytoplankton.

A diurnal plankton pulse has been observed in a polluted stream, apparently dependent upon and produced by midday sunlight which causes benthic forms to rise into the current and be carried downstream. Evidence that planktonic forms reproduce as they are carried downstream has been presented by various workers, but there remains the suspicion that much of the actual cell division occurs on the bottom and that the apparent increase in phytoplankton downstream is largely the result of more extensive nutrient beds there, and of denser populations of benthic individuals, many of which rise every day into the plankton. The vegetative dissemination of Spirogyra and Oscillatoria communities was observed by the author on warm summer days in 1952 and '53. These communities were especially characteristic of quiet shoals or bays of the stream. Here the algae remained on the bottom in contact with nutrient-rich silt deposits, as masses of filaments easily visible from a distance. The surface water of such shoals and bays is usually in slow circular movement set up by the main current of the stream, which by-passes the shoal or the bay in a tangent to the circular current which it produces there. At times of rapid photosynthesis, individual masses of the algal filaments are detached and buoyed upwards by trapped oxygen bubbles. Once the algal mass has quit the floor of such a shoal, it is carried slowly along in the eddying surface water. After moving for some time in this circular manner, it may eventually be picked up by the tangential current of the main stream which removes it definitively from the shoal. As the algal mass travels downstream, it disseminates live filaments along the way. The progress of these filaments is arrested on obstructions or on new shoal areas or other sediments downstream, which in this way are themselves colonized. The elevation of algal masses by entrapped bubbles can be observed from about noon until about 2-3 p.m. on sunny days in summer, and the movement downstream of these floating masses can be observed throughout an entire afternoon (Blum, 1956).

Under benthic algae are included both seasonal and perennial species. While a single alga may be dominant over relatively long reaches of the stream's course, it is more common to find a number of dominants with different parts of the stream having different dominant communities. In some streams the algal vegetation remains much the same throughout the year, whereas in others there are marked seasonal aspects. As with plankton algae, the seasonal variation may be summarized broadly as maximum development in warm months, followed by minimum development in cold months. But, many algae behave quite differently. To what degree these seasonal changes are due to some environmental factor, has not been established.

## DISTRIBUTION OF ALGAL POPULATIONS IN SPACE

Depth zonation.--The relation of depth to the algae of rivers has received relatively little attention. On the bank of the polluted Mulde River, Schroder (1939) has illustrated a series of algal zones with Spirogyra spp. just below the water surface, Stigeoclonium tenue and then Oscillatoria spp. further down, and Sphaerotilus spp. and Nitzschia spp. in the deepest water. In certain lower portions of the Meuse, Symoens has described a distinctive zonation consisting of Rhizoclonium sp. just above the usual water level, Bangia atropurpurea at the level washed by the water's edge, in a band 10 to 20 cm. wide, and finally Cladophora glomerata, below the water level and mixed with certain mosses (Symoens, 1957). It is probable that nearly all streams which maintain a given level for several weeks exhibit some zonation of the attached algae. Figure 1 illustrates a type of zonation, involving two or three species, which has been noted in southern Michigan streams. Scheele (1954) has recorded diatom zonation in drains (Schleusenwände) in Germany. The most outstanding difference with increasing depth was a decrease in numbers of Nitzschia palea. N. frustulum was found most commonly in a transition zone intermediate in depth and in available light.

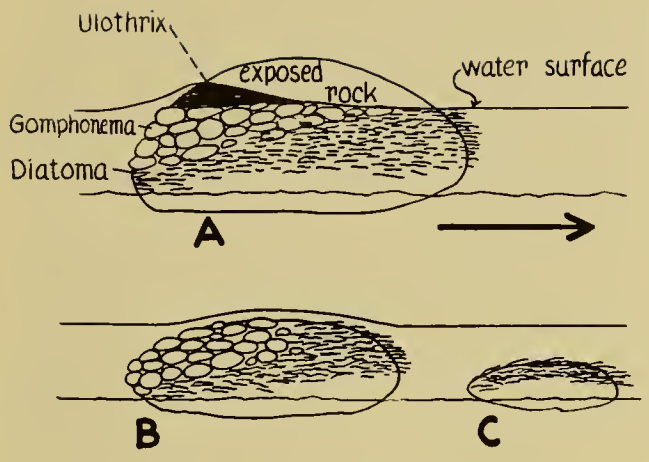


Fig. 1. Diagram of spring zonation of principal algae on rocks of shallows (average depth ca. 15 cm.) in the Saline River, southeastern Michigan. Dark area represents Ulothrix tenuissima filaments, present only on rocks in swift water immediately below the water surface where the current is broken by protruding emergent rocks as at A. Irregular oval patches represent Gomphonema olivaceum occupying the upstream face of rocks such as A and B in rapid current but slightly below the Ulothrix level. Irregular short lines represent the Diatoma vulgare community which is partly intermingled with the Gomphonema but is dominant at a slightly lower level. Rocks such as C, below a given depth are not colonized by massive Gomphonema colonies or by Ulothrix. Arrow shows direction of water movement. Note that this zonation is not uniquely related to depth, but is also influenced by current rate.

## DISTRIBUTION WITHIN THE BASIN AS A WHOLE

Most small streams consist of a series of alternating shallow areas ("shallows", "riffles") and deep areas ("pools"). The shallow areas naturally receive greater abrasion by the water and the molar agents it carries. The water flows faster here and in a thinner sheet, and significant chemical differences are to be expected between the shallows and pools, although little effort has been made to demonstrate them. Many algae are confined to shallow parts of headwaters streams, just as others are characteristic of the slower and deeper waters of pools or deeps. Peculiarities of the deeps and shallows as they alternate with each other on the stream profile determine the laws of distribution of the animal population as well. Succeeding riffles or shallows frequently carry the greater volume of water on alternating sides of the stream, so that erosion is greater first against the right bank and then against the left. This asymmetrical pattern results in asymmetric distribution of the benthos biocoenoses of the pools, with an accompanying break in their continuity at every riffle. Ulothrix spp., Stigeoclonium tenue and Diatoma vulgare are all characteristic of riffles and regularly drop out as massive components of the vegetation wherever pool conditions obtain. Spirogyra spp., Euglena spp. and other mostly unattached forms naturally collect where current is minimal. Within a riffle or shallows itself, certain areas are apparently much more favorable than others for the larger algae. Growth of Diatoma vulgare has been observed to be inhibited in the portions of the riffle downstream from large rocks, where water movement is relatively slow. (Fig. 2)

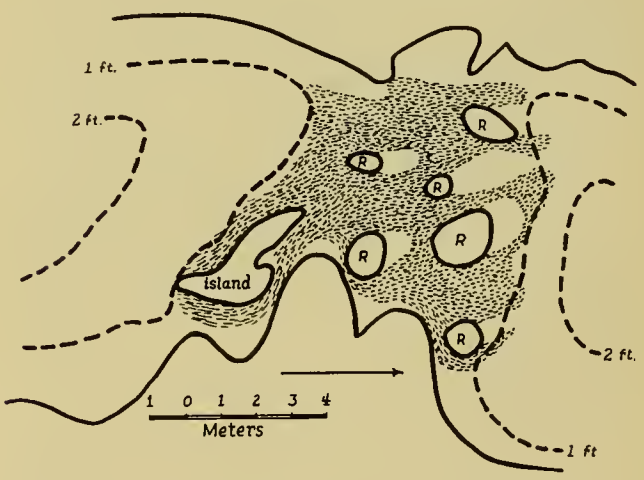


Fig. 2. Diagram of colonization by Diatoma vulgare in a shallows of the Saline River, Michigan. Heavy continuous line represents the water's edge. Heavy broken lines are isobaths at 1 and 2 ft. depths. R = large, emergent rocks; pattern of short lines represents the Diatoma vegetation. Note that this Diatoma is limited to shallow (swift) water, and that it is absent from shallow areas near the stream edge and from areas to the lee of emergent rocks where water movement is much slower. Arrow represents direction of water movement.

Eddy presented the view that the amount of plankton in river water is dependent upon the

length of time required for the water to pass downstream from headwaters sources, or, as he regarded it, as the "age" of the water. Thus there is an initial increase in plankton with time and distance going downstream. Certain streams (e.g., the Illinois River and the Rock River, both in U.S.A.) exhibit a headwaters area low in plankton and a middle region rich in plankton, followed by a consistent decline in plankton in the lower course. This decline is, however, not universal, and the phenomenon can not yet be correlated with a given length of course or degree of eutrophy. Conditions in a mature stream may be expected to approach those of late or middle-age ponds in the neighborhood of the stream whose waters have a similar chemical composition (Blum, 1956).

An ambitious attempt to compare the flora of different streams has been made by Budde (1930) who reviewed several published works on European and other rivers. Of the British streams studied by Butcher and his co-workers, the Itchen, Test, Bristol Avon, Hampshire Avon, and Lark seem to be somewhat similar physico-chemically and to have many parallels in their algal vegetation (Butcher, 1932). The work of Scheele on diatoms of the Fulda (Germany) has shown that the tolerant, ubiquitous species increase from source to mouth and that the species characteristic of springs show a corresponding decrease (Scheele, 1952).

The changes in benthic algae over the course of a stream from source to mouth have been investigated by many workers. One of the more exhaustive treatises of this nature is the work of Lauterborn (1910, 1916, 1918) on the Rhine vegetation. The great variability in size, profile, geology, degree of pollution, and other atrributes of the streams studied does not admit of many comparisons of their respective floras, and little information of general application can be drawn from these studies until an extensive analysis of the data contained in them has been assembled.

As an example of some of the changes which may be expected in the algal flora on the profile of a stream, Table 1, condensed from Butcher (1947) shows some of the changes as determined quantitatively in his work. The table shows the response

TABLE 1

(Condensed from Butcher, 1947)

ALGAL COLONIZATION ON BLANK GLASS SLIDES SUBMERSED IN A POLLUTED STREAM

Totals under the different algal species identified show average growth in numbers per square millimeter of slide surface on slides submersed in the River Trent (England) above and below outfalls from tar distilling industries. Collections were taken from slides at intervals of 20 days from April to October, 1938. The river water showed a pH of about 7.6. The station above the source of pollution, and that at 35 miles below this source of pollution may be considered oligosaprobic. Stations from 2-8 mi. below may be considered polysaprobic, 10-17 mi. below, mesosaprobic.

| Miles from pollution source | Stigeoclonium tenue | Nitzschia palea | Gomphonema parvulum | Stigeoclonium farctum | Ulvella frequens | Chamaesiphon spp. | Cocconeis placentula |
|---|---|---|---|---|---|---|---|
| above | 0 | 0 | 0 | 0 | 130 | + | 820 |
| below | | | | | | | |
| 2 | 30 | 130 | 20 | 0 | 30 | + | 0 |
| 3 | 190 | 680 | 130 | 0 | 20 | 20 | 0 |
| 5 | 1620 | 2380 | 600 | 0 | 20 | 0 | 0 |
| 8 | 15,300 | 5250 | 3390 | 20 | 40 | 0 | 0 |
| 10 | 50 | 620 | 690 | 1880 | 0 | 0 | 0 |
| 13 1/2 | 45 | + | 660 | 270 | 60 | 20 | 0 |
| 17 | 180 | 250 | 3000 | 300 | 260 | 330 | 0 |
| 35 | 220 | 150 | 1280 | 210 | 480 | 200 | 1480 |

of certain algae to pollution due to gas liquor and organic chemical wastes from tar distilling. The effluent from this industry included tar acids, cyanides, and ammonium. The totals, shown under the different algal species identified, represent average growth in numbers of plants per square millimeter of slide surface on slides submerged in the River Trent (England). Noteworthy here is the striking build-up in numbers of Nitzschia palea and Stigeoclonium tenue throughout the polluted (polysaprobic) portion from 2 to 8 miles below the source of pollution, and the great abundance at the lower end of this region. Also significant is the striking elimination of Cocconeis placentula from the flora with the accession of the chemical wastes, and the reappearance only after many miles of the stream's course had permitted sufficient reduction and oxidation of the wastes, dilution, and mixing adequate to return the water to a condition once more suitable for growth of the Cocconeis.

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# BIOLOGICAL DISTURBANCES RESULTING FROM ALGAL POPULATIONS IN STANDING WATERS

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Nowhere can there be found more striking examples of interactions between, and interdependence of organisms in nature than in the ramifying effects produced by algal populations. The roles which these plants play in aquatic biology and in limnology are clearly recognized but often incompletely understood. Whereas the place of algae in nature may involve some benefits to man and to other organisms, some of the effects produced are radically and offensively disturbing, sometimes costly and even lethal. The putrefying masses of algae in recreational lakes and in water supply reservoirs are aesthetically disturbing and economically aggravating. But of greater importance are the indirect and far-reaching effects algae have on other aquatic organisms, including their ability to kill.

It is from populations of algae in standing water, of course, that effects are most obvious and most disturbing because concentration of numbers of individuals is possible. To be sure, some of the same disturbances occur in slowly flowing waters which have ecological conditions conducive to bloom-forming algae. We are not primarily concerned with marine waters here, but it is appropriate to recall that some of the same effects are produced by algae in the sea (Red Tides; fish poisoning).

It is well-known that of the countless species of fresh-water algae, only a few produce disturbances which attract our attention. These belong almost entirely to three phyla, the Cyanophyta, Chrysophyta, and Pyrrhophyta. The species with which the present subject mostly deals are listed for reference. It will be recognized immediately that these are mostly so-called "bloom"-producing species.

I. Cyanophyta
*Microcystis aeruginosa*
*M. toxica*
*Coelosphaerium Kuetzingianum*
*Oscillatoria rubescens*
*O. lacustris*
*Anabaena circinalis*
*A. flos-aquae*
*A. Lemmermannii*
*Anabaenopsis Elenkinii*
*Nodularia spumigena*
*Aphanizomenon flos-aquae*

II. Chrysophyta
*Dinobryon sertularia*
*D. sociale*
*Synura uvella*
*Fragilaria* spp.
*Tabellaria fenestrata*
*Asterionella gracillima*
*Coscinodiscus* spp.
*Melosira granulata*
*Stephanodiscus Niagarae*

III. Pyrrhophyta
*Gymnodinium brevis*
*G. veneficum*
*Ceratium hirundinella*

When the algae guilty of causing disturbances are examined and their ecology analyzed, a few generalities appear:

1. Disturbances occur in waters which are enriched with phosphates and nitrates; usually the algae occur in bloom condition, and the disturbances arise in relatively shallow lakes where it is possible for nitrates and phosphates to be recirculated from bottom decomposition.

2. Excessive and troublesome growths can occur only in lakes which are amply supplied with $CO_2$ or with bicarbonates from which carbon dioxide necessary for photosynthesis can be extracted. Hence, hard water lakes support blooms, whereas soft water or acid lakes are spared.

3. Most of the adverse effects of algal blooms occur when one species dominates in a standing body of water. A population density involving one species is often followed by successions of other dominants in a repeating sequence.

4. The most drastic and often lethal effects of algal blooms appear in a curious distribution in both the Eastern and the Western Hemispheres.

5. The disturbing effects of algae are both directly and indirectly produced.

6. Effects are mostly related to algal physiology, but in some instances the simple morphology of algae is involved.

## ECOLOGICAL CONDITIONS

Some of these generalities can now be considered in limited detail to illustrate the interactions of those factors which are responsible for algal disturbances.

First to be considered are the conditions of the environment which are responsible for superabundant algal populations. Of all the nutritive elements and dissolved substances known to be used by algae, those which are most often controlling or critical are phosphorus and nitrogen. Phosphates and nitrates are essential nutrients for all chlorophyll-bearing organisms; but when these are present in unusual quantities, a lake can support tremendous populations of algae which recur year after year. Those who are familiar with the lakes in north mid-America know the relationship between polluted lakes and algal blooms. This relationship,

simply stated in the negative is: no phosphates and nitrates, no blooms; hence, no disturbances. We naturally inquire why, of all the dozens of species of algae in a lake, do only a few respond to a rich supply of nitrates and phosphates by explosive reproductions. From one Montana valley lake, for example, over one-hundred species of algae have been listed; yet only one, Alphanizomenon flos-aquae developed a bloom. It is difficult, if not impossible, to explain this "selectivity" partly because of the multiplicity of specific physiological requirements, the presence or absence of critical trace elements (so far undetermined), the inhibiting actions of antibiotics, and the presence of growth stimulators in the medium.

Most likely the response of some species by producing bloom populations, and no such response from others, is related in part to the high reproductive rate possessed by particular plants. For example, when conditions are favorable for both Dinobryon and Synura, they occur together especially in the early spring plankton of hard water lakes. But Dinobryon spp. quickly assume bloom proportions and vastly outnumber Synura because of the rapid, zoospore method of reproduction used by the former. The reproductive rate is usually paralleled by the speed with which nutrients are absorbed, as with Aphanizomenon, Microcystis and Anabaena among the Cyanophyta which develop superabundant populations in a short time. Also, this group of the algae possesses a more rapid photosynthetic rate than any other.

Among the blue-green algae there is at least one characteristic which explains why they develop especially well only in waters well-supplied with phosphates and nitrates. The protoplasm of most blue-green species is highly proteinaceous, more so even, than some animal protoplasms. Crude protein analyses (dry weight) show that Microcystis aeruginosa is 55.58 per cent protein; Anabaena flos-aquae is 60.56 per cent and Aphanizomenon flos-aquae is 62.8 per cent. Hence, their requirement for nitrates for the elaboration of proteins is much greater than that of green algae such as Spirogyra, for example, which is 23.82 per cent protein, or Cladophora 23.56 per cent. Nitrogen alone in Aphanizomenon amounts to 10.05 per cent (dry weight) as compared with 3.81 per cent in Spirogyra. Some other data give inferential evidence to support the thought that phytoplankton depends upon nitrogen for bloom production. Juday (1943) analyzed plankton of Waubesa Lake and found that it was composed of 49 per cent protein, 5 per cent fat, 6 per cent pentosans, 4 per cent crude fiber. The total annual plankton yield of this lake was 2592 pounds per acre, which incidentally supported 295 pounds of fish per acre.

It has been demonstrated in laboratory cultures and inferred from numerous water and plankton analyses that phosphorus is more critical than nitrogen in determining phytoplankton production. Phosphorus is also important in that it, in turn, facilitates the assimilation of nitrogen. In fertilization experiments where attempts are made to increase phytoplankton, it has been found desirable, or even necessary, to use a sulphate to fix iron and so prevent this element from taking up the phosphorus and thus lessening the well-known desirable effect of phosphate on plankton production. In respect to fertilizers, it is pertinent to mention here that such an operation frequently leads to disastrous upsets in a fish pond because superabundant growths of algae decay, the oxygen is depleted, and winter-kill results.

The work of Atkins illustrates the importance of phosphorus. He found that a pure culture of Nitzschia closterium developed a concentration of 300,000 organisms per cc. when Miquel's solution was used. All the P was consumed and it was determined that 1.12 mg. of $P_2O_5$ was used to produce $1 \times 10^9$ diatoms during the first phase of growth. One gram of P-pentoxide suffices for $9 \times 10^{11}$ diatoms. Krumholz (1954) found that Oscillatoria accumulated P 800,000 times that of the medium. These figures are derived from culture studies but it is reasonable to infer that they are applicable in a general way to situations in nature. Atkins (1923, 1925) found that in the sea one liter of water can produce 26.8 million diatoms for each 0.03 mg. of P consumed. Here, $P_2O_5$ -content at 37 mg. per cubic meter, the plankton removed all but 7.4 mg. per cubic meter. It is claimed that in the tropics where phytoplankton development is more uniform throughout the seasons the sea water is persistently low in $P_2O_5$ -content. Correspondingly, in the Arctic the $P_2O_5$ is high in winter and low in daylight periods, paralleling the action of plankton populations in taking up P. In the English Channel P and N are completely exhausted at the peak of phytoplankton production.

Rice (1953) and others have demonstrated the use and uptake of P by radioactive-P in laboratory cultures. Hasler (1958) has suggested that stratified lakes be given artificial circulation to bring phosphorus from the bottom layers into the photosynthetic zone so as to increase productivity.

The disappearance of nitrates and phosphates from standing water in summer and the increase again in winter is taken as evidence that these substances are taken up by phytoplankton. In fact, it appears clear that the exhaustion of $P_2O_4$ in a body of water by plankton uptake in late spring or in midsummer acts as a brake or increment and explains the plateau in population numbers. Therefore, it is only in those lakes which receive a continuous supply of phosphate that tremendous blooms of algae can appear and reappear throughout the entire 'growing' season. It has been noted that phytoplankton increases sharply after the decay of larger aquatic plants whereby substances are

returned in a soluble form to a lake.

It follows that lakes which have been enriched by various kinds of pollution from human habitats, run-off from agricultural lands, wastes from farm animals, etc. are the ones in which blooms appear. Accordingly, blue-green algae follow man about as he colonizes and pioneers, creating situations favorable for his worst aquatic pests. It is common knowledge that virgin lakes and lakes in unsettled areas rarely, if ever suffer from blooms and have no fish deaths caused by algae. Also, it is well-known that oligotrophic lakes are spared algal blooms since they are low in electrolytes, nitrates, and phosphates. Such lakes have a high $\frac{Na_2 - K_2O}{CaO - MgO}$ ratio (3.2) and support a desmid and planktonic chlorophycean flora together with such Pyrrhophyta as Peridinium Willei. Uncontaminated lakes, therefore, are not enriched, have a flora that is 62.4 per cent desmids and a diatom flora of 11.1 per cent; blooms do not develop. When the Na/Ca ratio is low (1.1) eutrophic conditions and a predominant blue-green flora exist. Two English lakes, for comparison, were found to have:

Lake Postherne - Nitrates .012;
Hardness 18.0
Chlorine 3.45;
Flora Myxophyceae

Lake Katrina - Nitrates .001;
Hardness 9.0
Chlorine .85;
Flora Desmids

Eutrophic lakes, on the other hand, by their chemical nature may be veritable garden spots for blue-green algae when phosphates and nitrates are plentifully supplied. Here at Pymatuning Lake, Tryon and Jackson (1952) have shown that blue-greens predominate in summer months, with 90 per cent of the plankton consisting of Microcystis, the remaining 10 per cent being made up of Chlorophyta and Pyrrhophyta. In their middle lake station $HCO_3$ varied from 35 to 20 ppm.; $CO_3$ from 15 to 25 ppm. This is the situation found, in general, in all basic, eutrophic lakes with a high pH.

It is purely conjectural, but at least worthy of mention, that trace-elements and/or growth stimulators are present in water where peak populations of a single species explode. In our ignorance it appears possible that there is an undetectable agent (or agents) present--some ingredient coupled with animal excretory matter, possibly $B_{12}$.

Numerous other studies and analyses, both marine and fresh-water, in the field and in the laboratory, have demonstrated the importance of N and P in plankton production. Reference might be made to numerous researches on this problem. Harvey (1940), for example, determined in laboratory studies that phytoplankton used 10.5 times more nitrogen than phosphorus, but of course this does not mean that a maximum amount of P is critical. Hasler and Einsele (1948) found that for every 1000 Kg of N in lake water, 20,000 Kg. of plankton material was possible but that P to the amount of 10 per cent of the available N was necessary before the N could be used.

As mentioned above, lakes well-supplied with bicarbonates provide a $CO_2$ reserve that makes blooms possible. The demand for carbon dioxide by such a mass of vegetation during photosynthetic hours is tremendous. Obviously $CO_2$ from respiration provides for some photosynthesis, but additional amounts from bicarbonates can support a mass of vegetation when, or if the dissolved $CO_2$ is reduced or depleted. Photosynthesis has been shown to be proportional to concentrations of bicarbonates. In distilled water, free, from carbonates, which has been saturated with $CO_2$ photosynthesis occurs but very little. Two- thousand six-hundred and fifty-four units of blue-green algae per cc. of lake water were found at the upper one-foot zone of a lake where carbonic acid was 1 ppm. Whereas, at the 23-foot depth where carbonic acid was 8 ppm., only 756 units per cc. were found. At 46 feet with carbonic acid at 16 ppm. there were only 120 units of algae per cc.

As Birge and Juday (1911) have pointed out, an ample supply of dissolved carbonates benefits aquatic plants in two ways: 1) by supplying $CO_2$ from the half-bound carbonates, and 2) in that mono-carbonates take on a greater amount of $CO_2$ from the atmosphere than would be absorbed otherwise. Further, they also take up and hold $CO_2$ of respiration in the water and thus retain the gas for subsequent use by the plants.

Plankton population in eutrophic lakes where electrolytes are abundant are characteristically greater than in oligotrophic where electrolytes and $CO_2$-content are low. For example, Rawson (1953) measured 10 to 40 Kg. of plankton per hectare in Canadian oligotrophic lakes as compared with 100 Kg. per hectare in eutrophic lakes. He refers to Lake Mendota (Wisconsin) in which Birge and Juday measured 177 Kg. of plankton per hectare.

An attending phenomenon in lakes which are well-supplied with calcium carbonate is the precipitation of lime on stems of submerged plants, stones, and other objects. When carbon dioxide is withdrawn by photosynthesis, mono-carbonates are formed. Lime may also be formed by oxidation of calcium bicarbonates. Hence, dense blooms of algae may lead to the formation of a thick layer of lime which directly or indirectly has a profound effect upon the limnology and the biology of an aquatic habitat. This layer becomes inhabited by lime-precipitating, benthic blue-green algae and the deposition is accordingly amplified. Marl weights down vegetation, thus forming bottom layers of dead and decaying vegetation and so

forms another link in

$$Ca(HCO_3)_2 + O_2 = H_2O + CaCO_3 + CO_2 + O_2$$

a complex chain of chemical-biological events.

A still unexplained aspect of algal blooms is the phenomenon of dominance and succession of species. (An arbitrary index of 33-1/3 per cent of the flora can be used to define dominance). When a bloom develops, invariably a single species is involved. Aphanizomenon flos-aquae, for example, is never in abundance, or scarcely present at all, when Microcystis aeruginosa is in peak production, and vice versa. Anabaena Lemmermannii completely takes over in some lakes early in the summer and after a week or two, disappears as Gloeotrichia echinulata assumes a bloom condition. Algal species, like other organisms have their own metabolic rate, time of development and maturation, and then have a decline. No doubt some of the sequences commonly observed of algal species in lakes are related to natural life histories. Also, dominance is related to specific rates of cell division. The fission method used by blue-green algae gives them an 'edge' over more slowly reproducing Chlorophyta.

But at the same time, there are incompatibilities between species which must be explained other than by differences in life histories. For example, two lakes within less than a hundred feet of one another may at the same time support in one a bloom of Microcystis, in the other a bloom of Anabaena. Or, as in the Yahara River system in Wisconsin, Lake Waubesa in the chain often has a purée of Aphanizomenon (as many as 16,000,000 filaments per L.), whereas Lake Kegonsa, about three miles below in the chain, supports a dense bloom of Microcystis. The masses of Aphanizomenon carried into the lower lake are completely eliminated, and one might say annihilated as determined by plankton sample counts from Lake Kegonsa.

Ideas are expanding in respect to the causes of and explanations for such incompatibilities and successions of species and dominance. Many points are difficult of clarification because of the highly refined chemical analyses that are called for. The best explanation of these phenomena is that there are extracellular by-products produced by one species which inhibit the growth of another, or of others. These by-products well may be classed as antibiotics and, indeed, there is ample evidence to indicate that they are. The sudden 'crash' of a dense population of a single species is due to its inability to tolerate its own growth-inhibiting substances. After a growth period when extracellular products have accumulated, it is thought that these act as deterrents and that the plant, in a sense, manufactures its own algacide. The extracellular substances (possibly of a toxic nature) have prevented the growth of selected or certain other species, and thereby the plant which begins its development first in a body of water quickly assumes a dominance (depending upon its inherent rate of cell division). With autodestruction comes a reduction in the inhibitor, thus permitting another species or group of species to develop. We say "selected" species because when one plant, such as Microcystis aeruginosa, for example, is in dominance, a combination of a few other phytoplankters, relatively sparse in numbers, occurs along with the bloom. The accompanying phytoplankters are mostly species in other phyla of algae, and the species which apparently are eliminated are members of the phylum to which the inhibiting alga belongs. Akehurst (1931) long ago pointed out the succession of oil-producing and carbohydrate-producing algae. The brown-pigmented algae undergo self-destruction by their own toxins and/or antibiotics, which in turn serve as stimulators to Chlorophyta. Although there is some variability from lake to lake, or region to region (as ecological factors vary), it has been noted that the same combination of associated species occurs and recurs along with the dominant. Species which are usually associated with Microcystis aeruginosa when this plant is in a bloom condition, are: Anabaena circinalis; Coelosphaerium Kuetzingianum; Stephanodiscus Niagarae; Asterionella gracillima; Tabellaria fenestrata; Ceratium hirundinella. With Aphanizomenon flos-aquae are usually found Anabaena Lemmermannii; Gomphosphaeria aponina; Lyngbya Birgei; Stephanosphaera Niagarae; Coscinodiscus spp.; Melosira granulata; Pediastrum spp.

As mentioned elsewhere, we do not know wherein antibiotics of algae differ from exotoxins, if indeed they are different. From the many studies which have been made, we know that extracellular substances occur in the dissolved organic matter of lakes. In Wisconsin, for example, dissolved organic substances vary from 2.9 to 39.6 ppm., whereas as much as 300 ppm. have been measured. Secreted nitrogenous and carbohydrate materials from algae have been detected (Bishop et al, 1954; Fogg, 1951). Some are liberated by autolysis (Aleyev, 1934). Complex proteins, peptides, leucine, aspartic acid, tyrosine, and many other substances have been found in the medium of the algae and in bottom deposits (Petersen et al, 1925; Wangersky, 1952). It is among these organic substances that we must search for specific antibiotics and toxins; growth stimulators and growth inhibitors, sometimes both, depending upon the concentration.

Laboratory experiments (Pratt, Akehurst, Proctor and others) have demonstrated incompatibility of two algal species and the inhibition of growth of one plant in the presence of another. Chlorellin from Chlorella vulgaris is a well-known

antibiotic to some other species of algae, and it has been found further (Pratt and Fong, 1942; Rice, 1954) that two species may be mutually antagonistic. Rice used varying concentrations of Nitzschia and Chlorella and by evaluating growth rates he determined that there were varying degrees of mutual inhibition. His findings, when applied to conditions in nature, could explain seasonal fluctuations. Sampaio (1946) discovered that Closterium acerosum produced an antibiotic to species of bacteria, especially Bacillus subtilis. Pandorine from Pandorina morum produces marked effects and abnormal shapes in Scenedesmus; whereas scenedesmine III inhibits completely the development of Pediastrum Boryanum. Not exactly pertinent, but of interest, is the fact that Elodea and Potamogeton foliosus in Wisconsin were found to produce antibiotics which inhibited populations of phytoplankton and rotifers.

Laboratory experiments by Proctor, and by Lefevre and his co-workers have clearly demonstrated antibiotic effects of algae. In Proctor's work (1957) two closely related species of green algae were employed--one inhibiting the growth of the other. His supposition is that antibiotics are located in unsaturated fatty acids liberated from the algae. The hypothesis is advanced that antibiotics interfere with oxidation of nutritive substances, and that they interfere with chlorophyll behavior.

Both Microcystis and Chlorella secrete substances active against Staphylococcus and Clostridium, and it is thought highly possible that many other species of algae have the same capacity.

Antibiotics may even have inhibitory effects on fish. In Europe it was found that the fish Gordonus rutilus was stunted in habitats dominated by Aphanizomenon.

If one allows a plankton collection dominated by Aphanizomenon to stand in the laboratory for a time, Ankistrodesmus, Pediastrum and Oocystis will flourish after the dominant has died. As long as the Aphanizomenon is alive, it completely inhibits development of the other plankters.

The idea has been advanced that by-products of one species, or the substances characteristically produced by one phylum of the algae, actually serve as stimulators to another species or to a group of species belonging to another phylum. Thus, the death and destruction of one protoplasmic mass frees substances that are veritable fertilizers for other algae. In the research on this facet, the surface film over a great sea of ignorance has been punctured only in a few places. Most of the studies on stimulators has been done with marine organisms and their ecology, but some investigations have been carried out using fresh-water algae. In any case, results and implications are applicable to fresh-water algae and their ecology, and to the determination of blooms.

The present state of our knowledge concerning growth stimulators has been derived largely from culture studies which, because of their artificiality, introduce an element of questionability when laboratory-derived concepts are applied to situations in nature. Possibly Pringsheim (1921) was the first to recognize and to publish on growth stimulators in the form of acetate-peptone and acetate-ammonium present in his soil-extract cultures of Polytoma spp. and other unicellular forms.

Since then Hutner and his co-workers including Provasoli, Lwoff and co-workers, Lewin, Starr and others have determined the existence of growth stimulators and have used microorganisms for their assay. These workers have found that $B_{12}$ is an essential requirement, especially for Dinoflagellata. In fact, the seeming universality of the demand for $B_{12}$ by algae has led Provasoli to postulate that this factor alone might be a determiner of algal blooms. Inferential but strong evidence for this is to be found in the appearance of marine blooms in coastal waters periodically following rainy seasons and floods which wash large amounts of $B_{12}$ (and other growth promoters) into the sea. Thus the death-dealing red tide may be related to $B_{12}$ increment because these blooms seem to follow periods of flooding.

Measurements have shown that $B_{12}$ occurs in marine waters such as Vinyard Sound (0.03 to 2.0 µg/L), near Nova Scotia (0.01 µg/L), at Millport Marine Station (0.005 to 0.01 µg/L). These amounts are apparently normally present (and well they should be if they are as critical as observations indicate; otherwise there would be no coastal phytoplankton). Robbins, Hervey and Stebbins discovered that in one fresh-water habitat the $B_{12}$ varied greatly seasonally from 0.1 to 2.0 µg/L. Hence in lakes receiving pollution or where $B_{12}$ analogues can be formed by bacteria, it is possible for blooms to develop. This would be especially true for species whose requirements for growth promoters are not highly selective. They would have, therefore, an advantage over competing organisms, the requirements for which are highly specialized.

It has been shown that there is a specificity for various analogues of $B_{12}$ which are produced by bacteria. Thus the activity of these microorganisms is caught up in the complex cycle of exchange of metabolites. Provasoli, for example, reports Amphora spp., Skeletonema sp. (diatoms), and Phormidium (a blue-green genus) to use all cobalomins, including factor B (one of the fractions of $B_{12}$). Two dinoflagellates were found to require factors A, H, $B_{12}$, artificial $B_{12}$ and $B_{12}$-III, but were not responsive to pseudovitamin $B_{12}$.

What such specificity means in respect to our present consideration is that the appearance and disappearance of algal populations and bloom-producers are related to growth promoters and vitamins. Thus, both the regular sequence of peak populations of different groups of algae, and also

the erratic and sudden appearances of blooms might be determined mainly by $B_{12}$ -content.

Doubtless very many instances of disturbances by algae have not been reported in the literature; so, we are ignorant of a true pattern of their distribution over the earth. But it is interesting and somewhat enigmatic that those cases which have been reported do form a pattern. In North America, especially, disturbances involving the death of animals are localized in the midpart of the continent. Almost all instances of cattle deaths have been reported from Michigan, Wisconsin, Iowa, Minnesota, the Dakotas, and south-central Canada. Further, it is from this general region that reports are recurrent. There is another area in Australia where lethal blooms of algae develop, and one in south central Africa. The author is aware of but one report of lethal blooms in Europe (Finland) and none from Asia or South America. Algal blooms occur in many parts of the world, of course, but it is the distribution of toxic effects which invites curiosity. Whatever the distribution of bloom-producing algae is, we can state, rather tritely to be sure, that waters in scattered parts of the earth have critical ecological factors in common: (high nitrate- and phosphate-content, an abundance of $CO_2$, a high pH and suitable temperatures).

It has been mentioned that disturbances attributable to algae are both directly and indirectly produced. These effects will be discussed later. By way of explanation, it may be mentioned here that direct effects are those arising from substances given off by certain objectionable algae, or through their physiology wherein nutrients necessary for the growth of less offensive forms are taken up; clogging of fish gills because of their concentration in shallow water; producing disagreeable tastes and odors in drinking water, etc. Indirect effects arise as a result of a chain reaction in modification of ecological conditions leading to oxygen depletion, formation of ptomaines as a result of bacterial decomposition, forming poisons in shellfish eaten by man, birds, etc.

Whereas most of the disturbances of algae are related directly to their physiology, it may be noted in the instances of cyanophycean trouble-makers especially, that their effects are related to morphology and to their habits. For example, the sticky sheath possessed by blue-green algae makes it possible for plants to adhere to one another, thus forming dense mats. Many blue-green algae, especially the trouble-makers, possess gas vacuoles (pseudo-vacuoles) which permit or force the plants to float high in the water. Because of their tremendous numbers, the mat is actually elevated above the water level. Thus, in intense illumination and in warm water layers, where oxygen is low in any case, a decaying blanket forms in which oxygen-consuming bacteria thrive. If the species involved were evenly distributed through the water, some of their objectionable direct and indirect effects would not occur. Species of Lyngbya, for example, are sometimes extremely abundant, as are some members of the Volvocales. But these forms do not have the mechanisms for producing sticky scums and for causing serious disturbances.

One instance of an apparent toxic effect has been noted in a subalpine lake just outside Glacier National Park. This is an unusual condition because all of the lakes in the area are relatively soft and are relatively unproductive of plankton. This particular body of water, about five acres in area, has received during the recent past drainage from a small farm and barnyard; sufficient drainage apparently to raise the nitrogen and phosphorus content so that a bloom of Aphanizomenon flos-aquae is supported. Each year that this bloom develops, mud-puppies (Necturus) die by the scores and those which are not dead are sluggish and roll about on the bottom.

Deaths of farm animals have been reported from Iowa, Minnesota, North and South Dakota, Wisconsin, Montana, Bermuda, and elsewhere (as mentioned previously). The greatest economic loss of cattle has been in central South Africa. Here in the shallow pans and reservoirs in the cattle-grazing country, one species of blue-green algae, Microcystis toxica dominates the phytoplankton, producing 'soupy' blooms. Cattle drinking these waters have died by the thousands. Symptoms include convulsions (similar to strychnine poisoning), paralysis, jaundice, constipation, loss of apetite, falling off in milk production, abortion, and skin sensitization. Cases can be acute, with death occuring in a few hours after animals have drunk from Microcystis-infested water, or chronic, wherein death does not occur until after some weeks or months. It is thought that the toxin contains two poisons, one of which is found in phycocyanin. This poison, after damaging the liver, is carried by the blood stream to the surface of the body where, being stimulated by ultra-violet light, it produces tenderness, dryness of the skin, and lesions. The other toxin is said to be a liver-neural type or a fucoin.

Also from Africa, a serious disease of sheep is reported which has now been referred to a photosensitizing toxin. Stewart et al (1950) found that cattle, dead from blue-green algal poisoning in Canada, had experienced lung hemorrhages and liver lesions. As reported by Olson (1951), serious outbreaks of animal deaths occurred in 1948 on Round and Fox Lakes in Minnesota where horses, cattle, hogs, and dogs were killed by drinking water infested with Microcystis aeruginosa (flos-aquae?) and Anabaena Lemmermannii.

Poisoning is not confined to mammals, however, for from several localities throughout central North America come authentic reports of deaths among water fowl, pigeons, and even song birds.

Botulina poisoning has been definitely excluded as a possible cause of these bird deaths. It seems highly possible that many instances of water fowl deaths in the past were not caused by botulina as claimed. In one year 7000 Franklin gulls and 60 mallard ducks were killed by Anabaena flos-aquae in Iowa.

In addition to these animal deaths, there are many instances on record of the killing of fish by algal toxins. Carl (1937) reported fish deaths in British Columbia from Anabaena flos-aquae. Prymnesium parvum secretes a toxin which kills gill-breathing organisms. Also, it has been found (Ryther, 1954; Lucas, 1936) that extracellular substances have a profound effect on the biology and feeding habits of microorganisms. Whereas these cannot be construed as highly detrimental, they do constitute examples of disturbances that may have far-reaching and ramifying effects on aquatic biota.

Mention should be made here of the critical studies of Abbott and Ballantine (1957) on the toxins of the dinoflagellate, Gymnodinium veneficum which plays a role in the wholesale death of fish. Material obtained from laboratory cultures of the flagellate was found to be toxic to the nervous system of animals by depolarizing nerve and muscle membranes.

Although in need of further study and confirmation, evidence indicates that fish are killed in Iowa lakes by toxins and scanty but strong inferential evidence is at hand to support the belief that epidemics of human intestinal disturbances are caused by them. (See Spencer, 1930; Tisdale, 1931). That there are not more cases of this sort of disturbances is related to the simple fact that domestic drinking water is seldom allowed to develop concentrated algal blooms. Persons would naturally refrain from drinking water directly from ponds which are obnoxiously overgrown with algae, especially blue-green species which produce disagreeable tastes and odors.

That toxins actually exist in extracellular excretions has been demonstrated by both laboratory and clinical tests. Fitch, et al (1934) are the first to have investigated waters after an epidemic of cattle deaths. The toxins with which they worked were produced by Aphanizomenon, Anabaena and Microcystis in Minnesota. They fed animals in the laboratory with algal material taken fresh from the lake and also administered injections. The animals died within a matter of minutes in some instances, or after 12 hours in others.

Mason and Wheeler (1942) also found that small animals were killed within three hours after being injected with Microcystis medium. Veterinarians in Iowa injected 10-15 cc. of a bacteria-free filtrate from Anabaena flos-aquae into guinea pigs which lived but 12 minutes. Only 0.04 cc. injected into a mouse caused its death in four minutes. They also found that after one year the filtrate was ineffective. Olson (1951) points out that only 0.02 cc. of raw algal material injected into a 20-gram mouse produced death in one hour. More time is required for death to occur when laboratory animals are given algal material orally. An interperitoneal injection of 10 cc. killed a chicken in 20 minutes, whereas 50 cc. fed to a chicken produced death one and one-half hours later according to Olson (l. c.).

Shelubsky (1951-b) obtained toxin from Microcystis by drying the plants and by precipitating it with phosaphotungstic acid at a pH of 2.0. Also, he demonstrated a toxin to be present in living cells experimentally and that it was not an antigen. Living Microcystis injected in several small animals, including carp, produced death.

The deaths mentioned above are supposedly caused by exotoxins present in the water in which algae have been living (in a bloom condition). These exotoxins have not been isolated. This is understandable since extracellular substances in quantities sufficient for analyses would have to be separated from the other substances in solution within the medium. Can they be filtered, or precipitated, or distilled? It is known that the exotoxins are alkali-labile, which indicates a possibility that a technique can be developed that will permit chemical analysis. Laboratory tests using water and/or algal material have determined that the toxins of Microcystis and Anabaena occur in the medium of healthy cells; that they are non-filterable; that like endotoxins, they are non-ionic, are not alkaloid, nor albuminoid, are non-volatile and are heat stable. They have a low molecular weight and may be soluble in alcohol. Exotoxins are not destroyed by air-drying, by freezing or by ultra-violet radiation. Louw (1950) claims to have detected two alkaloids from Microcystis, however. One of these has a suspected formula of $C_{10}H_{19}NO_2$. The other is a picrate and is the one that acts as a hepatotoxin.

Molecules of chlorellin are smaller than 15 Å in diameter. The exotoxin effects of Microcystis have been shown by Ashworth and Mason (1946) to be similar to the symptoms of poisoning by Amanita. From a marine alga a toxin has been identified as a form of polysaccharide.

Some workers have found that decayed blue-green algae are toxic, a fact which might be explained by the presence of endotoxins which have been released. Thus, it is clear that either living or in a decomposed state algal substances are toxic. Louw (1950), for example, found that filtrations of decayed algae were toxic, whereas fresh or living plants were non-poisonous to laboratory animals. It has been found also that the 'maturity' of the alga involved is related to variations in the strength of the toxin. Just what this age-time factor is, has not been determined, but

it is known that laboratory cultures several days old are not so potent as fresh material or young cultures.

Bishop, Anet and Gorham (1959) have succeeded in extracting an endotoxin from Microcystis aeruginosa (flos-aquae ?). The toxin was not isolated, but a syrup-like dialyzate was obtained and many properties were determined. It was found that the toxin is a non-volatile peptide, that it behaves as a non-ionic substance in electrophoresis in a pH of 2.2 but is negatively ionic at a pH above neutral. Five fractions were found to be present and only one (peptide No. 2) was proven toxic to laboratory animals. This component also included leucine and one other amino acid residue.

In addition to their lethal effects, some algae produce other types of pathological conditions, in human beings for example. Mention was made of intestinal disorders, epidemic in nature, as reported by Spencer and Tisdale (l.c.). Further, it seems clear that allergies, asthmatic conditions, and epidermal irritations result from algal toxins. Many persons experience skin irritations and stinging rashes after bathing in algal-infested waters. These symptoms are sometimes ascribed to cercariae and accordingly inflamations are regarded as the well-known swimmer's itch. Phycocyanin from Anabaena is one particular irritant. Gloeotrichia will cause similar irritation when it is in bloom condition.

Mention was made of the indirect effects produced by dense algal populations. Examples are to be found in fish deaths and in human poisonings from eating fish and shellfish. Because blue-green algae especially are high in proteins, when they decompose it follows that proteinaceous by-products are formed, some of which are poisonous. It has been demonstrated that when algal blooms are dense in shallow water, the decomposition products may be concentrated enough to kill fish. Large scale deaths of fish have been noted in East Okoboji Lake, Iowa during periods of dense Aphanizomenon blooms. Examination of the fish (by specialists) indicated that they had not died from suffocation nor from parasitism. Hence, poisoning was suspected. A large mass of algae was collected and allowed to decay in vats. A variety of fish, freshly seined from a nearby lake, were placed in two large concrete tanks and the D. O. adjusted and maintained at a safe level by introducing oxygen from oxygen tanks. Then the decayed algae were poured into the aquaria, and the behavior of the fish observed. After behaving erratic for a time the fish all died within a few hours. Chemical analyses of the decayed algae showed the presence of hydroxylamine in quantities theoretically sufficient to be lethal, and also 8.5 ppm of $H_2S$. A similar experiment to confirm these observations, and with similar results, was conducted by constructing lakeside ponds in which fish and decaying algae were placed. Adequate D. O. was maintained by allowing fresh lake water to flow into the ponds periodically.

Mackenthum et al (1945) likewise experimented by placing fish in aquaria with lake water in which Aphanizomenon had occurred and in which a decaying plant mass had developed. Perch and crappies were all dead at the end of a 34-hour period although the oxygen was maintained at 8.3 ppm.

It cannot pass unnoticed that disastrous effects of algal blooms are caused by those species in which pseudovacuoles occur. I suggest that there is more than a casual relationship and that research may show that pathological conditions are caused by substances contained in these vacuoles, possibly a gas. I have never read a discussion of this and apparently pseudo-vacuoles have never been investigated in this connection.

Another type of indirect effect from algal populations is experienced when human beings and other animals eat fish or shellfish that have fed upon phytoplankton in which the Pyrrhophyta are abundant (Gonyaulax, Ceratium, Prorocentrum). Shellfish, especially, store toxins in their digestive tract and liver in quantities sufficient to produce death, or at least to cause serious illness in animals who partake. The toxin appears to have no effect however, on the fish or shellfish.

Puffers (Tetraodon) store tetraodontoxin which forms a white, water-soluble powder after extraction. It has a suspected formula of $C_{16}H_{31}NO_{16}$. Like other exotoxins, referred to above, this one is non-alkaloid. It has produced death in 60 per cent of the reported cases of poisoning. Persons who eat moray eel (Gymnothorax) are poisoned but only 10 per cent of the cases are fatal. It is claimed that more than 400 Japanese soldiers died from eating fish in Micronesia, attributable to concentrations of Pyrrhophyta toxins.

Another indirect effect chargeable to algae is that of fish-kills by oxygen depletion. In such instances algae supply the basic food for bacteria which actively oxidize masses of dead plants. Dissolved $O_2$ is often reduced to zero (or to an amount too low to be read by usual oxygen tests). As the oxygen decreases below the threshold necessary for them, various groups of organisms are suffocated. In Iowa lakes the sequence of deaths in a necritic cycle appears to be first green algae and Protozoa; yellow-green algae and microcrustaceans; certain species of fish, including carp and sheepshead; then other and all species of fish; finally chironimid larvae and bottom organisms. In one observed climax in an Iowa lake the oxygen dropped from 4.5 ppm. at midday to zero the following morning at 1:00 a.m. Later the same day not a single living organism of any kind could be found in this lake. The lake shore was bordered by a 25-foot zone of carcasses, and the water was a

purée of microcrustacea, chironimids, and decaying algal masses. A few days later the dead fish floated to the surface and were washed ashore, forming a veritable "windrow" along several miles of lake front. Such events often occur, unfortunately when water temperatures are high (22 to 30 degrees C.) and when the oxygen-content is accordingly low. Rarely, blooms of algae, diatoms, and planktonic blue-greens decay under winter ice. This leads indirectly to fish deaths. Such events not infrequently follow fertilization experiments (as previously mentioned) due to oxidation of the surplus organic matter, as well as to the breakdown of the dense population of algae which has accrued as a result of the fertilization practice. A thick blanket of snow over winter ice decreases illumination so that a growth of winter-time plankton 'crashes' and decays.

Whereas blue-green algae are accountable for toxic effects, other disturbances are caused mostly by those organisms in which yellow and brown pigments predominate and in which oil and leucosin accumulate as food reserve. These are representatives of the Chrysophyta and the Pyrrhophyta, especially the former. Two classes of the Chrysophyta, the Chrysophyceae and the Bacillariophyceae contain several species which are infamous pests in aquatic habitats. Dinobryon spp. and Synura uvella become abundant enough to produce obnoxious odors and fishy tastes. These genera are often most abundant early in spring when silicon- and calcium-content are low, these elements having been exhausted by winter pulses of diatoms.

In suitable habitats (eutrophic lakes, pH 7.2-8.5) diatoms often develop dense blooms or form thick encrustments on submerged surfaces (Asterionella, Melosira, Fragilaria, Tabellaria). The 'fishy' odor of water is caused not by fish but by diatoms which either alive or dead, but especially after a peak development, release large quantities of oil into the water.

Chrysophyceae and the diatoms are physiologically suited to development in cold water and in weak illumination. As classes they often develop peak populations in the winter, or in early spring, following a fall dominance of blue-green species. For example, diatoms composed 100 per cent of the plankton during winter and spring months in Cleveland harbor of Lake Erie. Accordingly disagreeable tastes in domestic water supplies may appear during winter as well as in summer.

The Pyrrhophyta that cause trouble are mostly marine and are not of direct concern here. As mentioned previously, Noctiluca, Gymnodinium spp. and Gonyaulax are responsible for fish deaths (red tide) along shores. The former is reported to be especially troublesome in the eastern Pacific (East Indies). In fresh-waters only Ceratium hirundinella appears to form blooms sufficiently dense to cause objectionable tastes in drinking water. This species, although it does not form scums, may color water a distinct gray-brown with as many as 13,000,000 organisms per liter. To my knowledge, Ceratium hirundinella has never brought about fish deaths.

Another incidental way in which diatoms cause disturbances is by clogging sand filters in city water systems which use impounded water. This calls for additional work and expense for sanitary engineers. Diatoms are successful in this because of their persistent silicious walls and because of their large numbers which produce a dense film over a filter bed.

Some chlorophycean algae cause minor disturbances. Unlike the blue-green algae and the diatoms, they have a slower reproductive rate and seldom form superabundant growths. The product of photosynthesis is starch and not ill-smelling fats and oils. They have no pseudovacuoles and do not form surface scums. Even the Volvocales which frequently form conspicuous blooms cause no particular disturbance. They are excellent oxygenators and remain evenly distributed throughout the medium.

Such coarse filamentous forms as Rhizoclonium and Hydrodictyon, however, develop thick mat mats which do cause some minor troubles. Boating, fishing and vacation sites are spoiled by these and other species. The weight of filamentous blankets causes larger aquatic plants to sink and to undergo decay with attendant objectionable effects.

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# ALGAE AND METABOLITES OF NATURAL WATERS

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Increased attention has been given in recent years by investigators working in various aspects of algal ecology to the quantity, chemical nature, and source of the organic constituents of natural waters. This study of a long-ignored portion of the aquatic environment has resulted from a growing awareness of the role of organic substances in heterotrophic algal nutrition and from an appreciation of the possible effect of these substances in influencing the size and species composition of algal communities by stimulating or inhibiting the growth of particular species. Two important and comprehensive reviews have recently appeared on the subject of organic matter in natural waters. Vallentyne (1957) has brought together the available information on the organic compounds isolated or identified from water, seston, and sediments of lakes and oceans. Saunders (1957) has reviewed studies on the interrelationships between dissolved organic matter and phytoplankton pointing out four functions which dissolved organic substances may have for algae: (1) direct nutritional value, (2) source of accessory growth factors, (3) toxins or growth inhibitors, and (4) chelation of trace minerals.

In the present paper it is my intent to review primarily the evidence in support of the existence of organic metabolites produced by algae, to examine the properties of these substances, and to consider the possible role of such substances as inhibitors, stimulators, or regulators of growth in natural algal populations. The role of organic metabolites in direct nutrition of the algae and the production of substances toxic to fish and other vertebrates will not be discussed since these phrases have been or will be treated by other papers in this symposium.

Biological literature is replete with attempts to relate fluctuations in algal populations to variations in chemical constituents of the water or to physical factors of the environment. The problem is most difficult, and the answers least satisfactory, when attempts are made to explain the rapid development of bloom-producing species and the apparent decline of other species under environmental conditions which appear to be equally satisfactory for each organism. Hutchinson (1941, 1944) in discussing these problems some years ago pointed out that a clear-cut correlation between chemical conditions and the qualitative composition of the phytoplankton is not to be expected. He suggested the possible importance of accessory organic substances in development of specific populations.

As early as 1931 it was suggested by Akehurst (1931) that toxins produced by algae were responsible for fluctuations in phytoplankton populations. He theorized that changes in the rate of development of certain algal genera were in response to excreted toxins which could serve as "accessory food" and could inhibit or stimulate growth. Phytoplankton was divided into two groups, "starch" and "oil", according to the chemical nature of their food reserves. The autotoxins produced by the oil group were postulated as an accessory food of the starch group. It was thus possible to account by this theory for many successional patterns shown by algal communities.

This proposal was advanced with essentially no direct evidence for the production or existence of such toxic substances and was based almost entirely on observations of the sequential changes in phytoplankton populations over the seasons. Data from many investigators covering a variety of geographical locations were gleaned from the literature and analyzed in support of his proposal.

Direct evidence for the existence of such extracellular growth modifiers in natural waters has been slow to accumulate. Even now, most of the experimental data confirming the production of extracellular substances are based on results from investigations of algae in laboratory cultures. Physiologically active organic substances from algae may originate in nature in either of two ways: (1) by extracellular excretion from living algal cells or (2) from the decomposition of the remains of dead cells. While it may be possible to demonstrate the existence of such active substances in nature, it is almost impossible to determine the specific source of the active materials. Furthermore, even though many intensive studies have been carried out in the laboratory with certain algal species, little has been accomplished in determining the actual chemical composition of the active substances or in understanding the nature of the physiological response of algae to these substances.

## SOLUBLE EXTRACELLULAR SUBSTANCES

The excretion of soluble, extracellular substances by algae has received the attention of a number of investigators. Studies have been concerned with algae from various taxonomic groups, with the chemical nature of the excreted products, and with the environmental and internal conditions influencing excretion. Myers (1951) after reviewing the work of Krogh, Lange and Smith (1930) on Scenedesmus and of Myers and Johnston (1949) and Spoehr and Milner (1949) on Chlorella concluded

that "conservation of carbon and a minimal level of excretion is probably generally characteristic of the green algae". Fogg (1953), on the other hand, notes that the amount of extracellular carbon produced by species of green algae may be relatively high, with quantities as high as 12.5 per cent being given off by old cultures.

Studies of motile green algae, particularly species of Chlamydomonas, indicate that production of soluble organic materials in the media by these organisms is quite extensive. Lewin (1956) using cultures of eighteen species isolated mostly from soil samples, showed that all species tested liberated some polysaccharides into the growth medium. Chlamydomonas mexicana released as much as 0.1 gram per liter, equivalent to 25 per cent of the total organic matter produced by the cells of this organism. Allen (1956) similarly demonstrated excretion of oxidizable organic matter by six species of Chlamydomonas. Organic acids as well as polysaccharides were identified in the media. It was apparent from the results of the studies on Chlamydomonas that the appearance of organic substances in the media did not result primarily from decomposition of dead cells. Increased quantities of extracellular organic material in the media has been shown to parallel growth of the algae.

In mucilaginous species it is obvious that much, perhaps most, of the soluble polysaccharide arises from disintegration of the external portion of the enveloping sheath. Lewin (1956) states "a clear distinction cannot be readily drawn between a soluble liberated polysaccharide and diffluent sheath, between the latter and a firm capsule, or between a capsule and the cell wall itself".

The production of extracellular nitrogenous materials in the growth medium is characteristic of many blue-green species, for example: Nostoc spp. (Henrikson, 1951); Tolypothrix tenuis, Calothrix brevissima, Anabaenopsis sp., and Nostoc sp. (Watanabe, 1951) Anabaena variablis, A. gelatinosa (De, 1939); and A. cylindrica (Fogg, 1942). Filtrates from healthy cultures of these algae were shown to contain up to 50 per cent of the total nitrogen fixed by these organisms. These soluble nitrogenous compounds appear to be present largely as polypeptides, but amides (Fogg, 1952) and amino acids (Watanabe, 1951) have also been identified in the filtrates. Phormidium uncinatum has also been shown by Lefevre and Nisbet (1948) to produce quantities of soluble organic material in the medium (as determined by oxidation with $KMnO_4$), increasing in 40 days from 4.8 mg/l to 30.4 mg/l.

It has been suggested (Fogg, 1953; Fogg and Westlake, 1955) that extracellular nitrogenous compounds are liberated by most algae. Further evidence in support of this was supplied by Fogg and Boalch (1958) who showed that the brown algae (Ectocarpus confervoides) growing in a bacteria-free culture liberated sizeable amounts of nitrogenous material to the media. The ecological significance of the presence of extracellular nitrogenous compounds in natural waters, has been discussed by Fogg and Westlake (1955) and Fogg (1956) who have emphasized the possible role of excreted polypeptides in forming complexes with sparingly soluble salts and with toxic ions. The availability of the nitrogenous materials to organisms unable to fix nitrogen may also be of great ecological importance. It has been pointed out, however, that not only is Anabaena in bacteria-free culture unable to make use of its own excretion products, but that Chlorella sp. and Oscillatoria sp. are likewise unable to use these materials as sources of nitrogen.

Of ecological importance, at present not fully evaluated in the case of algae, is the production of extracellular substances capable of influencing growth of the producing organism itself or of associated species. Harder (1917) has been credited (Jørgensen, 1956) with the first report of the production of growth-inhibiting substances, in this case as autoinhibitor produced by Nostoc punctiforme. The classical work of Pratt and Fong (1940) established firm evidence for the production of an autoinhibitor by Chlorella vulgaris. Subsequent investigations by Pratt and his co-workers (Pratt 1942, 1943; Pratt et al 1944, 1944a, 1945, 1948) and by various other investigators over nearly a score of years has resulted in the accumulation of a great deal of information about this species. The inhibiting material, termed "Chlorellin" by Pratt has not been isolated nor chemically defined although it has been concluded that the active agent is probably an organic base.

Evidence for the production of extracellular inhibitory or stimulatory substances by algae has come largely from laboratory studies carried out in either of two ways: (1) by growing certain test species in cell-free media (filtrates) in which an alga had previously been grown or (2) by growing two species in mixed culture using a media which would permit satisfactory growth of either organism when grown alone.

The use of filtrates permits following the production of active substances throughout different growth phases of the culture. Mixed-culture techniques, while providing perhaps a closer approach to the natural situation than procedures using culture filtrates, produce no concrete data on the rate of production of active metabolites nor do they permit recognition and evaluation of responses resulting from interactions of the mixed strains in culture. Difficulties may arise, for example, when two organisms have different inherent growth rates under a given set of conditions. Active growth and rapid photosynthesis of one species may bring about the pH changes in the media which

can alter subsequent development of the other species. Such effects are difficult to separate from those resulting directly from the presence of metabolites. Various modifications of these basic procedures have been made, including the technique used by McVeigh and Brown (1954) in which two species were grown together in a flask but kept separate by a dialyzing membrane.

The effect most frequently observed in culture studies has been the modification of the rate of cell division which may result in reduction or, more rarely, in stimulation of growth of the population. Morphological modifications, particularly changes in cell size, have been noted along with increased accumulation of storage products within the cell. There appears to be such variability in the nature of the physiological action induced, and in the degree of response shown by various species belonging to the same taxonomic group that is often difficult to generalize from reported results. It is true of course that some of the variability reflects differences in experimental procedures. Diversity in culture media used, variations in temperature and light conditions under which the organisms were grown, age of the culture at the time of extraction of active substances, methods of extraction or concentration of the active materials, and physiological conditions of the test inoculum, all may influence the results and make comparison of the findings of several workers very difficult, even when the same algal species are being considered.

In an attempt to examine the results obtained with a number of different algal organisms in laboratory culture, observations reported by a number of different workers have been tabulated in Table I. (Table at end of paper) The most commonly observed effect of algal metabolites has been their influence on the rate of multiplication of cells. Relatively few authors (Pratt, 1942; Jørgensen, 1956; Lefevre and Jakob, 1949; McVeigh and Brown, 1954; Lefevre and Nisbet, 1948; Lefevre, Jakob, and Nisbet, 1952) have reported an increase in the rate of this process, most attention having been given to those interactions in which a reduction in the rate of cell division appeared. Reports of induced morphological changes have likewise been relatively few.

A certain amount of ambiguity exists in the terms used to describe the reactions of algae to the physiologically active substances. Lefevre and co-workers plainly distinguish in most cases between "toxic" substances which bring about the death of affected cells and "algostatic" substances which retard cell division but do not cause death of the organism. Cells affected by "algostatic" substances resume multiplication if transferred to fresh media. Other workers are not always so definite in their descriptive terminology and it is not always clear whether cells were actually killed or merely prevented from undergoing cell division.

It is apparent from Table I that the algal species which have been demonstrated to be producers of inhibitory or stimulatory substances represent a relatively few taxonomic groups. The division Chlorophyta is represented by several genera in the orders Chlorococcales, Volvocales, and Zygnematales. Chrysophyta is represented by diatom genera of the order Pennales and the Cyanophyta by various genera in Oscillatoriales and Chroococcales. The nature of the problem of studying growth modifying substances has demanded eliminating any possible effects of other organisms from the observed reactions. For this reason most of the studies have been carried out with bacterially-free algal cultures. The list of active organisms, therefore, reflects largely those species which may be easily cultivated in pure culture.

A few investigators (Lefevre, et al, 1950, 1951; Rice, 1954; Johnston, 1955; Proctor, 1957) have used natural waters containing high populations of particular species in their studies and have thus extended the laboratory observations to field conditions. Results from studies of this type carried out in fresh-water are grouped in Table II. (Table at end of paper)

To generalize as to the mode of action or the nature of the physiological processes concerned in the observed response is difficult from present data. The effect produced by one filtrate, for example, may vary from one test species to another. It may produce stimulating effects on some species, inhibitory effects on others, and have no observable effects on still others (Lefevre and Jakob, 1949; Jørgensen, 1956). The composition of the media may affect the interaction between species in mixed culture as shown by McVeigh and Brown (1954).

It is the opinion of many investigators (Denffer, 1948; Lefevre, and Nisbet, 1948; Lefevre, Jakob, and Nisbet, 1952) that inhibition of growth induced by algal metabolites results from interference with the cell division process and not, in most cases, with nutritional processes. The few morphological or cytological modifications which have been reported are in many cases apparently the result of the accumulation of abnormal amounts of storage products within cells which failed to divide.

## NATURE OF THE INHIBITING SUBSTANCES

It seems extremely unlikely that the variety of effects attributed by various investigators to extracellular algal metabolites are the result of a single chemical substance. Such widely differing, specific physiological effects as interference with the dark reaction in photosynthesis (Pratt, 1943), reduction of oxygen-consumption of bacteria

(Steeman Nielson, 1955, 1955a), blockage of mitosis (Denffer, 1948), interference with the filtering action of Daphnia (Ryther, 1954), as well as such ambiguous and all inclusive terms as "stimulation" and "inhibition of growth", have all been laid to algal metabolites.

A growth-modifying substance may, of course, be stimulating to a specific reaction in low concentrations and inhibitory in higher concentrations. The action of auxins is illustrative of this effect. Bentley (1958) has demonstrated that auxin-like substances are produced by a variety of algae and that these substances are excreted into the media. While 3-indoleacetic acid was shown to have a stimulating effect on algal growth, Bentley concludes that it is not the major natural auxin produced by algae. It should be noted that in the extraction and study of these materials the petroleum ether soluble fraction was extracted from the algae or the media and discarded in order to forstall any inhibitory effects by fatty substances. This latter group is perhaps most suspect as the agents active in inhibitory or antibiotic reactions among the algae. The work of Spoehr, et al (1949), for example, indicated that the antibacterial properties of chlorellin resulted largely from photooxidizable unsaturated fatty acids. Proctor (1957a) has shown that the inhibition of Haematococcus pluvialis by Chlamydomonas reinhardi resulted from fat-like extracellular substances. He suggested that the inhibitor produced by Chlamydomonas was probably a long-chain fatty acid or a mixture of such acids. Eight long-chain fatty acids were tested against six algae in culture, all of them showing varying degrees of inhibition. Considerable differences in sensitivity could be observed with Haematococcus being most sensitive, among the algae tested, and with Chlorella vulgaris and Scenedesmus quadricauda the least. Proctor is of the opinion that the release of the toxic substance is dependent largely on the death of the cell. Although there has not been sufficient evidence to establish this with other species, it is obvious that age of the culture is important in determining the activity of a filtrate. Phormidium uncinatum, for example, was found by Lefevre and Jakob (1949) to be stimulated by a filtrate from a young culture of Scenedesmus quadricauda but to be inhibited by a filtrate from an old culture. This does not appear to be a consistent reaction, however, since the filtrate of the young culture of this species brought about complete inhibition of Scenedesmus oahuensis. Proctor (1957a) has observed stimulatory effects of filtrates from young cultures of Chlamydomonas reinhardi and inhibitory effects from filtrates of older cultures when tested against Haematococcus pluvialis. This early stimulation, he feels, results from a conditioning of the medium, presumably by removal of heavy metals.

In the greater number of cases studied (Table I), strongest inhibition has been obtained in filtrates from old cultures. In only a few cases, however, has the production of inhibitor been followed through the growth period of a culture by quantitative procedures. In studies on Chlorella vulgaris, Pratt, Oneto, and Pratt (1945) have shown that almost as much of the inhibiting substance, chlorellin, could be extracted from two day old cultures as could be extracted from cultures that had attained their full growth. During the period of rapid growth, however, the chlorellin content decreased rapidly, increasing again as the rate of growth of the colony decreased. Whether similar conditions prevail in the case of inhibiting substances from other algae has not been adequately determined.

The higher concentration of inhibiting substances observed in old cultures may of course result from an increased rate of production by these cells; it may represent merely the accumulation of large quantities of the material from prolonged growth of the algae; or it may result from release of larger quantities of cellular substances through changes in permeability of the cell membrane or from actual autolysis of old cells.

## EFFECT OF DECOMPOSITION PRODUCTS

While attention has been directed thus far largely to the action of substances released during growth and development of algal organisms and presumably formed and excreted as a part of normal metabolic processes, another source of soluble organic substances must be considered. The vast quantity of material, both plant and animal, released in natural waters from organisms which undergo a seasonal decomposition by aquatic bacteria and fungi give rise to a multitude of degradation compounds which makes identification or quantitative estimation of active substances extremely difficult. Nevertheless it must be recognized that such substances, particularly when they result from the decomposition of highly concentrated bloom communities represent a potentially important source of soluble organic materials. Critical studies of the effect of such degradation products on algae in culture have been few in number.

Lefevre and Farrugia (1958) have recently reported the results of studies of the effect of decomposition products from the alga Cladophora glomerata and the gudgeon (Gobio fluviatilis) on the growth of seven algae in culture. Their experiments were carried out by adding various concentrations of the decomposition products from these organisms to distilled water and inoculating with pure cultures of algae. Results indicated that concentrations of the decomposition products as low as 1 mg./1. permitted growth of some algal species without the addition of mineral salts. Higher concentrations

resulted in stimulation of growth of certain species and in complete inhibition of others. Decomposition products of algae and of fish gave comparable results but affected cultures in different ways. Each had a specific effect and each demonstrated individual thresholds of activity for particular algae. The action was essentially unchanged whether the decomposition products were added in combination to distilled water or were added separately to mineral media. As noted previously in studies of substances interfering with cell multiplication, distinct cellular anomalies were observed in inhibited cells.

Preliminary studies of a somewhat similar nature are under way in our laboratories (Hartman and Demoise, unpublished). In these investigations, extracts have been prepared from large quantities of algal material collected from Pymatuning Reservoir at the end of the summer bloom. The species present in the material tested were mostly Microcystis aeruginosa and Coelosphaerium kuetzinganum. When water extracts, prepared by freezing, repeated centrifugation, and millipore filtration were added to cultures of Scenedesmus obliquus and Dictyosphaerum ehrenbergianum the growth rate of these organisms was approximately double that of control cultures, as measured by gravimetric or colorimetric techniques.

Extrapolation of generalizations based on results from laboratory culture studies to conditions existing in natural waters must always be made with an appreciation of the many difficulties involved. Excreted substances which remain in fairly concentrated form in confined culture media may become diluted below their limit of physiological activity in the great unconfined volume of the natural situation. Activities of associated fresh-water bacteria may result in rapid decomposition of substances which remain active for long periods in bacterially-free cultures. Active materials may also be rendered inactive in natural waters by formations of complexes with other substances. Finally, some consideration must be given to the fact that modifications of algal strains under conditions of the laboratory may have given rise to alterations in enzyme systems or to changes in metabolic pathways which differ from those in naturally occurring algal species.

The significance of these physiological active substances in the ecology of the algae of natural waters is difficult to appraise. Many workers since the time of Akehurst have leaned heavily on external metabolites in their attempts to explain such seasonal succession of species and the often-observed situation where a single species completely dominates a phytoplankton bloom community. Much of the efforts of Lefevre and his group have been attempts to explain these phenomenon by the action of algal inhibitors (Lefevre and Nisbet, 1948; Lefevre, Jakob and Nisbet, 1950, 1951; Lefevre and Farrugia, 1958). Lucas (1947, 1949, 1955) has brought together much of the findings from other areas of aquatic biology in support of the "exclusion theory" as applied to plant and animal relationships. From his interpretation of the available evidence he has suggested that many organisms have adapted themselves to tolerate or take advantage of the external metabolites of their neighbors and has postulated further that many "stimulating" and "inhibiting" ecological relationships may have arisen in this way.

The work of Proctor (1957) represents one of the few attempts to follow the presence of inhibiting substances in natural waters over several seasons and to relate the occurrence of these substances to community structure. Work of this type carried the criticism that the measurement of inhibition or stimulation is based on the reactions in the laboratory of a single organism, in this case Haematococcus pluvialis. The method is also open to criticism on the grounds that there is not direct evidence that phytoplankton populations are the source of the active substances being studied. In spite of such objections this type of approach combining seasonal studies on natural waters with laboratory culture studies of bloom-producing species, promises to be the most rewarding in terms of understanding the role of algal metabolites in influencing the structure and occurrence of many phytoplankton communities.

In summary, it would appear that ample evidence now exists to indicate that many species of fresh-water algae are capable of producing physiologically active metabolites which may function as toxins, growth inhibitors, or growth stimulators to themselves or to associated algae. Natural waters supporting bloom concentrations of algae have been shown to contain substances inhibitory to various species although evidence is not absolute that the active substances are derived directly from algae. Chemical and physical studies of the active substances have indicated that in a number of cases these materials appear to be related to fatty acids.

There remain many unsolved problems and unanswered questions: One is concerned with the chemical nature of the inhibiting or stimulating substance and the mechanism of physiological action on affected organisms. A second is concerned with the nature of the production of the active materials and its release from the source organism. Is it a metabolite produced and excreted during normal growth of the organism or is it produced only on death and subsequent breakdown of cells? Finally there remains the question most important perhaps to to the algal ecologist - Are these substances produced in physiologically effective concentrations in nature and do they remain at these concentrations for sufficient time to affect the growth of other algae?

TABLE I

Summary of laboratory studies on the production of physiologically active metabolites by algae in culture

| Test Organism | Interacting Organism | Observed Effects | Reference Source |
|---|---|---|---|
| Chlorella vulgaris | Chlorella vulgaris | Autoinhibition observed in culture filtrates | Pratt (1940) |
| C. vulgaris | Chlorella vulgaris | Stimulating effect of filtrate in low concentration | Pratt (1942) |
| C. vulgaris | Nitzschia frustulum | Mutual inhibition in mixed culture and inhibition in filtrate | Rice (1954) |
| C. vulgaris | Scenedesmus quadricauda | Inhibition in mixed culture | Proctor (1957) |
| C. vulgaris | Chlamydomonas reinhardi | Inhibition in mixed culture | Proctor (1957) |
| C. vulgaris | Haematococcus pluvialis | Inhibition in mixed culture | Proctor (1957) |
| C. vulgaris | Daphnia magna | Inhibited rate of filter feeding | Ryther (1954) |
| C. pyrenoidosa | Euglena deses | Inhibition in mixed culture | Lefevre and Nisbet (1948) |
| C. pyrenoidosa | Pediastrum duplex | Inhibition in mixed culture | Lefevre and Nisbet (1948) |
| C. pyrenoidosa | Pediastrum clathratum var. punctulatum | Inhibition in mixed culture | Lefevre, Nisbet and Jakob (1950) |
| C. pyrenoidosa | Nitzschia palea | Inhibition by filtrate | Jørgensen (1956) |
| C. pyrenoidosa | Scenedesmus quadricauda | Acceleration by filtrate | Jørgensen (1956) |
| Scenedesmus quadricauda | Phormidium uncinatum | Stimulation by filtrate of young culture | Lefevre and Jakob (1949) |
| S. quadricauda | P. uncinatum | Inhibition by filtrate of old culture | Lefevre and Jakob (1949) |
| S. quadricauda | Scenedesmus Oahuensis | Inhibition by filtrate of old culture | Lefevre and Jakob (1949) |
| S. quadricauda | Pediastrum boryanum | Inhibited by culture filtrate | Lefevre and Nisbet (1948) |

| Test Organism | Interacting Organism | Observed Effects | Reference Source |
|---|---|---|---|
| S. quadricauda | Cosmarium botrytis | Inhibited by culture filtrate | Lefevre and Nisbet (1948) |
| S. quadricauda | Nitzschia palea | Inhibited by filtrate | Jørgensen (1956) |
| S. quadricauda | Chlorella pyrenoidosa | Inhibited by filtrate | Jørgensen (1956) |
| S. quadricauda | Haematococcus pluvialis | Mutual inhibition in mixed culture | Proctor (1957, 1957a) |
| S. quadricauda | S. quadricauda | Inhibited by filtrate | Jørgensen (1956) |
| S. quadricauda | Daphnia magna | Inhibited rate of filter feeding | Ryther (1954) |
| S. quadricauda | Pediastrum clathratum var. punctulatum | Inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| S. quadricauda | Mesotaenium caldoriorum | Inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| S. quadricauda | Cosmarium lundellii | Inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| S. quadricauda | Cosmarium ochthodes | Inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| S. quadricauda | Phormidium uncinatum | Complete inhibition by filtrate | Lefevre, Jakob and Nisbet (1952) |
| S. quadricauda | Achnanthes microcephala | No action by filtrate | Lefevre, Jakob and Nisbet (1952) |
| S. oahuensis | Achnanthes microcephala | Inhibited in mixed culture | Lefevre and Jakob (1949) |
| S. obliquus | Microcystis aeruginosa | Inhibited in mixed culture | Hartman (Unpublished) |
| S. obliquus | Microcystis aeruginosa | Inhibited by filtrate | Hartman (Unpublished) |
| S. obliquus | Dictyosphaerium ehrenbergianum | Inhibited by filtrate | Hartman (Unpublished) |
| Pediastrum tetras | Scenedesmus obliquus | No effect of filtrate | Hartman (Unpublished) |
| Dictyosphaerium ehrenbergianum | Microcystis aeruginosa | Inhibited by filtrate | Hartman (Unpublished) |

| Test Organism | Interacting Organism | Observed Effects | Reference Source |
|---|---|---|---|
| Pandorina morum | Cosmarium lundellii | Inhibited by filtrate | Lefevre and Jakob (1949) |
| Pandorina morum | Scenedesmus ovalternus | Inhibited by boiled or frozen filtrate | Lefevre and Jakob (1949) |
| P. morum | S. ovalternus | Stimulated by untreated filtrate | Lefevre and Jakob (1949) |
| P. morum | Cosmarium spp. | Inhibited by filtrate | Lefevre and Jakob (1949) |
| P. morum | Micrasterias spp. | Inhibited by filtrate | Lefevre and Jakob (1949) |
| P. morum | Cosmarium obtusatum | Not inhibited by filtrate | Lefevre and Jakob (1949) |
| P. morum | Pediastrum boryanum | Not inhibited by filtrate | Lefevre and Jakob (1949) |
| P. morum | P. clathratum var. punctulatum | Inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| P. morum | Scenedesmus oahuensis | Inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| P. morum | Achnanthes microcephala | Inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| P. morum | Phormidium uncinatum | Good growth for 1 week then cells die | Lefevre, Nisbet and Jakob (1949) |
| P. morum | Mesotaenium caldariorum | Not inhibited by filtrate | Lefevre, Nisbet and Jakob (1949) |
| P. morum | Cosmarium lundellii | Cells accumulate reserve materials, fail to divide, die. | Lefevre, Nisbet and Jakob (1949 (1949) |
| P. morum | Cosmarium ochthodes | Cells accumulate reserve materials, fail to divide, die. | Lefevre, Nisbet and Jakob (1949) |
| Chlamydomonas chlamydogama | Haematococcus pluvialis | Stimulation or inhibition depending on media and time in culture | McVeigh and Brown (1954) |
| Chlamydomonas sp. | Chlorella sp. | Filtrates permit growth in situation where Chlorella could not otherwise grow | Allen (1955) |

| Test Organism | Interacting Organism | Observed Effects | Reference Source |
|---|---|---|---|
| C. reinhardi | Haematococcus pluvialis | Killed in mixed culture | Proctor (1957) (1957a) |
| C. reinhardi | Scendesmus quadricauda | Inhibited in mixed culture | Proctor (1957) |
| Haematococcus pluvialis | Chlamydomonas chlamydogma | Stimulation or inhibition depending on media and time in culture | McVeigh and Brown (1954) |
| H. pluvialis | Scenedesmus quadricauda | Mutual inhibition in mixed culture | Proctor (1957) (1957a) |
| Selanastrum minutum | Cosmarium impressulatum | No inhibition in mixed culture | Lefevre and Nisbet (1948) |
| Cosmarium impressulatum | Selanastrum minutum | No inhibition in mixed culture | Lefevre and Nisbet (1948) |
| DIATOMS | | | |
| Nitzschia frustulum | Chlorella vulgaria | Mutual inhibition in mixed cultures. Inhibition by filtrate | Rice (1954) |
| Nitzenhia palea | Nitzschia palea | Inhibited in filtrate | Jørgensen (1956) |
| N. palea | Chlorella pyenoidosa | Accelerated in filtrates | Jørgensen (1956) |
| N. palea | Asterionella formosa | Inhibited in filtrate and mixed cultures | Jørgensen (1956) |
| N. palea | Senedesmus quadricauda | Accelerated in filtrate | Jørgensen (1956) |
| N. palea | Pediastrum boryanum | Some multiplications, cells clumped | Lefevre, Jakob and Nisbet (1952) |
| N. palea | Scenedesmus quadricauda | Very few multiplications | Lefevre, Jakob and Nisbet (1952) |
| N. palea | Cosmarium lundelli | Inhibition by filtrate | Lefevre, Jakob and Nisbet (1952) |
| N. palea | C. obtusatum | Cells killed by filtrate | Lefevre, Jakob and Nisbet (1952) |
| N. palea | Nitzachia palea | Good development in filtrate | Lefevre, Jakob and Nisbet (1952) |
| N. palea | Phormidium uncinatum | Slight development in filtrate | Lefevre, Jakob and Nisbet (1952) |

| Test Organism | Interacting Organism | Observed Effects | Reference Source |
|---|---|---|---|
| Asterionella formosa | Nitzschia palea | Inhibition by filtrate and in mixed cultures | Jørgensen (1956) |
| A. formosa | Asterionella formosa | Accelerated in filtrate | Jørgensen (1956) |
| A. formosa | Nitzschia palea | Accelerate in filtrate | Jørgensen (1956) |
| A. formosa | Fragilaria crotonensis | No inhibition in mixed culture or filtrate | Talling (1957) |
| Fragilaria crotonensis | Asterionella formosa | No inhibition in mixed culture or filtrate | Talling (1957) |
| Phormidium uncinatum | Scenedesmus quadricauda | Inhibited by filtrate | Lefevre and Nisbet (1948) |
| P. uncinatum | Achnanthes microcephala | Stimulated in mixed culture and by filtrate | Lefevre and Nisbet (1948) |
| P. pachydermaticum | Phormidium uncinatum | Killed by filtrate | Jakob (1954) |
| P. pachydermaticum | Nostoc zetterstedti | Unaffected by filtrate | Jakob (1954) |
| P. pachydermaticum | N. verrucosum | Unaffected by filtrate | Jakob (1954) |
| P. pachydermaticum | Cylindrospermum alatosporum | Inhibited in mixed culture | Jakob (1954) |
| P. pachydermaticum | Nostoc gelatinosa | Mutually inhibitory on solid media | Jakob (1954) |
| Ph. uncinatum | Pediastrum boryanum | Inhibited by filtrate of old cultures | Lefevre, Jakob and Nisbet (1952) |
| Ph. uncinatum | P. clathratun var. punctulatum | Inhibited by filtrate of old cultures | Lefevre, Jakob and Nisbet (1952) |
| Ph. uncinatum | Scenedesmus oahuensis | Inhibited by filtrate of old cultures | Lefevre, Jakob and Nisbet (1952) |
| Ph. uncinatum | Ankistrodesmus falcatus | Stimulated by filtrate of young cultures | Lefevre, Jakob and Nisbet (1952) |
| Ph. uncinatum | Achnanthes microcephala | Stimulated by filtrate of young cultures | Lefevre, Jakob and Nisbet (1952) |
| Nostoc gelatimosum | Oscillatoria acutissima | Inhibited by filtrate | Jakob (1954) |
| N. gelatinosm | Anabaena cylindrica | Inhibited by filtrate | Jakob (1954) |
| N. gelatinosum | Nostoc verrucosum | Unaffected by filtrate | Jakob (1954) |
| N. gelatinosm | N. carneum | Unaffected by filtrate | Jakob (1954) |

| Test Organism | Interacting Organism | Observed Effects | Reference Source |
|---|---|---|---|
| N. gelatinosum | N. borneti | Unaffected by filtrate | Jakob (1954) |
| N. gelatinosum | Phormidium rubroterricola | Mutually inhibitory on solid media | Jakob (1954) |
| N. gelatinosum | Anabaena cylindrica | Mutually inhibitory on solid media | Jakob (1954) |
| N. zellerstedti | Nostoc Broneti | Little affected by filtrate | Jakob (1954) |
| N. zellerstedti | N. verrucosum | Little affected by filtrate | Jakob (1954) |
| N. zellerstedti | Phormidium pachydermaticum | Little affected by filtrate | Jakob (1954) |
| N. zellerstedti | P. uncinatum | Inhibited by filtrate | Jakob (1954) |
| N. zellerstedti | Oscillatoria acutissima | Inhibited by filtrate | Jakob (1954) |
| N. zellerstedti | Nostoc verrucosum | Mutually inhibitory on solid media | Jakob (1954) |
| N. zellerstedti | Nostoc borneti | Mutually inhibitory on solid media | Jakob (1954) |
| N. verrucosum | Cylindospermum alatosporum | Mutually inhibitory on solid media | Jakob (1954) |
| N. verrucosum | Nostoc zellerstedti | Mutually inhibitory on solid media | Jakob (1954) |
| N. borneti | N. zellerstedti | Mutually inhibitory on solid media | Jakob (1954) |
| Anacystis nidulans | Chlorella vulgaris | Inhibited in mixed culture | Proctor (1957) |
| A. nidulans | Scenedesmus quadricauda | Inhibited in mixed culture | Proctor (1957) |
| A. nidulans | Chlamydomonas reinhardi | Killed in mixed culture | Proctor (1957) |
| A. nidulans | Haematoccus pluvialis | Killed in mixed culture | Proctor (1957) |
| Microcystis aeruginosa | Scenedesmus obliquus | No effect in mixed culture | Hartman (Unpublished) |
| Microcystis aeruginosa | Scenedesmus obliquus | Slight inhibition by filtrate | Hartman (Unpublished) |
| M. aeruginosa | Dictyosphaerium ehrenbergianum | Inhibition by filtrate | Hartman (Unpublished) |
| Cylindropermum alatosporum | Nostoc verrucosum | Inhibition in filtrate | Jakob (1954) |
| A. cylindrica | Sarcina subflava | No appreciable affect by concentrated filtrate | Fogg (1952) |
| A. cylindrica | Corynebacterium faciens | No appreciable affect by concentrated filtrate | Fogg (1952) |

| Test Organism | Interacting Organism | Observed Effects | Reference Source |
|---|---|---|---|
| A. cylindrica | Rhodotorula rubra | No appreciable affect by concentrated filtrate | Fogg (1952) |
| A. cylindrica | Torulopsis utitis | No appreciable affect by concentrated filtrate | Fogg (1952) |
| Blue green spp. (Later identified as Fischerella) | No details | Production of antibiotic substances | Flint and Moreland (1946); Proc. La. Acad. Sci. 10:30-31. (1957) |
| Mesotaenium caldariorum | Phormidium uncinatum | No effect by filtrate | Lefevre, Jakob and Nisbet (1952) |
| M. caldariorum | Scenedesmus oahuensis | No effect by filtrate | Lefevre, Jakob and Nisbet (1952) |
| M. caldariorum | Scenedismus quadricauda | No effect by filtrate | Lefevre, Jakob and Nisbet (1952) |
| M. caldariorum | Cosmarium ochthodes | Little effect by filtrate | Lefevre, Jakob and Nisbet (1952) |
| M. caldariorum | Pediastrum clathratum | Complete inhibition by filtrate | Lefevre, Jakob and Nisbet (1952) |
| Ankistrodesmus falcatus | Euglena gracilis | No effect of filtrate | Lefevre, Jakob and Nisbet (1952) |
| A. falcatus | Scenedesmus oahuensis | No effect of filtrate | Lefevre, Jakob and Nisbet (1952) |
| A. falcatus | S. falcatus | No effect of filtrate | Lefevre, Jakob and Nisbet (1952) |
| A. falcatus | Pediastrum boryanum | No effect of filtrate | Lefevre, Jakob and Nisbet (1952) |
| A. falcatus | P. clathratum var. punctulata | No effect of filtrate | Lefevre, Jakob and Nisbet (1952) |
| A. falcatus | Cosmarium botrytis | Inhibition by filtrate | Lefevre, Jakob and Nisbet (1952) |
| A. falcatus | Mesotaenium caldariorum | Slight inhibition by filtrate | Lefevre, Jakob and Nisbet (1952) |

TABLE II

Summary of Studies of the Effect of Natural Waters containing bloom communities on the growth of algae in culture.

| Water Source | Interacting Organism | Observed Effects in Filtered Water | Reference Source |
|---|---|---|---|
| Canal water containing bloom of Aphanizomenon gracile | Cosmarium lundellii | Inhibition of growth | Lefevre, Jakob, and Nisbet (1950) |
| " " " | Micrasteris papillifera | Inhibition of growth | " " " |
| " " " | Pediastrum boryanum | Inhibition of growth | " " " |
| " " " | P. clathratum v. punctulalum | Inhibition of growth | " " " |
| " " " | Phormidium uncinatum | Inhibition of growth | " " " |
| " " " | Nitzschia palea | No effect | Lefevre, Jakob, and Nisbet (1952) |
| " " " | Micrasteris papillifera | No effect | " " " |
| Canal water containing bloom of Oscillatoria planctorica | Chlorella pyrenoidosa | Inhibition of growth | Lefevre, Jakob, and Nisbet (1950) |
| " " " | Cosmarium lundellii | Inhibition of growth | " " " |
| " " " | C. obtusatum | Inhibition of growth | " " " |
| " " " | Micrasteris pacillifera | Inhibition of growth | " " " |
| " " " | Pediastrum boryanum | Inhibition of growth | " " " |
| " " " | Phormidium uncinatum | Inhibition of growth | " " " |
| " " " | P. autumnale | Inhibition of growth | " " " |
| Canal water containing bloom of Oscillatoria planctorica | Scenedesmus quadricauda | Inhibition of growth | Lefevre, Jakob and Nisbet (1950) |
| Pond water containing bloom of Anabaena planktonica | Pediastrum clathratum var. punctulatum | Complete inhibition with death of cells | Lefevre, Jakob and Nisbet (1952) |
| " " " | P. Boryanum | Complete inhibition with death of cells | " " " |
| " " " | Cosmarium lundellii | Complete inhibition with death of cells | " " " |
| " " " | C. obstusatum | No effect | " " " |

| Water Source | Interacting Organism | Observed Effects in Filtered Water | Reference Source |
|---|---|---|---|
| Pond water containing bloom of Anabaena planktonica | Phormidium uncinatum | Inhibition of growth | Lefevre, Jakob and Nisbet (1952) |
| " " " | Scenedesmus quadricauda | Slight inhibition | " " " |
| " " " | Nitzschia palea | Slight inhibition | " " " |
| " " " | Chlorella pyrenoidosa | Inhibition of growth | " " " |
| Pond water containing bloom of Anabaena spiroides | Pediastrum clathratum var. punctulatum | Inhibition of growth | " " " |
| " " " | P. boryanum | Slight inhibition | " " " |
| " " " | Cosmarium lundelli | Complete inhibition with death of cells | " " " |
| " " " | C. obtusatum | " " " | " " " |
| " " " | Pediastrum clathratum var. punctulatum | " " " | " " " |
| Pond water containing bloom of Anabaena spiroides | P. boryanum | Inhibition of growth | " " " |
| " " " | Cosmarium lundellii | " " " | " " " |
| " " " | C. obstusatum | " " " | " " " |
| Pond water containing bloom of Microcystis flos aquae | Pediastrum clothratum var. punctulatum | Complete inhibition, with death of cells | " " " |
| " " " | P. boryanum | " " " | " " " |
| " " " | Scanedesmus quadricauds | " " " | " " " |
| " " " | Cosmarium lundelli | " " " | " " " |
| " " " | C. obstusatum | " " " | " " " |
| " " " | Chlorella pyrenoidosa | " " " | " " " |
| " " " | Phormidium uncinatum | Inhibition of growth | " " " |
| " " " | Nitzschia palea | Slight inhibition | " " " |
| Pond water containing bloom of Ceratuim hirundinella | Pediastrum clathratum var. punctulatum | Increased growth over control | " " " |
| " " " | P. boryanum | Complete inhibition with death of cells | " " " |

| Water Source | Interacting Organism | Observed Effects in Filtered Water | Reference Source |
|---|---|---|---|
| Pond water containing bloom of Ceratuim hirundinella | Cosmarium lundellii | Complete inhibition with death of cells | Lefevre, Jakob and Nisbet (1952) |
| " " " | C. obstusatum | Inhibition of growth | " " " |
| " " " | Phormidium uncinatum | " " " | " " " |
| " " " | Nitzschia palea | Inhibition of growth | " " " |
| " " " | Scenedesmus quadricauda | " " " | " " " |
| " " " | Chlorella pyrenoidosa | " " " | " " " |
| Pond water containing bloom of Spirogyra (sp) | Pediastrum clathratum var. punctulatum | " " " | " " " |
| " " " | P. boryanum | " " " | " " " |
| " " " | Nitzschia palea | " " " | " " " |
| " " " | Cosmarium lundellii | " " " | " " " |
| " " " | Phormidium uncinatum | Complete inhibition with death of cells | " " " |
| Pond water containing bloom of Pandorina | Chlorella vulgaris | Inhibition of growth | Rice (1954) |
| " " " | Nitzchia frustulum | Inhibition of growth | Rice (1954) |
| Pond water containing bloom of Anabaena spiroides and Microcystis aeruginosa; also blooms of Cryptomonas sp., and Lyngbya aesturaii | Haematococcus pluvialis | Variable Inhibition | Proctor (1957) |

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# ALGAE IN RELATION TO OXIDATION PROCESSES IN NATURAL WATERS

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Agencies concerned with management of water quality are especially interested in the following oxidation processes that occur in natural waters: (a) stabilization by microorganisms of natural or introduced organic matter, (b) respiration by the aquatic biota, and (c) direct oxygen demand of chemical substances. Each process to some extent draws upon the oxygen resources of the water so that replenishment must occur to prevent serious and lasting oxygen depletion. Replenishment occurs chiefly by reaeration from the atmosphere and through photosynthesis in algae and other aquatic plants. As will be shown, algae are involved in several ways in both the oxidation processes and the broader oxygen relations in natural waters.

## Direct Algal Oxidation of Organic Matter

Algae have direct as well as indirect effects upon oxidation processes. It has been shown that a number of algae are able to use organic substances directly for energy and growth (Samejima and Meyers, 1958; Eny, 1951; Wilson and Danforth, 1958). A recent summary by Saunders (1957) lists 32 species of phytoplankton that possess this capability. Also listed is a wide variety of the organic substances known to be used, grouped as carbohydrates, aldahydes, ketones, alcohols, esters, fatty acids, organic acids, amino acids, and related compounds.

Raw and treated sewages have not generally been analyzed thoroughly for their content of organic compounds of the groups cited above. The origin and history of domestic sewage leave little doubt, however, that many if not all of these organics are present, along with others not mentioned. Presumably, select members of the phytoplankton in natural waters utilize some of these organics if they are present. When such organics in polluted waters are utilized by phytoplankton, the resulting reduction in their concentration would be beneficial to water quality in the same sense that bacterial oxidation is beneficial. In this sense the phytoplankton participate directly in the oxidation processes that occur in natural waters, although the extent of such action has not been determined. From casual observation it would appear that the over-all effect of this type of algal activity upon both the dissolved organics content and the stream's oxygen resources would be of little consequence. The algae would be merely competing with and perhaps to some extent displacing the oxidation activities of the customary assemblage of heterotrophic organisms that stabilize such pollutants.

Shallow waste stabilization ponds are becoming popular for treating municipal sewage and some industrial wastes and have recently received considerable study (Gotaas, Oswald and Ludwig, 1954; Oswald and Gotaas, 1955; Hermann and Gloyna, 1955; Gloyna and Herman, 1956; Anon., 1957; Bartsch and Allum, 1957). Successful operation depends essentially on bacterial decomposition of the waste organics and algal photosynthesis to provide dissolved oxygen for the aerobic processes. Observations on many sewage stabilization ponds have shown that ability to reduce organic matter, as measured by the B.O.D. test, is not seriously impaired during winter in spite of cold weather conditions that generally impede biochemical processes. B.O.D. reduction persists at 70% or more, in spite of anaerobic conditions under semiopaque ice cover 9 to 30 inches thick. Undoubtedly, a combination of processes makes possible this continued reduction. Much of the phytoplankton gathers on the bottom, but appreciable quantities of Scenedesmus dimorphus, S. quadricauda, Chlorella sp., Euglena sp., Phacus longicauda, and other algae have been observed suspended in the water, motile if flagellated, and obviously active. With extremely low light intensity below the ice, the presence and active condition of these algae raise the question of heterotrophic use of organic substances as a contribution to the stabilization performance. Interestingly, these algae are still active in the prolonged absence of dissolved oxygen as shown by the Winkler procedure and by the presence of sulfides up to 117 ppm. Such activity of Chlorella, Chlamydomonas, and Euglena in the presence of hydrogen sulfide has been observed also by Abbott (1951). The full significance of these observations is not presently apparent, and further study is needed to establish clearly the role of algae along with other more obvious processes in the winter performance of waste stabilization ponds.

Along this same line, studies are in progress at the Sanitary Engineering Center to determine the ability of specific algae to utilize sugars in spent sulfite liquor. The study was designed to explore possibilities that algae may serve in treating this

industrial waste.

## Provision of Dissolved Oxygen

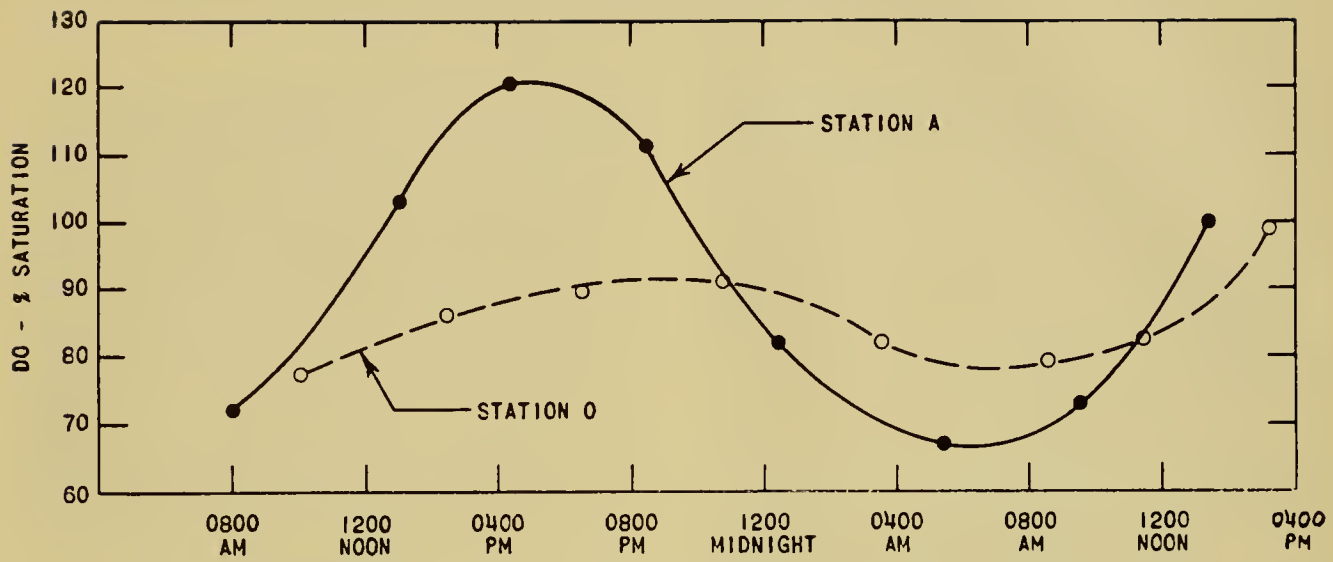
In relation to water quality management, the most valuable contribution of phytoplankton is provision of oxygen for use in oxidation by the total aerobic biota. Oxygen is vitally important because it affects the capacity of streams to receive and oxidize sewage and other organic wastes without unduly impairing water quality. Multiple use of water resources is increasingly necessary as water-hungry population and industries grow while the total water supply remains constant. Use of water for waste disposal presently is inescapable. It is therefore appropriate to point out some of the fundamental oxygen relationships related to waste disposal. Aside from the natural cyclic effects of phytosynthesis by aquatic plants, significant changes in the dissolved oxygen content of a stream result primarily from the oxidation of organic matter entering as waste. A combination of available dissolved oxygen and suitable biota - especially aerobic bacteria - results in progressive oxidation and stabilization of the organic matter. It has been postulated (Streeter and Phelps, 1925) that the rate of biochemical oxidation of organic matter is proportional to the remaining concentration of unoxidized substance measured in terms of oxidizability. Unpolluted water tends to hold in solution the maximum amount of oxygen it is capable of holding at the existing temperature and partial oxygen pressure of the atmosphere, but, when organic pollutants are introduced, use of the dissolved oxygen supply in progressive satisfaction of the demand tends to reduce the oxygen content below this saturation value. Sources of oxygen available for this use are (1) that initially dissolved in the water, (2) that absorbed from the atmosphere by partially deoxygenated water, and (3) oxygen made available through photosynthesis of aquatic plants. These interrelations are shown graphically in a recent publication (Bartsch and Ingram, 1959). At present, our interest is directed to the last oxygen source only.

Equations have been developed and used widely to elucidate the dynamics involved in the oxygen resources of streams (Streeter and Phelps, 1925). Although others have taken a modified approach and added refinements to evaluating these relations, photosynthetic oxygen production, unfortunately, has remained elusive of precise quantitative expression as a part of the over-all picture. That photosynthesis in aquatic plants affects dissolved oxygen levels in surface waters has been known for a long time. General relationship of photosynthesis to stream sanitation, however, is a more recent interest. Studies of Potomac River plankton and rooted plants in 1913 by Purdy (Cumming, 1916) included measurement of oxygen changes caused by photosynthesis. The importance of plant-covered flats below the District of Columbia in accelerating recovery from pollution originating at that point is discussed at some length. Oxygen production in one area was shown to be 17.7 pounds per acre per day, mainly by submerged aquatic plants growing on flats outside the river channel.

Calvert (1933) studied diurnal fluctuations of dissolved oxygen in the White River below the Indianapolis sewage treatment plant in relation to varying B.O.D. load and amount of sunshine. Daytime oxygen concentrations frequently reached 5 or 6 ppm. but usually dropped to zero at night, showing the importance of algal photosynthesis in maintaining a daytime supply of dissolved oxygen and preserving desirable aerobic conditions.

Since the early observations cited, diurnal variations in dissolved oxygen have been noted and reported many times. Typical variations are shown in Figure 1 for two stations on French Creek near Meadville, Pennsylvania, where a study was made in August, 1955, to determine stream conditions and assimilation capacity characteristics. Obviously, monetary oxygen data, such as might be obtained by sampling as chance and convenience bring the technician and the stream together, have little value in showing stream character. If station A had been sampled only at 5:00 p.m., the early morning depression would have been missed; if sampled only at 8:00 a.m., the period of supersaturation would not have been detected. The situation would have been the same at station O but the differences less pronounced. Sampling deficiencies such as these are discussed thoroughly by Gameson and Griffith (1959). It is obvious that stream technicians cannot properly discern oxygen conditions in surface waters by restricting themselves to field sampling and analyses only during the usual working hours between 8 and 5.

Newly developed equipment that automatically analyzes and records dissolved oxygen (Macklin, Baumgartner, and Ettinger, 1959) now makes collection of diurnal data both less tedious and more accurate. Desired oxygen data are now obtainable with less nocturnal sampling under the adverse conditions of darkness. Figure 2 shows dissolved oxygen records for a two-day period obtained with such equipment at an experimental sewage stabilization pond. The record for both days shows dissolved oxygen was absent at sunrise. Factors affecting photosynthesis and oxygen retention by the water obviously were more favorable on November 19 than November 14 according to dissolved oxygen levels attained. The periodic irregularities resulted from variation in illumination. It is sometimes taken as a "rule of thumb" in reference to algal photosynthesis that the higher the oxygen level climbs above saturation in the




DISSOLVED OXYGEN VS. TIME OF DAY IN FRENCH CREEK PENNSYLVANIA. AUGUST 17, 18, 1955

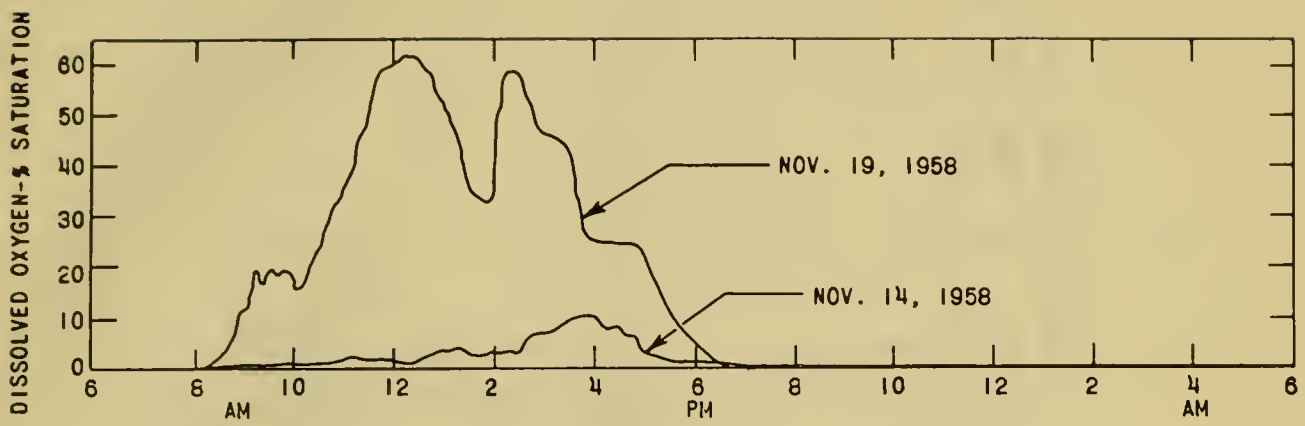
Figure 1

daytime the lower it is likely to drop below it at night. The record for the Little Miami and Ohio Rivers (Figure 3) seems to substantiate this "rule" when compared with the stabilization pond in Figure 8. For the period shown, the Ohio deviated only slightly from air saturation whereas the Little Miami, with more numerous attached algae and a habitat more favorable for photosynthesis, exhibits a little more vigorous photosynthetic oxygen production.

Recently, a six-month record of dissolved oxygen has been obtained for a small polluted stream in England (Gameson and Griffith, 1959) using photographic equipment to record the dial readings from a dropping mercury electrode (Briggs, Dyke, and Knowles, 1959). Data for the six-month average diurnal oxygen curve in Figure 4 were obtained in this way. While obviously the daily irregularities and extremes have been eliminated, the curve shows the times of day when the maxima and minima might be expected for this stream. About two-thirds of the maxima were found to occur between noon and 6:00 p.m.; 85 per cent of the minima, between 7:45 p.m. and 7:45 a.m. This curve is not unusual; it agrees generally with the much shorter term observations on other waters where the minimum commonly has been noted around 3:00 to 5:00 a.m. and the maximum around 4:00 or 5:00 p.m.

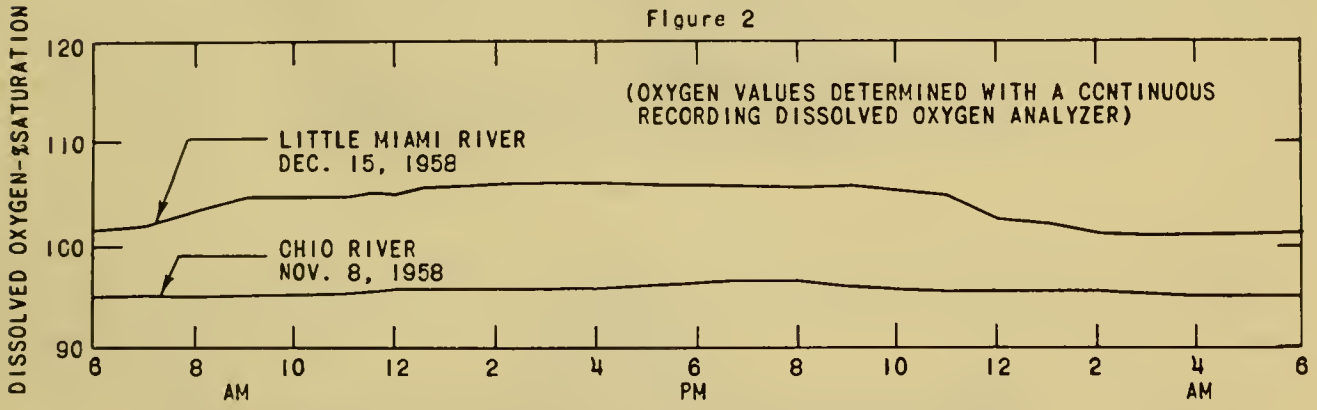
A number of approaches have been made to detect and evaluate the influence of algal photosynthesis on oxygen resources in streams. For a given reach of a hypothetical stream, Odum (1956) has attempted to interrelate existing oxygen concentration, provision of oxygen by inflowing water, the atmosphere, and photosynthesis with losses through respiration and diffusion (Figure 5). Three main daily processes are noted to affect the oxygen and carbon dioxide concentrations of water flowing between two established points. These are release of oxygen into the water during daylight through photosynthesis (A in Figure 5), absorption of dissolved oxygen by all organisms (B), and oxygen exchange between water and air (C). Interplay of these three processes determines the rate of oxygen change (D) and the resulting oxygen concentration (E) that would appear in a homogeneous medium.

Toward assessing the effect of large quantities of blue-green algae on oxygen resources in Wisconsin's Lower Fox River, Wisniewski (1958) modified the customary B.O.D. procedure to measure algal respiration and photosynthesis. Results for a sample from one station are shown in Figure 6. When incubated for five days at a light intensity of




DIURNAL VARIATION IN DISSOLVED OXYGEN IN A SEWAGE STABILIZATION POND
LEBANON, OHIO

Figure 2

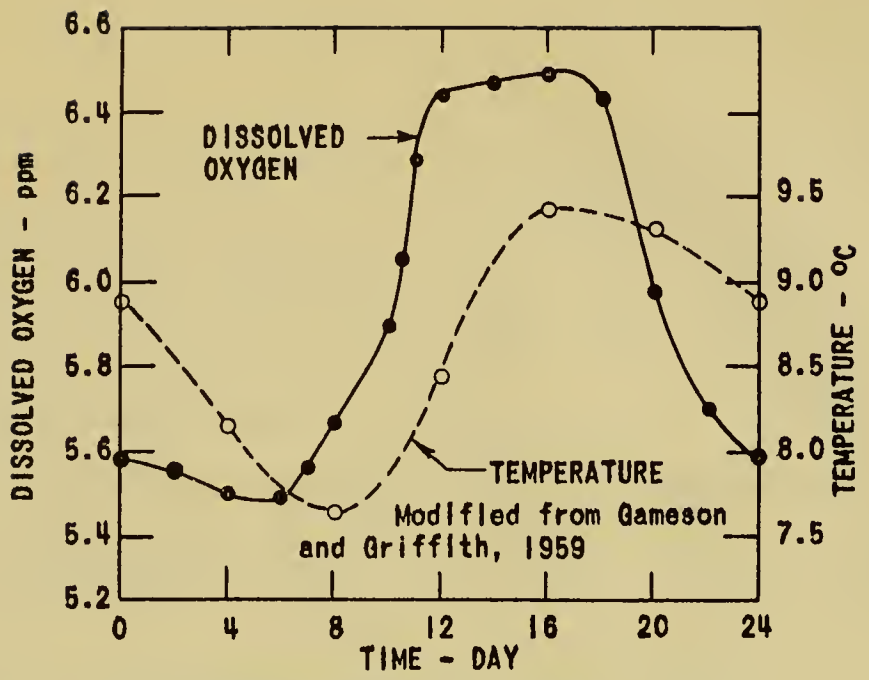



DIURNAL VARIATION IN DISSOLVED OXYGEN IN TWO OHIO RIVERS

Figure 3

300 foot-candles: 2.8 ppm. algae produced 3.4 ppm. oxygen; 11.0 ppm. algae produced 7.9 ppm. oxygen; and 13.7 ppm. algae produced 18.4 ppm. oxygen. It was also noted that oxygen production in relation to algal density, expressed as suspended solids, increased as samples were collected progressively downstream. Concurrently, the blue-green planktonic algae from Lake Winnebago, from which the Fox River flows, were replaced by algae more suited to the flowing water habitat. How the laboratory results are to be applied to river conditions was not determined.

A third approach used the "light and dark bottle test" to measure photosynthesis and respiration simultaneously. Although raw sewage stabilization ponds are not natural waters, the intensity of processes occurring there makes studying them especially instructive in relation to other waters. In general essentially all photosynthesis occurs in the surface water layer that absorbs 99% of the light. For this reason water transparency must serve as a guide in determining desirable depths at which to expose light and dark bottles within the euphotic zone. Typical relations between oxygen production and oxygen use in such ponds are shown in Figure 7. During mid-morning with intense light and abundant carbon dioxide accumulated during nocturnal bacterial decomposition, photosynthetic oxygen production reaches a high rate of more than 2 grams per square meter per hour. At the depth where light intensity is 20% of the surface value, oxygen production proceeds at 70 times the rate found at only 1% of surface value intensity. Because the density of phytoplankton is much greater than that commonly found in other surface waters, extinction of light is rapid and the euphotic zone is only about 120 centimeters thick. In the Ohio River, on the other hand, turbidity is caused largely by inorganic suspensoids, but they are sufficient to absorb 99% of the light in about 130




SIX MONTH AVERAGE DIURNAL CURVES FOR RIVER HIZ, ENGLAND

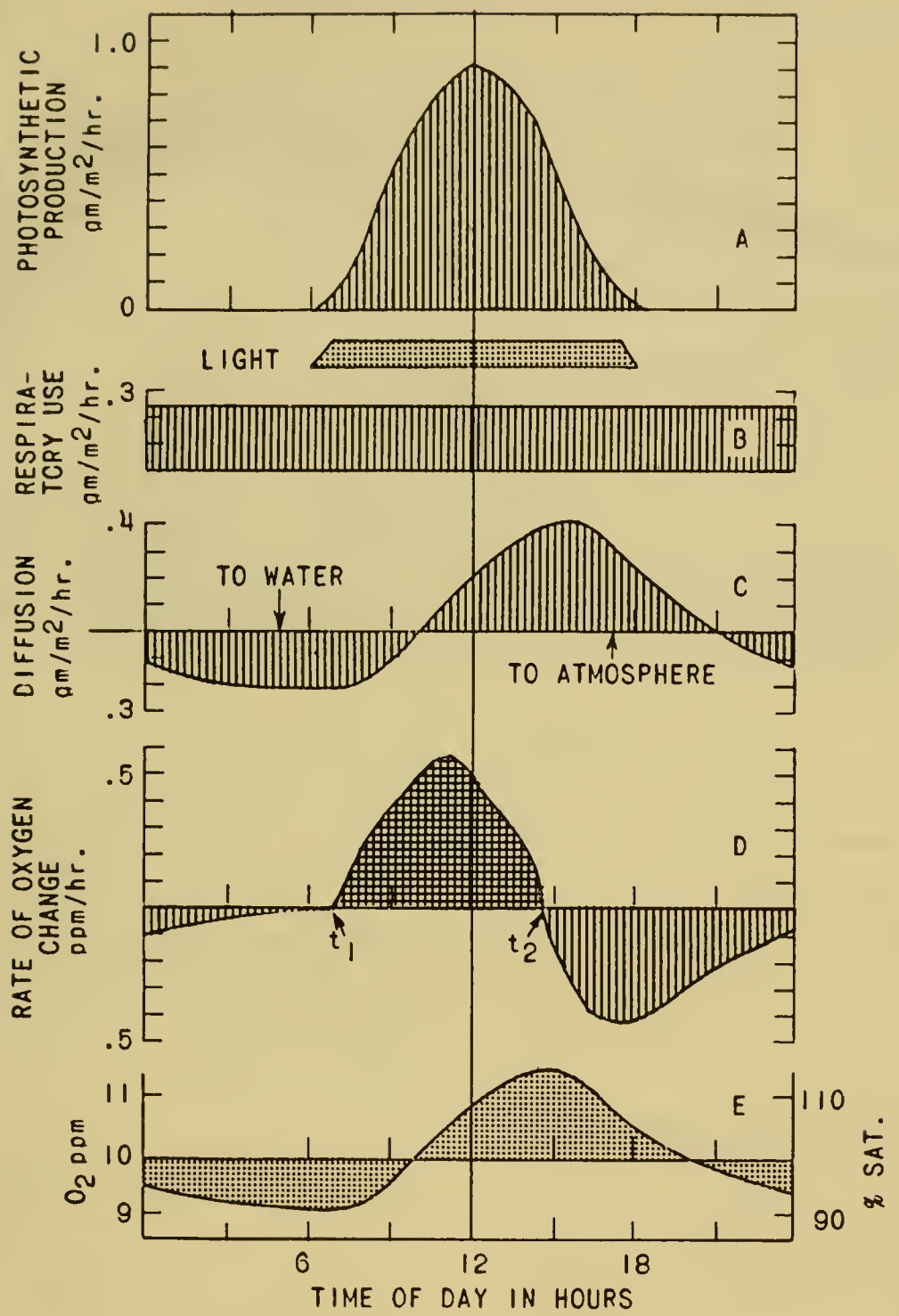
Figure 4

centimeters. Here, however, with a much lower phytoplankton population, the oxygen production rate never exceeded 1 gram per square meter per hour for the period studied.

The quantity of oxygen produced by photoplankton can be both impressive and important. In raw sewage stabilization ponds in the northern part of the country, production of as much as 26 grams per square meter per day by a biological system using 19 grams per square meter per day for total respiration (a P/R of 1.4) assures a favorable oxygen balance during illuminated periods. It also provides a surplus that can be mixed by wind and convection currents into deeper, less illuminated strata so that they also can participate in aerobic stabilization. Some dissolved oxygen persists into the night, but usually it is completely used before the next period of illumination. Although the quantities of oxygen in question may seem small, the customary daily addition to these ponds of oxygen-demanding material in the form of sewage is only about 1-1/4 grams per square meter. Even in rivers, unless their pollution load is unusually heavy, an oxygen production rate of 7.4 grams per square meter per day and respiratory rate of 5 grams per square meter per day (P/R of 1.5), as found in the Ohio River, leave a surplus sufficient for the usual total demand.

Both in sewage stabilization ponds and in streams, surplus oxygen produced by phytoplankton is not efficiently used and may be unavailable for oxidizing organic matter within a short time after it is produced. During periods of intense oxygen production the water becomes supersaturated and oxygen escapes to the atmosphere. Surface agitation by wind accelerates such loss as suggested by the lower oxygen concentrations in stabilization ponds on windy days than on calm days (Figure 8). In quiescent, organically rich waters such as these, oxygen concentration decreases rapidly with depth, but in rivers with their constant movement, such gradients are rare. Nocturnal oxygen depletion through continued intense respiration may become serious - more so in ponds than in rivers.

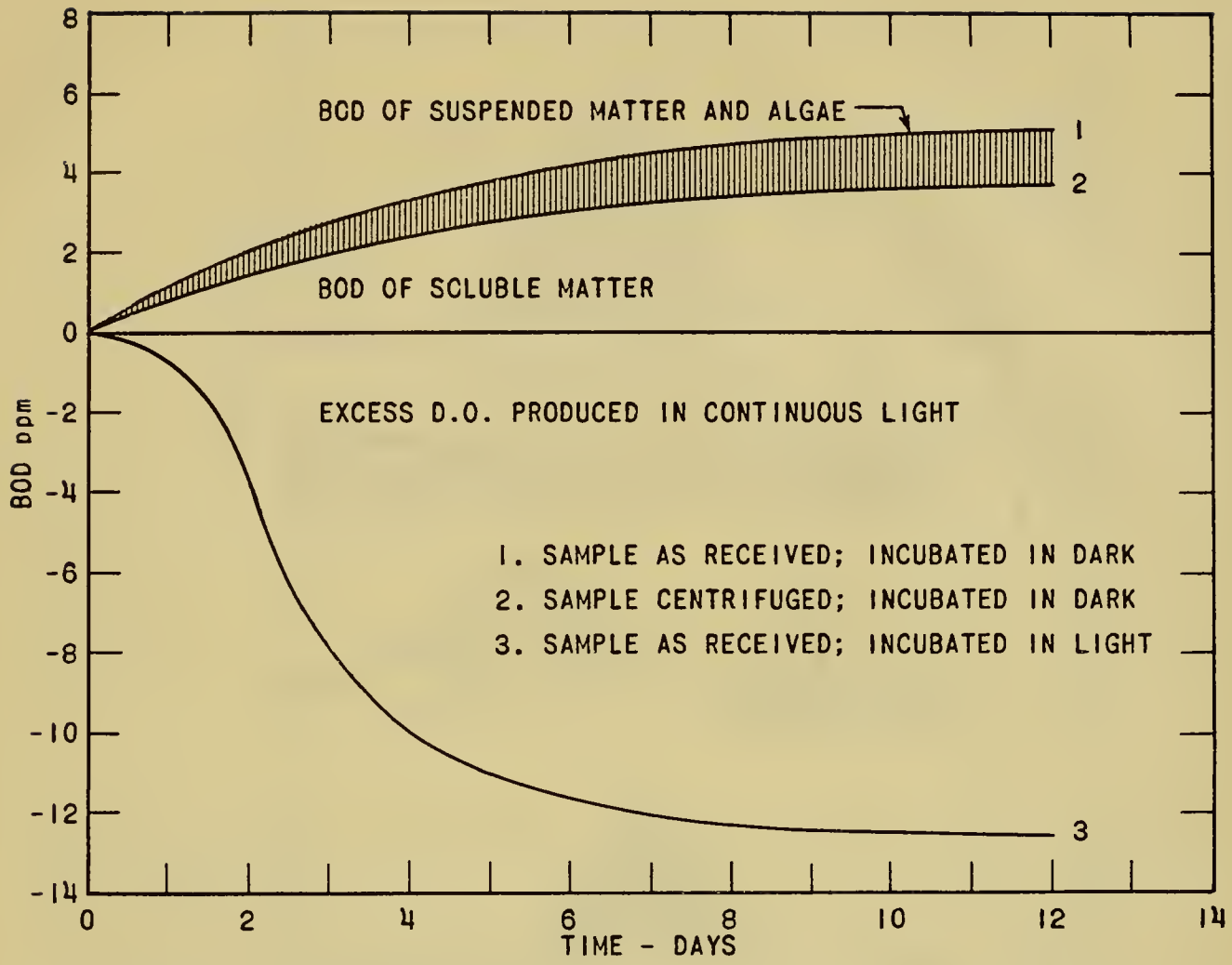
While no one disclaims the importance of photosynthetic oxygen, the present state of knowledge does not show how such oxygen may be utilized more efficiently. The whole subject of phytoplankton in relation to oxygen production and use has not yet reached a state of development permitting practical application to the analysis of oxygen resources or use in the oxygen sag equation. Even when data are available on momentary rates of photosynthesis in relation to phytoplankton density, light intensity, limits of the euphotic zone, and other factors, it is not clear how such data can be applied to practical problems. There is need for wider and more intensified study in this area. Also, there is need to relate photosynthetic potential of the waters to some numerical expression of the phytoplankton, whether this is given as pigment concentration, number of cells per volume, packed cell




DIURNAL OXYGEN RELATIONS IN A HYPOTHETICAL STREAM SECTION

Figure 5

Modified from Odum, 1956




EFFECT OF NATURALLY OCCURRING PHYTOPLANKTON ON RATE OF OXIDATION IN A FOX RIVER SAMPLE STATION C. JULY 25. 1956

Figure 6

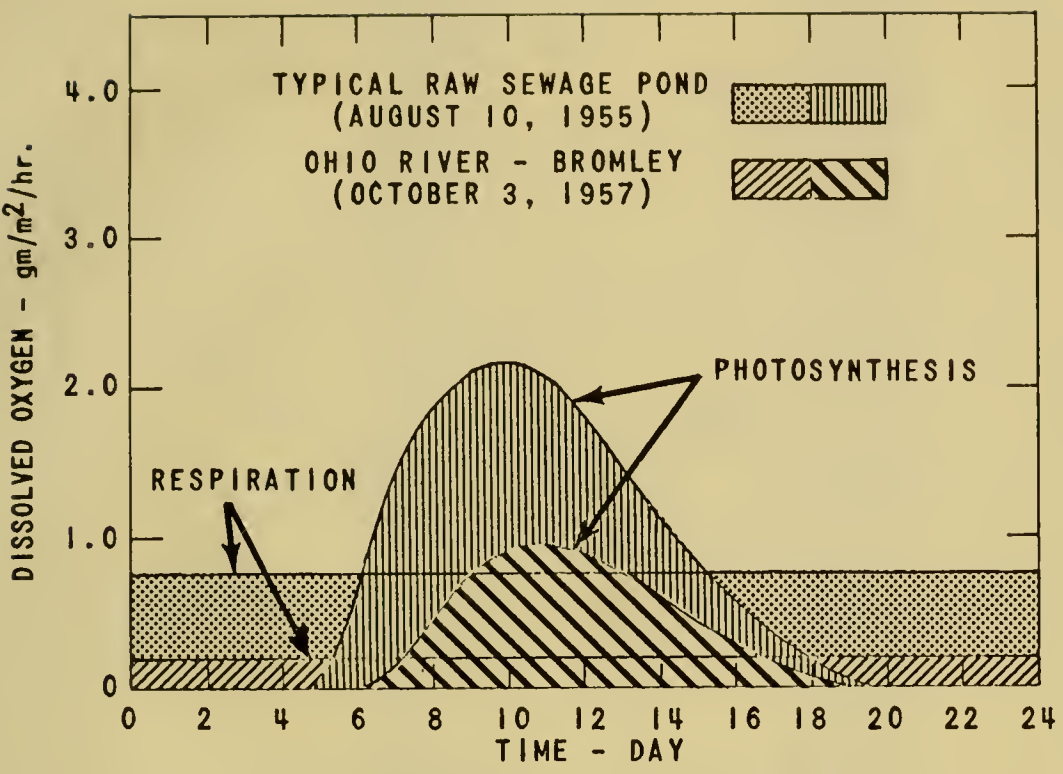
Modified from Wisniewski, 1958

volume, or some other expression. Toward this end the Sanitary Engineering Center is continuing to study photosynthetic oxygen production in rivers and sewage stabilization ponds and also is cooperating with the Tennessee Valley Authority on similar studies on rivers immediately below large impoundments.

### Costs of Dissolved Oxygen from Algal Photosynthesis

Oxygen production by algae is not a free benefit to the aquatic habitat. At times, in fact, it is probable that the benefits do not outweigh the costs.

Stimulation of algal production by nutrients derived from sewage, other organic wastes, and their decomposition products can lead to formation of a mass of organic matter greater than that of the original waste (Renn, 1954). This result is demonstrable in the laboratory. It was also observed by Renn at two study stations on the Potomac River that, during the bright light period, rise and fall of B.O.D. concentrations were parallel with rise and fall of dissolved oxygen. This observation was interpreted to indicate accelerated production of algae that became a part of the total B.O.D.




OXYGEN RELATIONS IN SURFACE WATERS

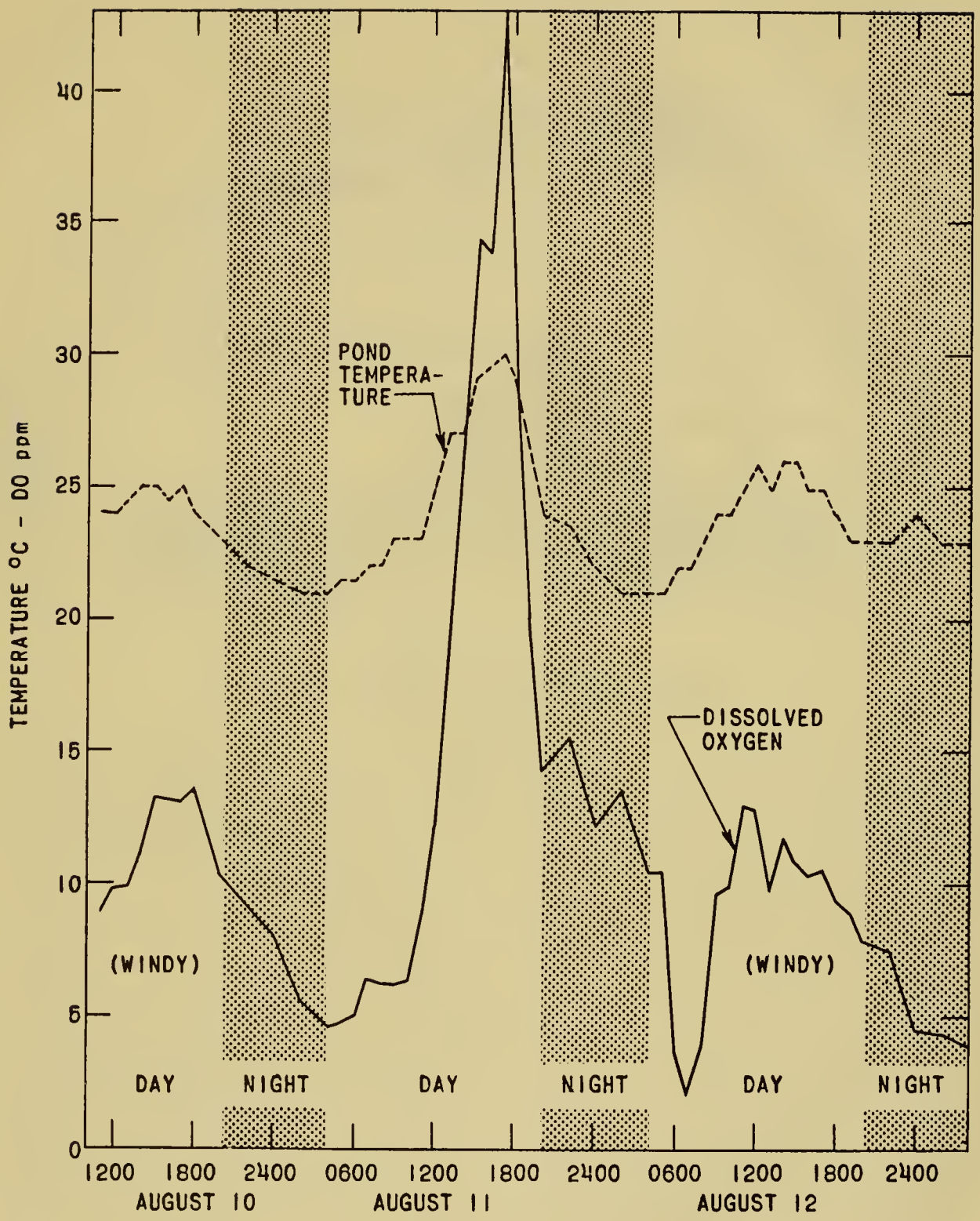
Figure 7

In sewage stabilization ponds algae are important for the oxygen they produce. California pilot plant studies of such ponds (Gotaas, Oswald and Golueke, 1954) showed an average yield of 1.65 pounds of photosynthetic oxygen for each pound of algae produced. In spite of this benefit, the fact remains that an algal residue is left and must be disposed of. In the pond, or in other receiving waters, the algae continue to draw upon available oxygen for their respiration while alive and are oxidized by bacteria when dead. Experience to date indicates that algae in such effluents generally die and decompose at a sufficiently slow rate that their deoxygenating influences are spread over a long stream reach and, consequently, do not produce acute oxygen depressions. In theory, however, an equitable materials balance must show not only the oxygen produced in growing the algae, but also the oxygen required to oxidize them to a stable state. In such a balance, the benefits of algal photosynthesis appear less attractive.

The necessity to include in evaluations of the oxygenating benefits of algae the oxygen required to decompose them is shown strikingly by recent developments in Lake Washington (Sylvester, Edmondson and Bogan, 1956). Domestic eutrophication has stimulated an increasing abundance of algae. It has been noted that oxygen consumption in the hypolimnion has increased greatly during the past 22 years because of the dropping down of increasing quantities of algae and organic derivatives from above. In 1933, 825 tons of oxygen were removed per month; in 1950 the rate was 1400 tons, and in 1955, 2190 tons. During the same period the minimum dissolved oxygen concentration near the bottom decreased from 6.4 ppm. to 3.5 ppm.

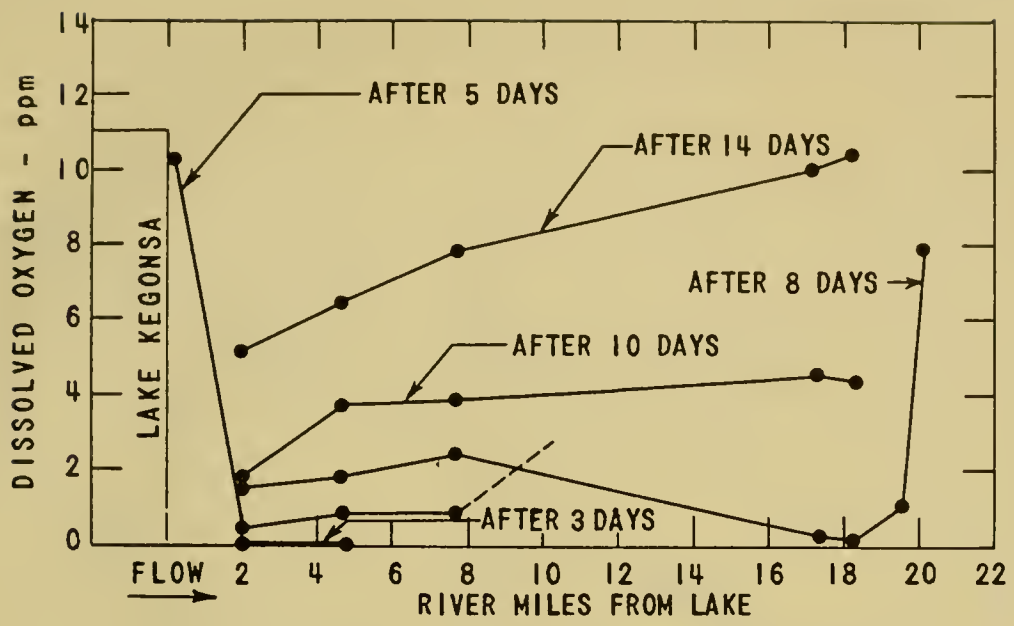
Elsewhere in lakes, severe odor problems have been encountered where planktonic blue-green algae, having abandoned their more uniform dispersion in the water, clump together in windrows and accumulate as a surface scum in protected areas. As they decay, such concentrated algal masses soon exhaust the available oxygen in the immediate surroundings so that gaseous products of anaerobic processes befoul the air.

In polluted water conducive to intense algal photosynthesis, super-saturation which so commonly occurs is wasteful, inefficient, and apparently even reportedly dangerous. In sewage stabilization ponds, oxygen concentrations exceeding 400% saturation have been observed. Because the rate of the B.O.D. reaction unfortunately is not accelerated by increasing the oxygen concentration, the surplus oxygen cannot be used for more rapid




EFFECT OF WIND ON DISSOLVED OXYGEN IN A TYPICAL RAW SEWAGE POND

Figure 8




EFFECT OF LAKE KEGONSA ALGAE ON YAHARA RIVER OXYGEN RESOURCES

Figure 9

waste stabilization and is readily lost to the atmosphere. This type of loss applies in principle to rivers also but usually occurs there at a much lower degree of intensity. For waste stabilization it would be far better that oxygen be available continuously at a lower but constant level than the "feast and famine" situation that occurs naturally.

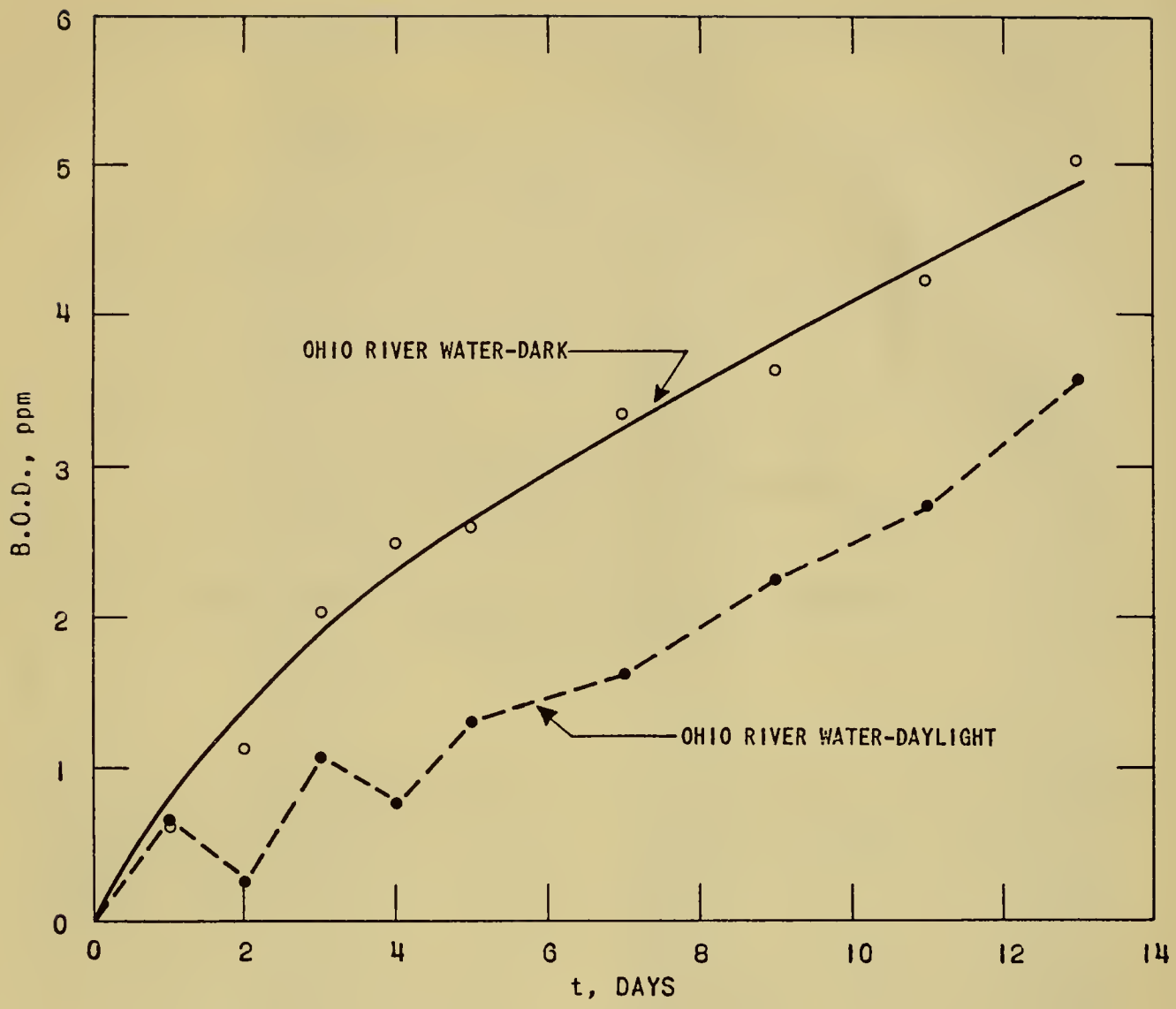
Death of fish has been reported in natural waters where high concentrations of dissolved oxygen were produced by a bloom of Chlamydomonas (Woodbury, 1941). At oxygen concentrations of 30-32 ppm., characteristic lesions of the fish consisted primarily of gas emboli in the gill capillaries and gas bubbles in the subcutaneous tissues. Although not tested, the gas was believed to be oxygen. Occurrences such as this are not common.

The Lower Fox River mentioned above is an example of a stream that receives its principal flow from a lake (L. Winnebago). During late summer and early fall, tons of algae produced in the lake enter the river and move downstream. Prominent in the phytoplankton are large quantities of Anabaena, Aphanizomenon, and Microcystis, which are more characteristic of standing than flowing water. These algae are reduced in numbers progressively downstream, apparently by dying off. Field and laboratory study showed that seasonal decomposition of such algae is a serious factor in decreasing the assimilation capacity of the river, which receives residual wastes from several cities and from pulp and paper mills.

Another more explosive example of excessive use of oxygen resources by algae occurred in the Yahara River in Wisconsin (Mackenthun, Herman and Bartsch, 1945, Published in 1948) where, in October of 1946, tremendous quantities of blue-green algae, almost entirely Aphanizomenon flos aquae, entered from Lake Kegonsa. Decomposing as they passed, wave-like, downstream, they depleted the oxygen supply, thus causing the death of tons of fish. Although algal toxicants were present, oxygen conditions were sufficiently severe to account for the fish mortality. The pattern of progressive oxygen depression and recovery that accompanied the mass movement of algae is shown in Figure 9. Fish mortality occurred 3-1/2 miles from the lake three days after algae entered the river, at 6 miles after five days, and at 18 miles after eight days.

### Effects of Algae on the B.O.D. Determination

The oxidation processes of algae that continue to occur in the absence of light in nature persist also in the laboratory in conduct of the widely used standard B.O.D. test (Anon., 1955). Ordinarily, the test result is used with other data to estimate the oxygen conditions that will occur at selected stream points in response to waste loading at others. Sufficient numbers of algae in stream samples collected as a step in such stream analysis affect applicability of the B.O.D. result because: (a) incubation in darkness for the standard five-day



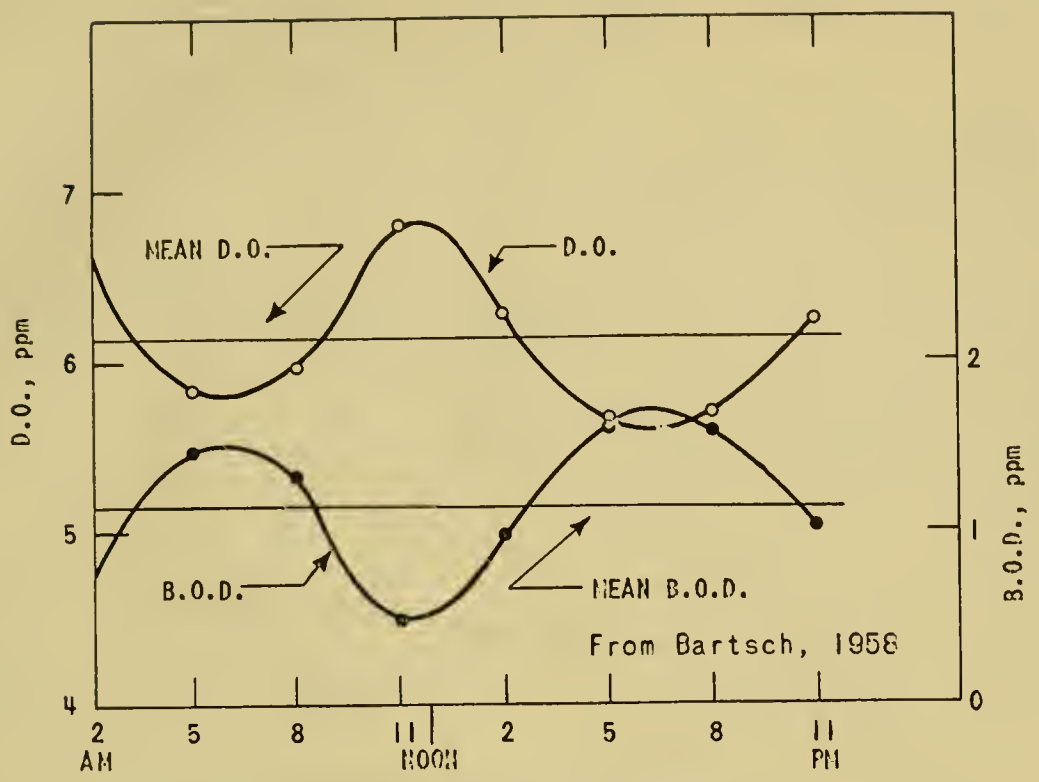

INFLUENCE OF ILLUMINATION ON B.O.D. OF SAMPLE CONTAINING ALGAE
AUGUST 26, 1955

Figure 10

period may give an unduly high value because of algal respiration, death, and decay; (b) incubation with illumination as an attempt at improvement may give unduly low or negative B.O.D. (Figure 6) because of the addition of oxygen by photosynthesis; and (c) incubation with intermittent illumination, although superficially attractive, does not sufficiently simulate stream conditions in terms of temperature, illumination, availability of nutrients, and other factors.

A number of attempts have been made to modify the B.O.D. procedure to make the result more meaningful with samples containing algae. Abbott (1948) incubated duplicate samples in B.O.D. bottles for 48 hours, one bottle of the pair in darkness and the other exposed to light at a north window. Test results were expressed as a ratio, $\frac{O_L - O_D}{O_O - O_D}$, when $O_O$ is initial dissolved oxygen, $O_L$ and $O_D$ are concentrations after light and dark incubation respectively. Later, the procedure was modified further (Abbott, 1952) to measure the light energy during incubation with a hydrogen iodide actinometer.

In studying the influence of blue-green algae on the B.O.D. result, Wisniewski (1958) modified the test to determine separately the influence of living, dead, and variable concentrations of algae. Further studies on this problem are in progress at the University of Wisconsin, the Sanitary Engineering




INFLUENCE OF DIFFUSED DAYLIGHT ON B.O.D. SAMPLE CONTAINING ALGAE - AUGUST 26, 1955

Figure 11

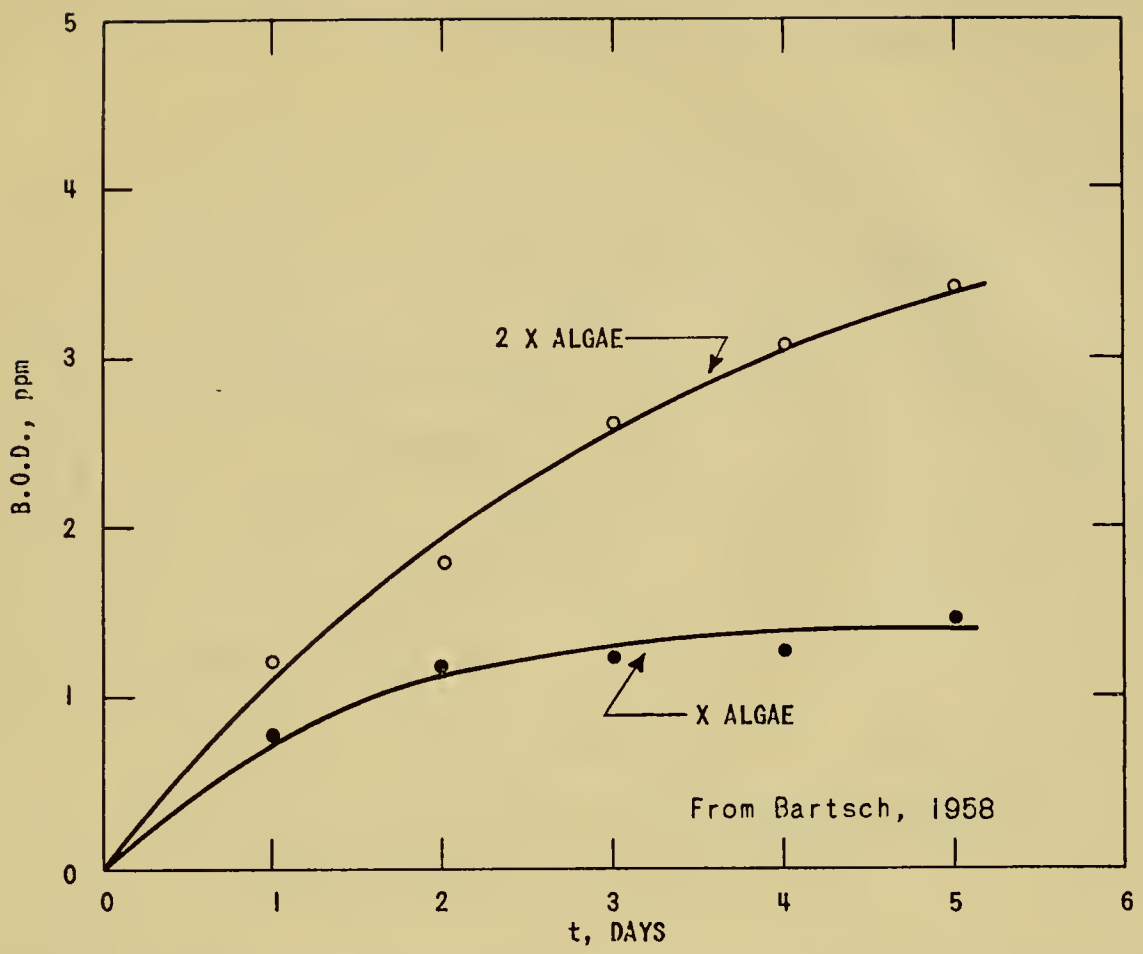
Center, and perhaps elsewhere.

Three areas of relationship between algae and the B.O.D. test are of particular interest. They are: (a) the effect of illumination, (b) quantity of algae, and (c) the influence of dead as opposed to living algae. Water from the Ohio River containing limited quantities of phytoplankton was incubated in an uncovered water bath at 20°C at an east window. Half the bottles were exposed to daily fluctuations in natural light; the others were covered with opaque material. At the same hour each day, duplicate bottles of each group were processed for B.O.D. concentration. The 13-day record is shown in Figure 10. The five-day B.O.D., after more closely simulating natural conditions, is only about half that derived by closer adherence to the standard procedure. The fluctuation in B.O.D. that occurred during the first five days resulted from intermittent sunny and cloudy weather. With continuous illumination, there was generally an excess of dissolved oxygen in the sample. In another test, when replicates were incubated in the dark for 19 hours, the B.O.D. was 2.77 ppm., but with natural light it was only 1.15 ppm. Furthermore, as shown in Figure 11, the mean B.O.D. of samples processed only at noon would have been, for those illuminated, 0.55 ppm., but for those in the dark, 2.10 ppm. - a difference of 400%! It is apparent that incubation in neither continuous light nor continuous darkness can effectively adjust for the photosynthetic and oxidation reactions related to algae.

To explore the influence of plankton quantity, B.O.D. samples were prepared so that one contained the phytoplankton removed from 20 volumes of Ohio River water, the other the quantity from 40 volumes. Three samples are referenced "X algae" and "2X algae" in Figure 12, which shows increasing amounts of algae result also in increasing B.O.D.

In the standard B.O.D. test with its five-day incubation in the dark, respiratory oxygen demand of living algae gradually is replaced by an oxygen-consuming attack by bacteria, protozoa, and other organisms as the algae die. The extent and rapidity of such transition in bottled samples is not known although, as pointed out, the same situation in principle has been observed repeatedly in natural waters.

Chlorella variegata and sewage were added to standard dilution water to give a fixed sewage concentration of 0.5% and an algal density of 1.22

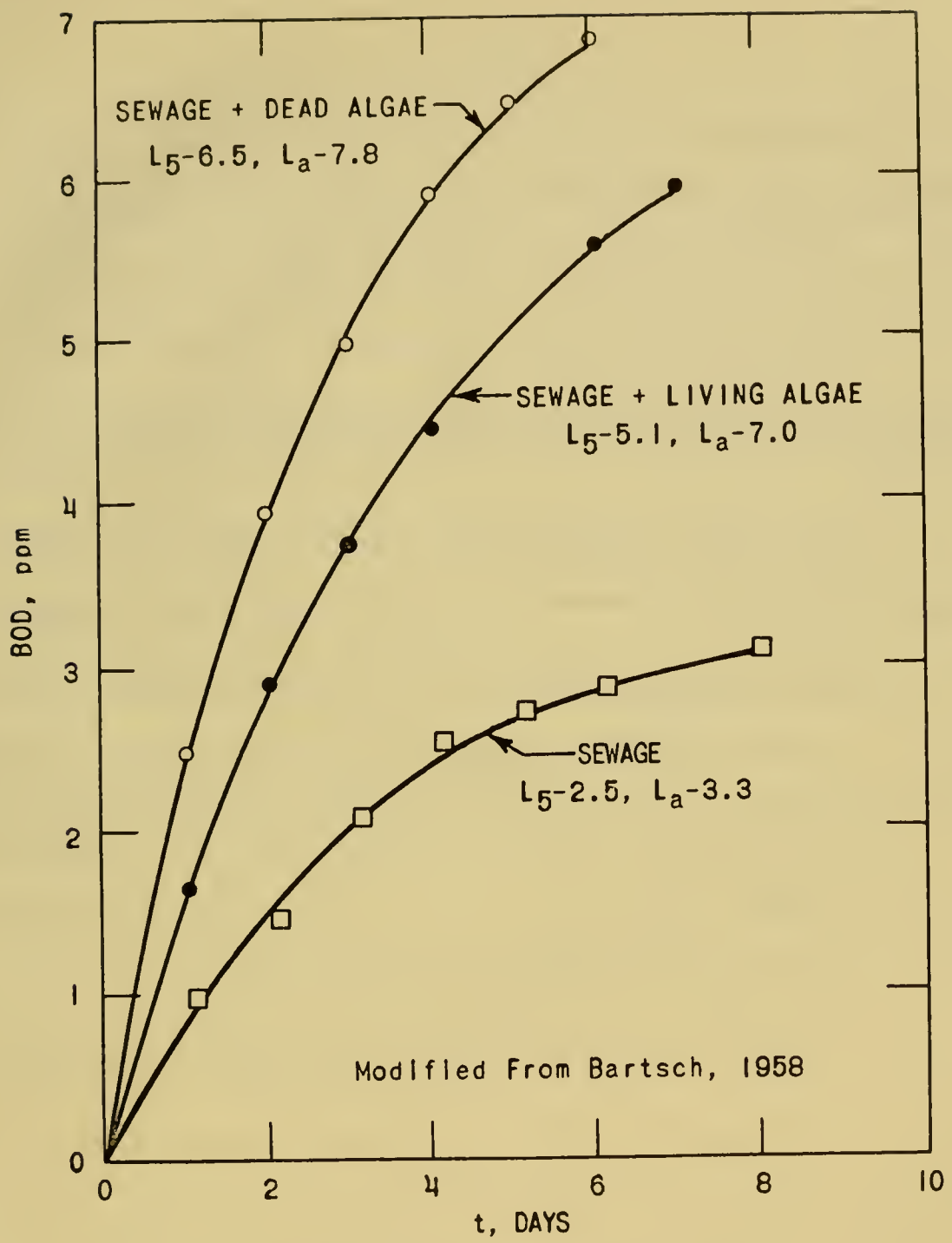



INFLUENCE OF QUANTITY OF ALGAE ON B.O.D.
AUGUST 26, 1955

Figure 12

million cells per ml. The algae added to one portion of the dilution water-sewage mixture were killed by short exposure to heat at 70°C; the others were living. B.O.D. values with living and dead algae were compared with values obtained when the medium contained sewage alone (Figure 13). Although oxygen demand with dead cells was a little greater, it is believed that the ultimate first-stage B.O.D. ($L_a$) in either case would have been about the same. Under identical laboratory conditions, when dead Chlorella cells were added to samples in relative quantities of 1, 10, and 66 (18,330, 183,300, and 1,222,000 cells/ml. respectively), the resulting B.O.D. values were 3.21, 3.73, and 7.74 ppm. respectively. On the basis of the Chlorella tests, it appeared that the contribution to the five-day, 20°C B.O.D. approximated 0.2 ppm. per billion cells.

Some workers believe that dense populations of algae in polluted waters are always beneficial and desirable. This view undoubtedly is supported by the common observation of high dissolved oxygen concentrations in their presence and failure to note the nocturnal depressions. Concurrent rapid production of a more stable mass of organics in the form of algae from readily putrescible wastes in effect postpones satisfaction of the oxygen demand to some later time at a different place. When conditions are favorable, such postponement may be desirable; when unfavorable, as in examples cited, algae make existing pollution conditions even worse. The oxygen released to the water in algal photosynthesis is momentarily beneficial in spite of the frequent inefficiency in its use. To evaluate equitably the algal source of oxygen, the processes by which algae along with the other biota also consume oxygen must be included in the tally.




INFLUENCE OF ALGAE ON BOD

Figure 13

Acknowledgment

The data used in preparation of Figures 10-13 were obtained jointly with Dr. W. M. Ingram, Dr. E. C. Tsivoglou, and Mr. D. G. Ballinger, all of the Sanitary Engineering Center. Their willingness to allow the information to be used here is gratefully acknowledged.

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# ORGANIC PRODUCTION BY PLANKTON ALGAE, AND ITS ENVIRONMENTAL CONTROL[1]

John H. Ryther
Woods Hole Oceanographic Institution

Roughly three quarters of the earth's surface is inhabited by an algal flora. Only on land and in a few shallow water areas do the higher plants prevail. Does it follow, then, that the non-vascular plants dominate the earth in terms of the biomass and productive capacity? In some coastal waters there are vast beds of giant seaweeds representing several thousands of grams of organic matter per square meter (i.e. Blinks, 1954), some of the densest stands of vegetation known. However, over 99% of the oceans are too deep to permit the growth of attached plants and the flora is represented almost entirely by the microscopic, unicellular algae, the so-called phytoplankton. The sole exception to this is the sporadic occurrence of floating seaweeds which may accumulate locally, as in the Sargasso Sea, but are quantitatively insignificant in the oceans as a whole.

There have been but few attempts to measure directly the standing crop, in terms of biomass, of the phytoplankton. But there have been a large number of measurements of their photosynthetic pigments, particularly during 1957 - 1958, as part of the oceanographic program of the International Geophysical Year. Chlorophyll a values for the upper, illuminated layers of the Atlantic Ocean were found to range usually between 0.1 and 0.5 $mg/m^3$, averaging perhaps 0.25. If we may assume a chlorophyll a:dry weight relationship of 1:100 in phytoplankton (Harris and Riley, 1956) the dry weight/$m^2$ of phytoplankton to a depth of 100 meters averages no more than 2.5 grams. Adding a generous 0.5 grams to allow for the richer coastal waters and to include the benthic algae, the oceans support an average standing crop of about 3.0 $g/m^2$ or, for the $361 \times 10^6 km^2$ of the hydrosphere, a total weight of some $1.1 \times 10^{12} kg$.

From various sources (Schroeder, 1919; Fawcett, 1930; Show, 1949; Brown, 1956) we may divide the land surface into the following relative proportions:

| | | |
|---|---|---|
| 1. | Wasteland (desert, artic regions, mountains) | 50% |
| 2. | Cultivated land, grasses, sedges, brush, etc. | 20% |
| 3. | Forests | 30% |

Let us assume that the first category, half of the land area, supports a negligible fraction of its vegetation. From various agricultural statistics, crop yields were found to average about 1000 $g/m^2$/year. According to Pearsall and Gorham (1956) natural stands of grasses, sedges, bracken, etc. produce from 400 to 1400 $g/m^2$/year, about the same as cultivated land. Since these crops are seasonal in most of the world, the average standing crop on an annual basis would be less, perhaps 50% of the annual production. The mean standing crop of this second category may be taken, then, as 500 $g/m^2$ over an area of roughly $30 \times 10^6 km^2$ for a total biomass of $1.5 \times 10^{13} kg$.

Ovington and Pearsall (1956) have estimated the annual production of forest trees in Great Britain from the weight of selected samples and the age of the trees. Working backwards from their data, the standing crop of their forest trees was found to range from 10,000 to 40,000 $g/m^2$, an average of 25,000 $g/m^2$. Extropolating this to the $45 \times 10^6 km^2$ of the world's forests, we may estimate a crop of trees of $1.1 \times 10^{15} kg$.

Summarizing these standing crop estimates, we have:

| | | |
|---|---|---|
| Oceans | | $1.1 \times 10^{12} kg$. |
| Land | | |
| | Wasteland | 0 |
| | Crops, grasses, etc. | $1.5 \times 10^{13} kg$. |
| | Forest | $1.1 \times 10^{15} kg$. |

Thus it appears that the higher plants, occupying no more than 1/8 of the area inhabited by the algae, maintain a biomass more than 1,000 times greater. Crude though these figures may be, they probably minimize the contrast between the two plant groups, since the values for the aquatic plants, if anything, have been exaggerated, while the terrestrial stands were probably underestimated.

What of the productive capacity of the land and sea? Does it follow that the algae are equally insignificant in the annual production of the earth's organic matter? There have been several recent studies of organic production in restricted marine areas on an annual basis (Steemann Nielsen, 1937, 1951; Riley, 1956, 1958; Ryther and Yentsch, 1958; Menzel and Ryther, in press), and many more scattered single observations in different parts of the world's oceans (i.e. Riley et al, 1949; Steeman Nielsen and Jensen, 1957). From these sources, we may place the mean value for net oceanic

[1]Contribution No. 1049 from the Woods Hole Oceanographic Institution and Contribution No. from the Bermuda Biological Station. Supported in part by contracts AT(30-1)-1918 and AT(30-1)-2078 with the U.S. Atomic Energy Commission and by NSF Research Grant G-3234.

production (that part of the plant's production in excess of its respiratory requirements) at approximately 100 g/$m^2$/year or 3.6 x $10^{13}$kg/year for the oceans as a whole.

Returning to our earlier consideration of land plants, it was estimated that the category of agricultural crops, grasses, etc. produce about 1000 g/$m^2$/year or 3.0 x $10^{13}$kg/year over its alloted 30 x $10^6$$km^2$. Making another bold assumption that the average forest tree is 50 years old (actually, this could be halved or doubled without greatly affecting the end result) the crop of 1.1 x $10^{15}$kg of trees would yield an annual production of 2.2 x $10^{13}$kg/year. This is equivalent to about 500 g/$m^2$/year, roughly the lower limit of the values obtained by Ovington and Pearsall (1956) for 20 - 40 year-old forests.

Summarizing the production estimates, we have:

| | |
|---|---|
| Ocean | 3.6 x $10^{13}$kg/year |
| Land | |
| Wasteland | 0 |
| Crops, grasses, etc. | 3.0 x $10^{13}$kg/year |
| Forests | 2.2 x $10^{13}$kg/year |

Calculations of this second type have been made before, but it is of some interest and value to repeat them using independent methods and data. Schroeder's (1919) value for the land (3.8 x $10^{13}$ kg/year) and Steemann Nielsen and Jensen's (1957) value for the oceans (2.4 - 3.0 x $10^{13}$kg/year) are both somewhat lower than those reported above, but are nevertheless in surprisingly good agreement with them.

The interesting fact which emerges from all this is that the annual rate of organic production on land and in the sea is about the same despite the fact that the latter is accomplished by a flora less than one thousandth the biomass of the terrestrial vegetation. The explanation for this is that most of the bulk of land plants is in the form of slowly growing, non-photosynthetic structural tissue. If we were to consider the relative quantities of photosynthetic pigments in the two environments, the results would be quite different. In fact, it has been proposed by Gessner (1949) that the highest concentration of chlorophyll that can be attained in nature (1 - 2 g/$m^2$) is actually the same for both water and land. What does this mean in terms of the photosynthetic potential of the land and sea? This depends not upon the amount of chlorophyll per se, but upon the amount of sunlight absorbed by this pigment. Steemann Nielsen (1957) correctly points out that the chlorophyll which lies below the illuminated or "euphotic" zone of lakes or oceans has no bearing on the productive potential of the area. In this connection, however, it would be difficult to estimate the chlorophyll in a comparable "euphotic zone" of a forest, where all of the pigment is probably never illuminated at any one time.

This difficulty can be circumvented if we look at the problem from another viewpoint and consider a situation in which all of the sunlight falling on a unit of the earth's surface is effectively absorbed by photosynthetic pigments. Stipulating these conditions in both a terrestrial and aquatic environment, how much of the solar energy will be converted to organic matter in each case? This, of course, depends upon the efficiency of the photochemical process, the quantum yield of photosynthesis.

The quantum yield of photosynthesis has been one of the most thoroughly studied aspects of plant physiology. Reviews by Rabinowitch (1951) and others reveal that, under similar conditions, approximately the same number of quanta of light are required to reduce one mole of $CO_2$ to carbohydrate by a wide variety of plant types; the process, in other words, seems to be largely species independent.

We have recently attempted to calculate the quantum yield of photosynthesis under completely natural conditions, considering the efficiency of utilization of light falling on a square meter of the earth's surface (Ryther, in press). The assumption was made that all of the light, except that lost by reflection and backscattering, was absorbed by photosynthetic pigments and that all other conditions for photosynthesis were optimal. In this treatment it was necessary to take into account the spectral composition of daylight and the photosynthetic utilization of light of this mean spectral composition. Particularly important was a consideration of the effects of light intensity. Photosynthesis is proportional to intensity up to about 1,000 foot candles, approximately 10% of full sunlight. Above this, the process becomes light saturated, and at still higher intensities may be actually depressed. Obviously, photosynthetic efficiencies decrease rapidly as plants are exposed to increasing intensities above the saturation point. At the same time, however, the higher intensities are effective in illuminating organisms deeper in the water of a planktonic community or the leaves farther down in a thick forest. Thus, while efficiencies are decreasing, production continues to increase with higher intensities of solar radiation. Individual algal cells or leaves become light saturated but entire plankton communities or forests do not. Figure 1 shows the striking similarity between plankton algae (Ryther, 1956a) and pine trees (Kramer and Clark, 1947) in this respect.

The resulting efficiencies which were obtained after corrections for respiratory loss, were equivalent to a yield ranging from 8 - 19 grams of dry organic matter/$m^2$/day for radiation values of 200 - 400 langleys/day (the range normally encountered over most of the earth). These theoretical

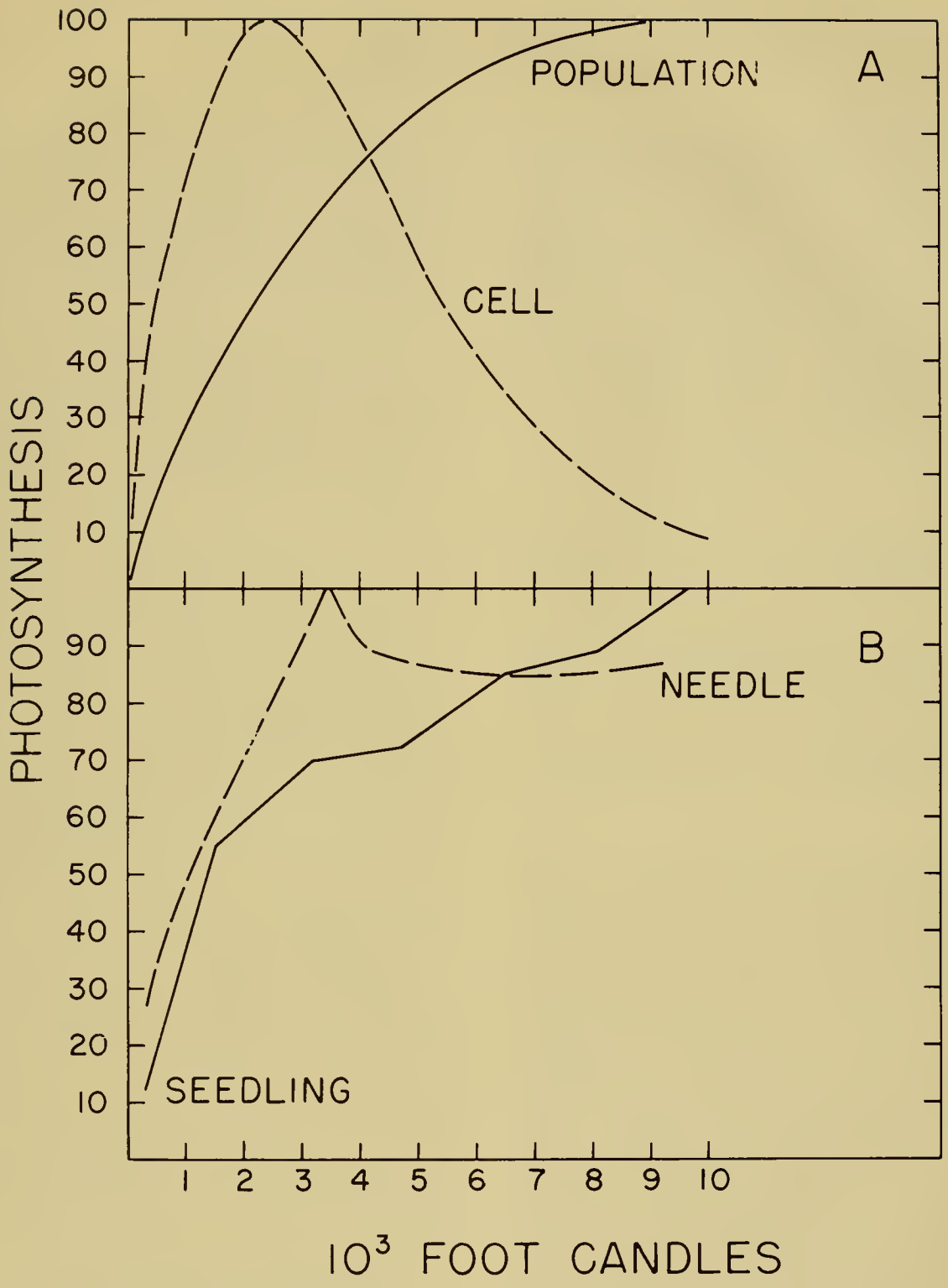


Figure Legends

Figure 1. Photosynthesis-light intensity curves for (A) individual phytoplankton organisms and entire phytoplankton populations (from Ryther, 1956) and (B) individual pine needles and entire seedlings (from Kramer and Clark, 1947).

Table I

Net organic production of various natural and cultivated systems in grams dry weight produced per square meter per day (from Ryther, in Press).

| | |
|---|---|
| A. Theoretical potential | |
| average radiation (200 - 400 langleys/day) | 8 - 19 |
| maximum radiation (750 langleys/day) | 27 |
| B. Mass outdoor Chlorella culture (Tamiya, 1957) | |
| mean | 12.4 |
| maximum | 28.0 |
| C. Land (maxima for entire growing seasons) (Odum, 1959) | |
| sugar cane | 18.4 |
| rice | 9.1 |
| wheat | 4.6 |
| spartina marsh | 9.0 |
| pine forest (best growing years) | 6.0 |
| tall prairie | 3.0 |
| D. Marine (maxima for single days) | |
| coral reef (Odum and Odum, 1955) | 9.6 |
| turtle grass flat (Odum, 1954) | 11.3 |
| polluted estuary (Ryther, et al, 1958) | 8.0 |
| Grand Banks (April) (Ryther and Yentsch, unpublished) | 6.5 |
| continental shelf (May) (Ryther and Yentsch, 1958) | 3.7 |
| Sargasso Sea (April) (Ryther and Menzel, in press) | 2.8 |

values were compared with some of the highest yields of organic matter which have been observed in nature. The data are reproduced here as Table I and they reveal that in cultivated crops and natural communities, on land or in the sea, in algae or in the higher plants, the maximum rate of production is very nearly the same and may closely approach the theoretical potential. As in laboratory quantum yield experiments, organic production in nature appears to be species independent and determined primarily by a photosynthetic potential common to all plants.

But the ideal conditions necessary for the attainment of this potential are seldom met in nature. Rather than producing 5 kg/$m^2$/year, the land averages no more than 10 - 20% of this, the sea perhaps 2 - 3%. The low mean production of the land is not hard to understand. Soils are frequently poor in quality or essential nutrients. Adequate moisture is often lacking. Carbon dioxide is thought to be usually, if not always, limiting to land plants, their yields being increased 2 - 3 fold when this gas is artificially introduced in greenhouse experiments (Maximov, 1930). Finally temperature, or climate, accounts for much of the discrepency between the potential and observed production of the land. A large portion of the culturable land on earth is found in temperate or semi-tropical climates where the plants have a limited growing season. An agricultural yield of 500 - 1000 g/$m^2$ may be achieved in such regions in 4 to 6 months.

But what of the ocean? Here there are no substrate problems; no lack of moisture. Carbon dioxide is probably never limiting due to the great reservoirs of dissolved carbonates and bicarbonates. Oceanic temperatures are always favorable for the growth of some species of phytoplankton. In the words of Henderson (1913) "No philosopher's or poet's fancy, no myth of a primitive people has ever exaggerated the importance, the usefulness, and above all the marvelous beneficence of the ocean for the community of living things." This ideal environment yields an average crop of organic matter which is almost two orders of magnitude less than the biotic potential of its producers. Only because of its vast area does the ocean equal the land in its over-all organic production.

The reasons for the apparent paradox lie in the restriction and limitations which the planktonic e existence impose upon plants with respect to two basic requirements, light and nutrients. We shall consider these separately.

Though the oceans are some three miles deep, over 95% of their waters are in virtual darkness, uninhabitable by photosynthetic organisms. The light intensity at which photosynthesis balances respiration, the "compensation intensity", varies somewhat with the species and physiology of the plant, but is of the general order of 100 foot candles. This is equivalent to about 1% of full noon sunlight incident to the surface. Below this compensation intensity, no growth of plants is possible. Thus the oversimplified but useful concept has come into use of a "euphotic zone", the depth through which plant growth can occur, which has as its lower limit the depth of penetration of 1% of the incident surface radiation. In the clearest ocean water the euphotic zone extends to about 120 meters.

But this clearest ocean water contains no plants, so a euphotic zone of 120 meters is hypothetical. Let us introduce an algal population into this clear solution which we will further stipulate to be adequately enriched with all the vital plant nutrients. Immediately, the plants themselves will absorb and scatter the light, and the euphotic zone will become progressively shallower as the population grows. Thus, the phytoplankton, shutting out its own light, becomes self-limiting. But as the euphotic zone becomes shallower, a progressively larger fraction of the light is absorbed by the plants, a proportionately smaller fraction by the water itself. Consequently, organic production increases with a decreasing euphotic zone. However, this relationship holds only as long as the decreasing euphotic zone results from the increase of living plants. Not all turbid waters are highly productive, for the turbidity is frequently caused by non-living particulate or dissolved matter. In Long Island Sound, for example, Riley (1956) estimated that two thirds of the incident radiation was absorbed by such material.

We may conclude, then, that photosynthesis in the ocean is not only restricted to a shallow surface layer, no more than 100 meters deep, but that for the process to proceed at its maximum potential rate, the plants must be concentrated in the upper five meters or less of otherwise clear ocean water. Let us now turn to a consideration of the nutrients available to support this production. For a convenient example we shall take nitrogen.

The highest concentrations of inorganic nitrogen in the oceans are present as nitrate at intermediate depths of approximately 1000 meters, and range from about 40 to 60 μg A $NO_3$ - N/l or .56 - .84 $gN/m^3$. Water from this depth rarely reaches the surface. The highest known concentrations of nitrogen within the euphotic zone are found in restricted areas where upwelling or divergences of water masses bring water from several hundred meters of depth to the surface. Rudd (1930) for example, reports values of about 40 μg A $NO_3$ - N/l in the surface layers of the Antarctic, one of the most fertile oceanic regions.

In most temperate and northern waters, the surface layers are enriched by winter cooling and mixing to depths of some 300 - 500 meters. The highest concentrations of nitrogen brought to the surface in this way range from 10 to 20 μg A $NO_3$ - N/l (R. F. Vaccaro, unpublished data for the North Atlantic). Let us assume that winter mixing has resulted in a surface enrichment of 15 μg AN/l or 210 mg $N/m^3$. In clear ocean waters with a 100 meter euphotic zone, the phytoplankton will have a reservoir of 100 m x 210 mg $N/m^3$ or 21,000 mg of nitrogen per square meter to draw upon. However, as the population grows, it not only depletes this supply, but creates in the process a progressively shallower euphotic zone with a correspondingly smaller reservoir of nitrogen. By the time the phytoplankton have increased to a population containing 10 mg of chlorophyll/$m^3$, production is limited to a euphotic zone of about five meters (Riley, 1956). This water contained an initial amount of only 1050 mg N and, of that, 500 mg were utilized in producing the population (assuming the plants to contain about 1% chlorophyll and 10% nitrogen). We may calculate the daily rate of production from the chlorophyll and depth of the euphotic zone for a day of average incident radiation, (300 langleys) according to the equations of Ryther and Yentsch (1957). From these calculations it is estimated that 1.8 grams of organic matter will be produced and about 180 mg nitrogen concurrently consumed within the five meter euphotic zone each day. Thus, after a population equivalent to 10 mg chlorophyll/$m^3$ has developed, there is sufficient nitrogen remaining in the resulting shallow euphotic zone to sustain production for less than three days. Clearly in a static situation such as we have pictured, high levels of organic production approaching the potential discussed above can scarcely be attained, much less maintained.

The oceans as a whole are not, of course, a static environment, but their surface waters are highly variable in this respect. In certain restricted areas hydrographic or meteorological forces bring water from intermediate depths to the surface. This happens when two water masses diverge, as in the equatorial Pacific (Sette, 1955), and most notably along the West Coasts of continents, where prevailing offshore winds produce a surface current which moves seaward, this water being replaced with upwelling, rich, deep water. It is for this reason that the coastal waters off Peru and parts of West Africa are among the most fertile regions of

the sea. However, if one were to follow a given parcel of water as it is brought to the surface and subsequently is transported horizontally, one would probably observe the same sequence of events which we discussed above. Steemann Nielsen and Jensen (1957) have described this for the coast of Africa, pointing out that the freshly upwelled water, though rich in nutrients, is poor in phytoplankton. It is "new" water, in which the plants have not had time to grow. As one moves seaward, following the path of the surface current, the plankton becomes more and more dense, passes through a maximum, and then decreases ultimately to a very sparse population by the time the water has reached mid-ocean. The time course of this sequence is probably not very different from that of a perfectly stable water mass which is enriched by winter mixing. The difference is that high production in an upwelling area is maintained at a given geographical location. Sette (1955) has described a similar geographical sequence "downstream" from the mid-Pacific equatorial divergence.

In contrast to these dynamic situations, which are comparatively rare in the oceans as a whole, we described above a static system in which the surface waters are enriched by winter mixing. The term "static" refers here to the absence or minor effects of horizontal advection, not to the absence of vertical water movements. Let us now return to this situation and consider it in more detail.

During the winter in temperate and northern regions, surface waters cool sufficiently to destroy the summer thermocline, and the waters become mixed to 300 - 500 meters, several times the depth of the euphotic zone. Not only are the nutrients from below the euphotic zone brought up and mixed with the impoverished surface layers, but the plankton algae are transported downward and spend a considerable fraction of their time in darkness. As a result, though nutrients are plentiful, production is severely curtailed due to the limitation of light.

With the return of spring, the surface waters begin to warm up, a seasonal thermocline develops, and the euphotic zone becomes stabilized against vertical mixing. At the same time, radiation increases. Those phytoplankton which find themselves in the euphotic zone are held there and suddenly have access to both light and nutrients. The stage is set for the "spring bloom", a feature characteristic of the temperate oceans. We have shown on the previous pages the succeeding events, terminating in the exhaustion of the nutrient supply. During a period of fine, calm weather in March or April, the whole process may run its course within a week or two. More typically, the formation of the summer thermocline is interrupted by storms, periods of cold weather, etc. and the spring flowering may then be prolonged, at a lower level, for a period of one or two months. But its days are numbered by the supplies of nitrogen, phosphorus, and the other essential elements which are limiting to plant growth in the sea. As we have seen, the amounts of these substances brought to the surface by mixing are small to begin with, and they are quickly consumed.

What happens next is a matter of some controversy. Some believe that most of the nutrient-deficient plants sink, their density increasing with old age. Evidence for this is the accumulation in summer of relatively high concentrations of chlorophyll at or near the lower limit of the euphotic zone. Others hold that the ultimate fate of the plants is to be eaten by the animal members of the planktonic community (i.e. Harvey et al, 1935; Cushing, 1958). Whatever happens, the spring maximum soon gives way to a summer minimum during which time production proceeds at a very low level which is probably maintained by the complete recycling (assimilation, death or consumption, and regeneration) of a small fraction of the winter nutrient budget within the surface layers.

In the fall, when cooling again destroys the seasonal thermocline, there may be minor and irregular outbursts of plant growth as the surface layers are alternatively cooled and mixed to a slight degree and then restabilized. These small blooms terminate with the final disappearance of thermal stratification and the return of winter conditions.

At the semi-tropical latitude of Bermuda, in the Sargasso Sea, the annual cycle of organic production is much the same as that pictured above, with the major difference that production persists at a relatively high level throughout most of the winter. This is due to the fact that winter cooling and mixing is less pronounced than in more northern waters, and never, in fact, extends below the depth of the permanent thermocline at 300 - 400 meters. In addition, incident radiation is higher at these latitudes, and the water is exceptionally clear. As a result of this combination of factors, the plants are never carried down out of the light for sufficiently long periods to prevent their growth. Figure 2 shows the seasonal cycle of organic production in the Sargasso Sea off Bermuda. The accompanying seasonal profile of temperature in the upper 700 meters will illustrate how hydrographic conditions influence plant growth. Note that high production is correlated with a well-mixed, largely isothermal layer in the upper 400 meters; low production, with the thermally-stratified summer conditions.

The nutrient supply available to the plants in this 400 meter deep reservoir in the Northern Sargasso Sea is extremely low--an order of magnitude less than that present in the temperate seas. Nitrate, for example, seldom exceeds 1.0 µg AN/l in the euphotic zone. Consequently, plant

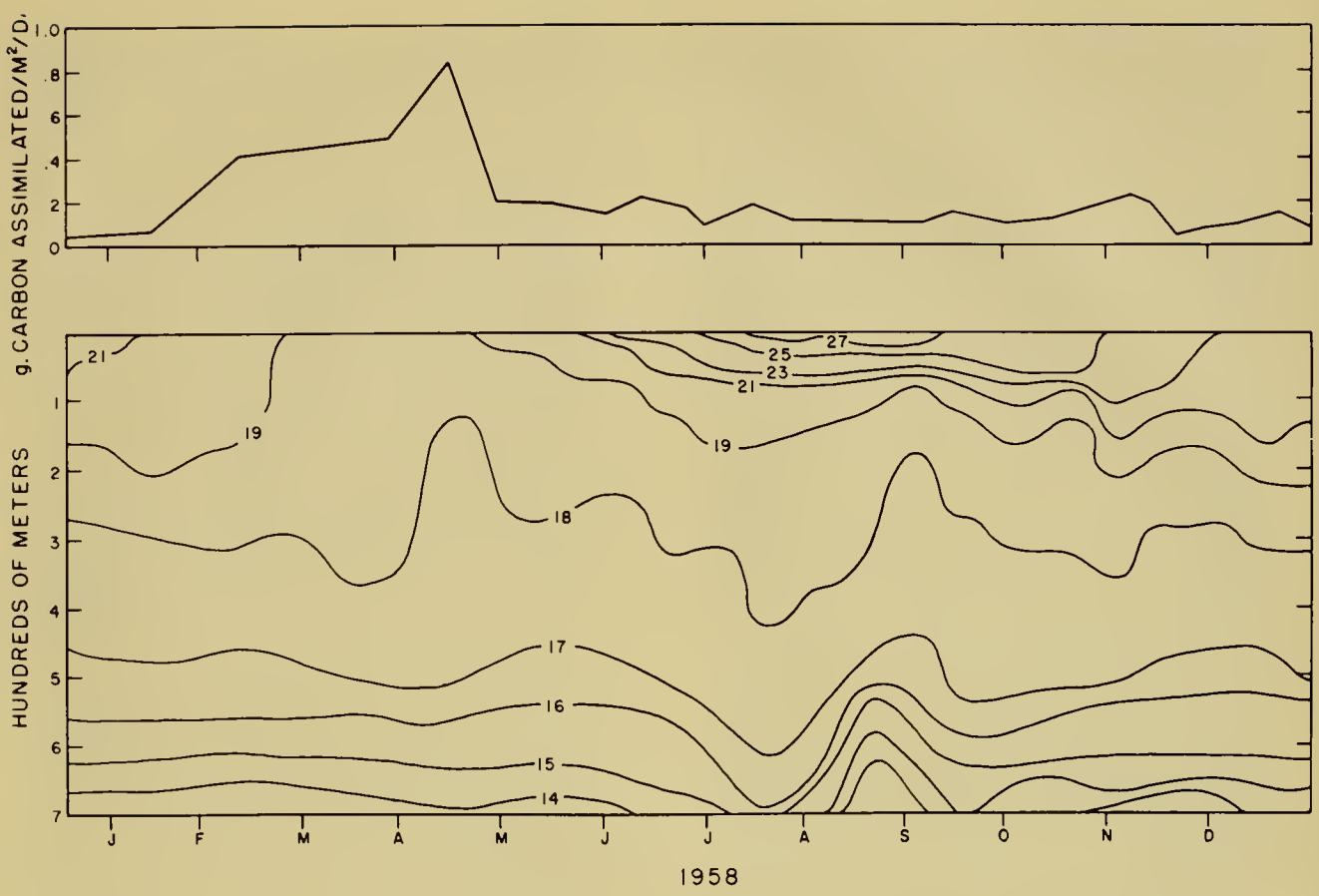


Figure 2. The seasonal profile of temperature to 700 meters and net organic production in the Sargasso Sea off Bermuda for the period January - July, 1958 (from Menzel and Ryther, in press).

production per unit volume is never high--the process cannot build up towards its biological potential. In contrast to a dense flowering in the upper five meters of temperate seas, the Sargasso Sea plankton grow over a euphotic zone which is never less than 50 - 100 meters deep. What maintains this organic production in the face of such poverty of nutrients? Low as they are, the concentrations of nitrate, phosphate, and silicate (to name a few essential elements which have been studied) never change appreciably in these surface waters. We don't yet understand this, but it appears that in these relatively warm waters the whole cycle of assimilation, consumption, death or excretion and remineralization occurs very rapidly. The amounts of plants and animals and minerals are small, but the metabolic wheels turn fast.

No study of the seasonal cycle of organic production has been made in tropical waters, but we can imagine what it must be like. In the true tropics there is no winter cooling of the surface waters, no mixing, no replenishment of the euphotic layer with rich aphotic waters. The seasonal thermocline of temperate and semi-tropical latitudes becomes a permanent, thermal barrier to vertical mixing.

On March 1, 1959, a short oceanographic section was made by Research Vessel Crawford between 24° and 35° North latitude at the longitude of Bermuda. At that time of year, the surface water temperatures in the North Atlantic Ocean are at their seasonal minimum; vertical mixing is most pronounced. Actually the 1959 season was somewhat early. Our seasonal study at Bermuda revealed the beginnings of warming and stratification by March 1, and the spring phytoplankton pulse was in full bloom on that date. Figure 3 shows the temperature profile and values of organic production for this section running from North to South. It is remarkably similar to the seasonal cycle at Bermuda. The well-mixed, almost isothermal, highly productive stations at the northern end of the section merge into stratified, low productive stations to the South much as the spring flowering

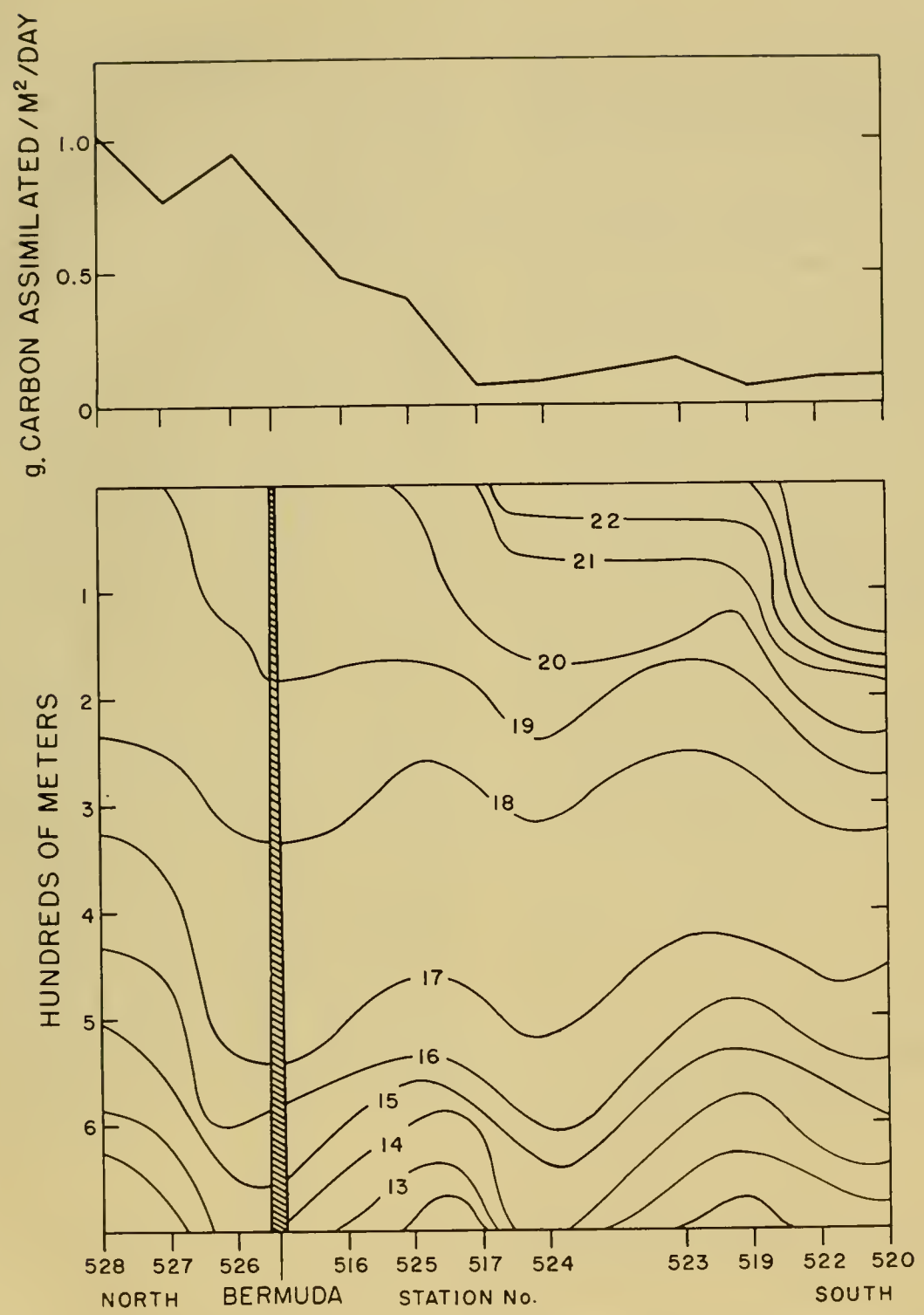


Figure 3. The profile of temperature to 700 meters and values for net organic production as measured on a section between 35° and 24° N. latitude at the longitude of Bermuda in March, 1959.

declines to the summer minimum at Bermuda. One can assume from this alone that organic production in the tropics is maintained at a low, rather steady rate throughout the year, probably maintained by the recycling of nutrients entirely within the euphotic zone, perhaps occasionally stimulated into brief minor outbursts of growth by the limited mixing action of storms.

It is clear, then, that organic production in the oceans is limited most of the time by either light or nutrients. Both are available in plentiful supply in the oceans as a whole, but both seldom occur together. At the few times and places where neither of these factors is limiting, production may proceed at rates comparable to the highest levels of production observed on land.

As mentioned earlier, the concentrations of nutrients in the tropics and semi-tropics are far less than those present in the high latitude seas. As a result much smaller populations of plants can develop. Yet, due to the rapid turnover of these materials, a low to moderate rate of production can be maintained throughout a deep euphotic zone. If one integrates production over the entire water column, the annual rate beneath a square meter of sea surface is as high or higher than that of presumably far richer waters. As an extreme example of this, let us compare the vertical profile of photosynthesis in the Sargasso Sea during a period of peak production (April 19, 1958) with that of a shallow, highly enriched sewage oxidation pond in South Dakota (from Bartsch and Allum, 1957). The chlorophyll concentration of the former averaged less than 1 $mg/m^3$, the latter some 450 $mg/m^3$. The oxygen production values of Bartsch and Allum have been converted to carbon fixation assuming an assimilatory quotient of 1.25 (Ryther, 1956b), and the depth curve of photosynthesis has been rather subjectively extrapolated to the surface. Examination of the depth profiles of daily production from the sewage oxidation pond and the Sargasso Sea (Figure 4) reveals an interesting fact. In the oxidation pond, the euphotic zone is two orders of magnitude smaller while production per unit volume is two orders of magnitude greater than in the Sargasso Sea. If one integrates the two curves, organic production beneath a square meter for the two areas is found to differ by less than 20%. Actually, the value for the oxidation pond is probably somewhat low, for the measurements were made from 10:00 a.m. to 3:00 p.m. rather than for an entire day. But even if this figure is increased by 25% - 50%, the similarity between the two situations is striking.

This may appear to contradict the earlier statement that production per unit area may be expected to increase with a decreasing euphotic zone. It should be reiterated here that such is true only in cases where living plants alone contribute to the turbidity of the water. In a pond receiving raw sewage wastes, this would hardly be the case.

But the point which I wish to make in comparing these two situations is this: that the daily rate of production of organic matter, as it is currently defined by ecologists, is very nearly the same in two bodies of water in which the amounts of living plant material are respectively of the order of 100 grams and 0.1 grams per cubic meter.

What, then, does this rate of primary production actually mean? One looks in vain for evidence of it in the clear, blue waters of the Sargasso Sea. The major fisheries of the world are located in the temperate or high latitudes, or in the few regions of divergences and upwellings which we discussed above, not in places like the Sargasso Sea. Is it realistic to compare fertility of northern and tropical seas, of the ocean and the land, of a plankton bloom and a cornfield, all on the basis of their relative rates of natural photosynthesis?

In modern, dynamic ecology, it has become unfashionable to speak of the "standing crop" of organisms. The important question is not "how much is there?" but "how fast is it being produced?" There is no doubt that this concept has opened up new and extremely interesting avenues of ecological research. But the population ecologist or fisheries biologist should beware of these values. The sociologist who compares the productive capacity of the land and sea may be sadly deluding himself. For animals eat food, not photosynthesis. What is the significance of organic matter which is produced, consumed, decomposed and remineralized almost simultaneously? Why add up a daily production which is daily expended into a non-existent annual total. Is this comparable to a barn full of corn? The study of the rate of organic production has already and will continue to reveal fundamental physiological and ecological principles. But the person who examines these data with the hope of feeding an overpopulated earth on marine resources would do well to remember, when he picks a pound of beans from his kitchen garden, that to get the same weight of rather undigestable and unappetizing plankton algae from the open sea, he would need to filter some five million gallons of water.

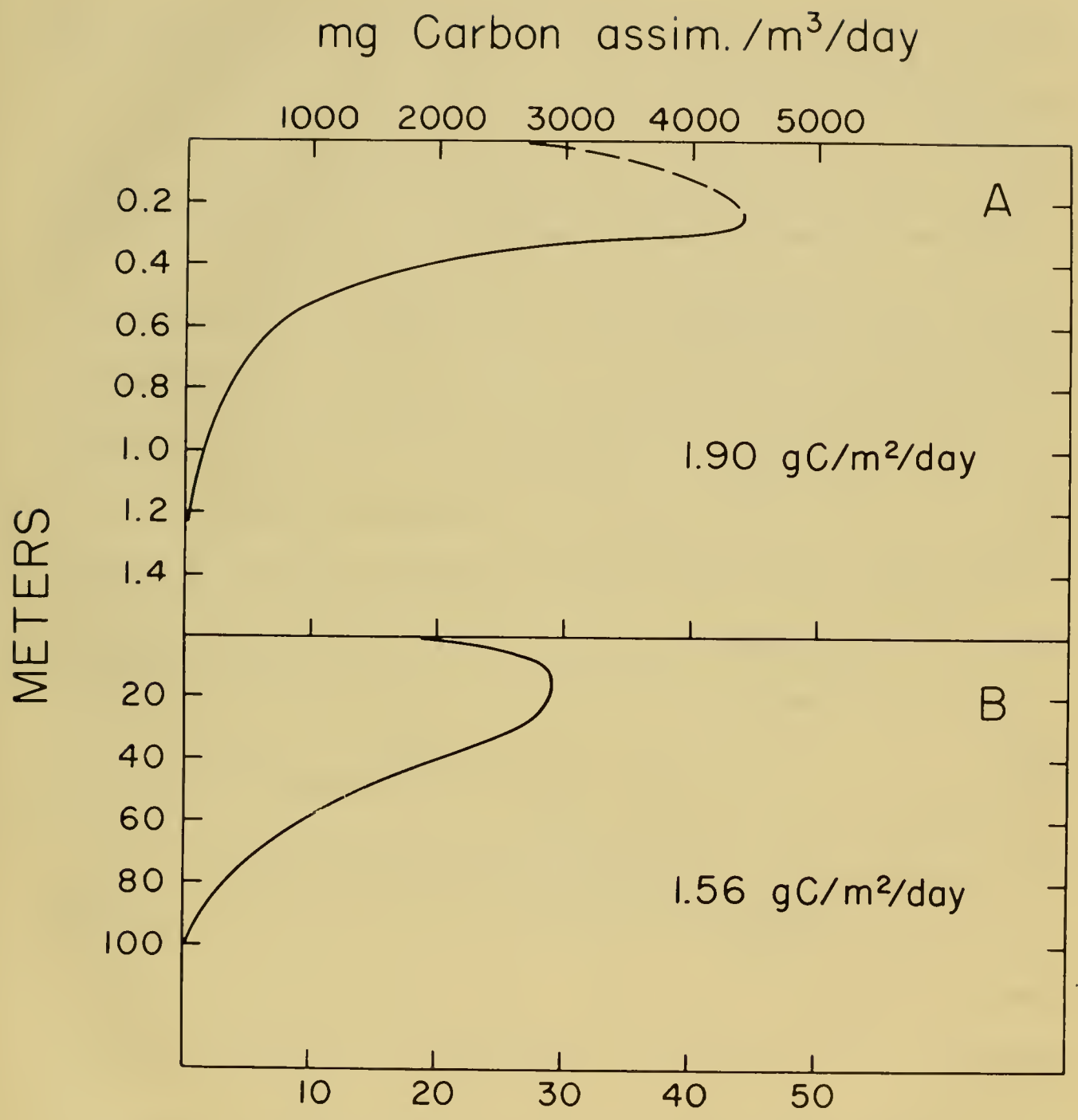


Figure 4. The vertical profile of daily photosynthesis in (A) a sewage oxidation pond in Lemmon, South Dakota (from Bartsch and Allum, 1957) and in the Northeastern Sargasso Sea (from Menzel and Ryther, in press).

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# ARTIFICIAL MEDIA FOR FRESH-WATER ALGAE: PROBLEMS AND SUGGESTIONS[1]

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Fresh-water algae seemingly are ubiquitous: as knowledge increases the same species are recorded from all continents. Because most fresh-water habitats are ephemeral, most species survive only if they can form stages resistant to dessication (Evans, 1958). Overcoming this ecological disadvantage permits species to be transported by high winds and birds all over the globe as constant inocula in search of opportune environments.

It is also well known that fresh-waters, unlike the almost homogeneous marine environment, display a wealth of environments and algal flora. The distribution of algal species in fresh-waters depends on one hand on the selective action of the chemico-physical environment and, on the other, on the organism's ability to colonize a particular environment and to compete with the other species living in the same ecological niche. The ecologist and the sanitation scientist seeking a rapid way to distinguish various environments rely more and more on biological markers, the indicator species which typify each environment more subtly and precisely than can laborious chemical analysis.

This knowledge is being extended and refined continuously. Meanwhile the ecologist would like to know more precisely the physico-chemical characters and boundaries of the ecological niches. A frontal chemical attack is difficult at present because many organic and inorganic substances are biologically effective and present in waters in extreme dilution. Another way to define the chemical environment is to study the nutritional needs of the indicator species. These, because of their restricted occurrence and narrow fit to their environments, are, presumably, outstandingly exacting for at least some important parameters (stenobionts). Few, if any, of these species have been cultured aseptically, the only way to determine their nutritional requirements. Ridding the algae of the unwanted microbial flora, though often tedious and delicate, is only occasionally the main obstacle. Agar-plating, washing and dilution techniques combined with the use of antibiotics are well-established methods [Pringsheim (1946); Provasoli et al., (1951), Lewin (1959), Provasoli and Holz (in press)]. The main difficulty is to design media which will support the aseptic growth of algae whose nutritional requirements are still unknown. A practical approach, when the indicator species of a specialized environment do not grow in present media, is to study the nutrition of a species of the same environment which can also live in closely related environments. An analysis of their nutritional requirements may show how to construct media adequate for the indicator species. Selection and construction of media would perhaps be facilitated if one had: a) data on the relative importance of the chemical components of the waters, b) data on the nutritional requirements of the different groups of algae, c) some guideposts in solving some of the problems of designing artificial media.

## Relative Importance of the Chemical Factors of the Environment

In dealing with organisms of unknown nutritional requirements one can obviously benefit greatly in mimicking as closely as possible the chemical environment.

Due to the diverse composition of fresh-waters one should know the following parameters in order of importance: a) total solids, b) the prevailing major ions, c) pH, d) main trace metals, e) ratios of monovalent/divalent cations and of Ca/Mg, and f) content of organic matter (which may act as trace metal solubilizers or as growth factors).

The pertinence of these considerations is emphasized by Rodhe's success (1948) in studying in detail the mineral requirements of Ankistrodesmus falcatus. At the end of his studies he compounded a medium in which all the major minerals were added at optimal concentration. This medium is very close to the composition of the waters of Lake Erken, where Ankistrodesmus blooms, and the average composition of the waters of many Swedish lakes. This medium proved to be satisfactory for most of the algal species inhabiting Lake Ekren and would probably sustain some growth of all the species when enriched with vitamins (at the time it was not known that many algal species require vitamins).

The medium of Rodhe might have been quite different had he chosen to study an eurybiont and not a stenobiont. The eurybiont, depending upon the species selected, would have led to construct several media specific for the species in question, and therefore very different from the natural waters.

The most important parameter, especially for oligotrophic algae, seems to be the total-solids

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concentrations. Chu (1942) succeeded in growing several oligotrophic fresh-water algae by simply diluting the well known old medium of Benecke. Later, by studying the mineral nutrition of several oligotrophic diatoms and chlorophytes, he compounded a medium (Chu 10) of wide use for oligotrophic organisms. The media experimentally designed by Chu, Rodhe and us (Provasoli and Pintner, 1953) are very similar. Further studies (Provasoli, McLaughlin and Pintner, 1954) confirmed that oligotrophic species in general require very low total-solids concentrations and are stenobionts toward this parameter. In the same analysis it was found that algae may prefer a mono - or divalent ion, but that they are in general far less exacting toward the Ca/Mg or Na/K ratio, and that, within large limits, the two monovalent and two divalent ions are interchangeable when the lower limits of the preferred ion have been satisfied, (Provasoli, McLaughlin, Pintner 1954, Droop 1958). However, it is possible that organisms living in dystrophic lakes may show the need for special ratios of major elements besides total solids concentrations.

Trace-metal content and pH are related factors because the solubility and availability of trace-metals varies with pH. Iron and Mn seem to be the two trace-metals ions which are quantitatively important. Zn, Co, Cu, Mo, and V are also important because required for growth, even though they are generally present in waters in far lower concentrations. They are generally present as impurities of other chemically pure salts and, depending upon the concentrations of major elements employed (especially in sea water media), one of them may be introduced in media at concentrations approaching inhibition if not toxicity. The use of metal chelators may be of advantage in chelating these metals and in giving the limited steady supply allowed by the dissociation constant typical for each divalent ion. Hence in mimicking natural conditions one should try to estimate the kind and content of organic substances in the water. "Humates" in alkaline or peaty waters are trace-metal chelators less strong in their chelating power than the chelator most used in artificial media (EDTA), but apparently more versatile in that they can be employed at different ph"s without causing toxicity (i.e. soil extract can be employed at pH's form 5-8.5). "Pollution" is generally thought beneficial to algae as a source of N and P. However, in the degradation of organic matter by microorganisms organic acids may be produced, especially amino acids, many of which are good trace-metal chelators. This is especially important in alkaline waters when the solubility of heavy divalent metals is almost nil. Iron was found by Uspenski and Uspenskaja (1925) to be the important trace-metal for several Volvox species. They were the first to introduce chelators (citrate) in fresh-water media to keep the iron available to the algae in neutral and alkaline media, because they suspected that this was the mechanism operating in nature. Volvox generally blooms in waters rich in organic matter. Further investigations on the nutrition of Volvox (Pintner and Provasoli, 1959) show that V. globator and V. tertius have scant heterotrophic abilities and that they do not need any preformed organic compound as sources of energy; the only organic compound needed is vitamin $B_{12}$. Their colonizing of water rich in organic matter is therefore due to the need of finding vitamins and, perhaps even more, to the need of having Fe solubilized by the "organic acids".

## Generalities on the Nutritional Requirements of Different Algae

The known requirements of the algae have been recently reviewed (Krauss, 1958; Provasoli, 1958). We will therefore consider only a few points.

a) Some nutritional requirements seem predominant or even unique in some algal groups. Silica is important and often a limiting factor for diatoms and perhaps also for the chrysomonads bearing silica plates. High Fe and trace metals are important for many euglenids and cryptomonads colonizing acid waters. Sodium and/or potassium are essential elements for blue-green algae. Diatoms have calciophilic and calciophobic species. Most algae utilize nitrates preferentially but the euglenids, so far cultured, can only utilize ammonia, and, some, amino acids as N sources. Many blue-green algae utilize atmospheric nitrogen.

b) Organisms in the same ecological niche often have common nutritional features. Most oligotrophic algae cannot withstand total solid concentrations above 100-200 ppm.; many are stenohaline. Algae in environments high in some substances (as the high concentrations of Fe and trace metals in bogs and ditches and the high S in heavily polluted waters) may merely be withstanding these conditions; some actually require them. The organisms of barnyards and sewage oxidation ponds in general withstand ammonia even at alkaline pHs.

c) Algae lacking photosynthetic pigments obviously need non photosynthetic sources of energy. Acetate, glutamate, aspartate, glycine, and glucose seem to be the preferred carbon sources for many. So far we know no chemotrophic algae.

However the possession of photosynthetic pigments does not exclude the possibility of heterotrophy, indeed, they may prefer it. While perhaps most pigmented algae are phototrophic, many are heterotrophic.

The taxonomic position of a species to be cultured offers indications of the probabilities.

Elsewhere (Provasoli, 1956) we have enumerated some of the nutritional hints derivable from the phyletic tendencies of algae so well described by Fritsch (1934). The Chlorophyceae and the Diatoms have strong vegetal tendencies; most of them can be expected to be phototrophic. Heterotrophy of the osmotrophic type is to be expected in genera which contain colorless species. The Chrysophyceae have a strong rhizopodial tendency even among the pigmented species and the Eugleninae and Dinophyceae have a strong tendency toward loss of pigments; many species can be expected to be heterotrophic or even phagotrophic. Two examples of the nutritional versatility of pigmented algae are Ochromonas and Euglena. Ochromonas malhamensis (Hutner et al., 1953; Aaronson and Baker, 1959), though pigmented and able under opportune conditions (high $CO_2$) to live photoautotrophically, is preferentially an heterotroph. In rich organic media, Ochromonas through many divisions, and until the organic nutrients are reduced to a low level, synthesizes only suboptimally its photosynthetic pigments: the cultures appear white and the large chloroplast is reduced to an anterior faint brown spot. However, when the organic solutes are low and nutrient particles (bacteria) are offered, it becomes a phagotroph (Aaronson and Baker, 1959). While Ochromonas can utilize three methods of nutrition the Euglena gracilis group is less versatile, but its photoautotrophy is as efficient as its osmotrophic heterotrophy. Therefore deductions based solely on the presence of photosynthetic pigments and type of environment may be misleading. Species living in, or restricted to polluted waters, may colonize these waters for entirely different needs and are not necessarily heterotrophs: Volvox grows there because it needs soluble iron and vitamins, while Ochromonas prefers heterotrophy and Euglena, besides its heterotrophic abilities, needs and tolerates $NH_4$ in alkaline waters.

c) Many algae require vitamins; a detailed tabulation is given in a recent review (Provasoli, 1958). No correlation has been detected between need in vitamins and source of energy employed or any particular environment; photosynthetic or colorless species, species living in oligotrophic or polysaprobic environments may need or not need vitamins. However, the incidence of auxotrophic species differs in various algae groups. (Table I). The Chlorophyceae and Bacillariophyceae have the lowest number of species requiring vitamins. The great majority of the Dinophyceae and Chrysophyceae needs vitamins and all the species so far studied of the Eugleninae and Cryptophyceae require vitamins. The need for vitamins seemingly predominates in algal groups having strong animal tendencies; most species of the algal groups having strong vegetal tendencies (Chlorophyceae, Bacillariophyceae, and probably Cyanophyceae) do not need vitamins. Although the sampling is very small, the data should be valid because the recently studied species (all except the Chlorophyceae) were not preselected by the choice of

Table 1. Vitamin requirements of algae

| Algal group | Number of Species | No Vitamins | Require Vitamins | $B_{12}$ | Thiamine | Biotin | $B_{12}$ + Thiamine | Biotin + Thiamine | $B_{12}$ + Biotin + Thiamine |
|---|---|---|---|---|---|---|---|---|---|
| Chlorophyceae | 40 | 25 | 15 | 6 | 8 | | 1 | | |
| Eugleninae | 9 | 0 | 9 | 2 | 1 | | 6 | | |
| Cryptophyceae | 9 | 0 | 9 | 2 | 2 | | 5 | | |
| Dinophyceae | 18 | 2 | 16 | 11 | | | 1 | 1 | 3 |
| Chrysophyceae | 13 | 1 | 12 | 3 | 1 | | 5 | 1 | 2 |
| Bacillariophyceae | 37 | 20 | 17 | 10 | 3 | | 4 | | |
| Totals | 124 | 46 | 78 | 34 | 15 | | 22 | 2 | 5 |
| Total requirement for single Vitamins | | | | 61 | 44 | 7 | | | |

isolation media (i.e. they were isolated from nature in media containing a mixture of known water-soluble vitamins).

It is remarkable that all the species which have photosynthetic pigments and the related colorless forms require only three vitamins; in order of decreasing incidence, vitamin $B_{12}$, thiamine, and biotin (Table I). Only the phagotrophic flagellates (e.g. Peranema) seem to need other vitamins and "building blocks".

## Problems in Designing Artificial Media

It is not generally realized that the classic media for algae (Knop, Beijerinck, Detmer) have been designed before 1900. No pH is mentioned, nor the type of phosphate (mono-, di-, tri-basic), and, often, if the salts are anhydrous or not. Unfortunately even the more recent media (up to 1920 and in some cases later) have the same defect. It is not surprising therefore that there exist different interpretations and modifications of these formulas. These solutions were apparently meant to be employed at their natural pH (i.e. pH 5 for Knop, 6.2 for the Detmer and 7.2 for the Beijerinck); they precipitate in the more alkaline pHs, yet they have been used successfully even at different pHs. These solutions are in general too concentrated and it was soon found that dilutions of 1:2 to 1:20 permitted growth of many more species. Most Chlorophyceae and many diatoms grow in these media.

In most algal media, especially the old ones, the only trace metal added is iron. This does not mean that the other trace metals are not needed: it is well known that algae need Mn, Zn, Co, Cu, V, Mo. It only reflects the degree of impurity of the major mineral constituents of the medium. It is worth noting that the industrial methods of purification of the "chemically pure" salts have undergone many changes since Knop's time resulting in an ever-changing sets of impurities. Besides, with the advent of plastic containers in industry we may expect increasingly pure salts and therefore possible nutritional deficiencies. New deficiencies (and perhaps toxicities) will develop when the normal glassware of the laboratory will be substituted by plastics with consequent absence of the impurities leaching from glass.

The addition of trace metal-chelate mixtures of media offers the possibility of minimizing the impurities (toxic or favorable) as well as furnishing a metal pool of available trace metals. Therefore chelated media should be more reproducible and withstand fluctuations in impurities of the chemically pure components of media. A further advantage is that chelators help in preventing precipitation - one of the main goals for reproducibility. A number of chelated media have been developed for a few species of fresh-water algae: Euglena (Hutner et al., 1956); Ochromonas malhamensis (Hutner et al., 1957); O. danica (Aaronson and Baker, 1959). These media were designed for maximal growth and rapid cell division. To obtain this goal, the media have been enriched, often up to the limits of osmotic tolerance, with as many preformed key metabolites as possible, to spare the organism most of the work of synthesis. Since the success of these media depends upon their exploiting all the externally accessible synthetic pathways (i.e. permeability) and any useful potential and tolerance of the organism in question, they become so highly tailored that seldom are they suitable for other organisms. Only a few chelated fresh-water media are of more general, though still restricted, use, as the Kratz-Myers (1955) medium for Anacystis nidulans, Anabaena variabilis, and Nostoc muscorum, which is also good for other blue-green algae (Phormidium autumnale, Synechococcus cedorum, Anabaena cylindrica), and the medium for Chlamydomonas (Hutner and Provasoli, 1951) which serves for its colorless counterpart Polytoma (Cirillo, 1957) and a few other colorless flagellates (Chilomonas, etc.). Perhaps the flexibility of these media is due to their being designed for photoautotrophic nutrition, while the others are for heterotrophic nutrition and for eurybionts. Good heterotrophic media contain several amino acids and often other building blocks, some with strong chelating abilities, therefore the trace-metal mixtures developed for these media are too high in metals for the photoautotrophs.

The prerequisites for reproducible and versatile media for photoautotrophic algae can now be specified:

a) total-solids concentrations.

b) concentrations of major elements to suit the prevalent ions required.

c) adequate sources of N, and growth factors.

d) sources of P and avoidance of precipitates in alkaline pHs.

e) pH buffering

f) trace-metal buffering

As stated in section I, the best chances to fulfill a) and b), at least for devising media for isolation and moderate growth, are to mimic as closely as possible the conditions of the natural waters in which the organisms normally bloom. Natural conditions in respect to carbonates are hard to reproduce because carbonates, during sterilization disintegrate releasing $CO_2$ with resultant alkalinization and precipitation of the medium. Filter-sterilized carbonates or $CO_2$ can be added aseptically. Fortunately, carbonates can be substitutes by other anions such as Cl, $SO_4$, $PO_4$, and $NO_3$. It is opportune to introduce as little as possible of chlorides and sulphates by employing as much nitrate as is compatible with the organism. Therefore the prevalent ions, often Ca, can be introduced as

nitrate as Knop did.

Most algae utilize nitrates except the euglenids which, so far as we know, prefer ammonia or amino acids. Ten to 20 mg.% nitrates are in general well tolerated. Ammonia tends to become toxic above 3-5 mg.% in alkaline media except for eurybionts living in polluted waters. Since it is difficult to know priori if an alga needs vitamins, it is advisable to include in the isolation media the three vitamins required by most auxotrophic algae: vitamin $B_{12}$ 0.01 µg.%, thiamine 10 µg.%, and biotin 0.05 µg.%. Mineral phosphates should be avoided because they cause precipitates, especially in alkaline media. In a recent survey (Provasoli, McLaughlin, Pintner, (in Provasoli, 1958, p. 294) we have found that glycerophosphate was utilized by all the algae tested. Glycerophosphates have the advantage of forming far more soluble salts with the divalent anions than the phosphates, thus precipitation is often avoided even in slightly alkaline media or in the absence of chelators. Concentrations of 0.5-3 mg.% are generally adequate.

The problem of avoiding precipitates while supplementing the necessary trace-metals was partly solved by Uspenski and Uspenskaja (1925) by using opportune ratios of citric acid and Fe. But chelation of Fe with citric acid does not prevent precipitates in alkaline media. Hutner (1948) and Hutner et al. (1950) explored the possibility of employing stronger chelating agents to study the essentiality and role of trace metals. The criteria for selection of a "good" chelator for studying essentiality were that: a) it should form very stable complexes; b) it should be a bulky non-penetrable molecule so that chelation will take place in the external medium and not within the cells; c) it should be photostable and thermostable; d) it should be non- metabolizable and non-toxic for reasons unconnected with its metal-binding properties. Ethylenediamine tetracetic acid (EDTA) met all these specifications. EDTA, or other chelators tried, could not, as hoped, serve to determine the essentiality of single trace metals, but were very effective in providing a non-precipitable metal pool of trace metals, thus approaching the goal of metal-buffering (pM), and became of general use. No difficulties were found for fresh-water eurybionts (Euglena, Ochromonas, etc.) and for some marine fungi (Vishniac, 1955). Enormous quantities of EDTA (50-100 mg.%) were employed at first and the trace metals had to be raised to correspondingly high and unphysiological levels probably to prevent the excess EDTA from binding other necessary ions such as Ca and Mg. It was soon found that most organisms (even the marine algae) could not tolerate the high content of free trace metals and free chelator resulting from the equation: $K = \frac{[MY]}{[M]\,[Y]}$. Provasoli et al. (1957) found that artificial synthetic marine media are suitable for the greatest variety of organisms when the trace metals pool is low and the trace metals are offered as a mixture slightly overchelated (ratio of chelator/trace metals = 1:1 to 3:1). Since these mixtures (PI, PII, and TMII are the most widely used for marine organisms) are over-chelated, Droop (1959) has rightly raised the point that when the salinity of the marine media is varied and lowered (by lowering the Ca, Mg, Na, K concentrations) it might be better to employ citrate as a metal buffer because citrate has less affinity for Ca and Mg than EDTA. The free EDTA (of the over-chelated mixture) binds these cations more strongly and will reduce drastically their availability, especially at the lowest salinities. In fact it is possible to employ these over-chelated mixtures even for fresh-water organisms. One can substitute with 1-2 ml/100 of PII mixture the metal mixtures which had been experimentally found to compensate for the various chelations employed in the media for Phacus pyrum, Volvox globator, V. tertius, and Woloszynskia limnetica. But this does not hold for Synura media which are low in Ca and Mg (respectively 0.4 and 0.05 mg.%) while the other media contain from 2-4 mg.% of Ca and from 0.4 to 2 mg.% of Mg. This seems to confirm Droop's assertion. Experiments with Synura are in progress to see whether increased Ca and Mg will allow the use of over-chelated mixes; this might not be feasible because of the very poor tolerance of Synura toward total solids.

The other important difficulty in fresh-water media is pH buffering. Inorganic phosphate can not be employed because: a) most fresh-water algae, especially those of oligotrophic waters, cannot withstand the concentrations of phosphates needed for buffering (phosphates often become toxic above 5-20 mg.% except for organisms living in polluted waters and often for blue-green algae); b) heavy precipitates result because the fresh-water media are generally rich in calcium. Several amines have been employed successfully for buffering in the alkaline range. Of these the most useful are tris (hydroxy-methyl) aminomethane (TRIS) and triethanolamine (TEA); ethanolamine is in general more inhibitory. TRIS is a very good buffer for marine media: most marine algae are not inhibited by 100 mg.% and several withstand much higher concentrations. However, TRIS and other amines are toxic to certain pathogenic bacteria. MacLeod and Onofrey (1954) found that the toxicity of amines could be counteracted by the addition of K, Ca, Mg, and Na, alone or in combinations. This is probably why Tris is not toxic at useful pH buffering concentrations in media rich in those ions, like the marine media. TEA and TRIS can be employed for fresh-water algae fairly resistant to high total-solids concentrations so that it is possible to raise the cations and counteract the inhibition of the amines without approaching osmotically inhibitory

concentrations of salts. Different species of algae react differently toward the various amines. The toxicity of TEA is counteracted by increasing concentrations of Ca for Volvox globator and by Ca and Mg for V. tertius and Woloszynskia. The toxicity of TRIS for Cyanophora paradoxa can be removed by additions of Mg. and K; Ca and Na are of no use and even in presence of TRIS, they can be dispensed with in media allowing optimal growth. Since the amines heighten requirements for the major cations, they may be used to reveal the predominant necessary ions. But the amines can be toxic through other, unknown mechanisms: the toxicity of TRIS (20 mg.%) for Volvox globator cannot be counteracted by Ca, Mg, Na, K or trace metals.

Another pitfall of TEA, at least for Volvox, is that minor variations in pH (from 7.0-7.6) are immediately reflected in a change in availability of trace metals, particularly iron: the same concentrations of iron act at one pH as if Fe were available in excess (toxic) and at a slightly different pH as if Fe were lacking. A similar inflexibility toward the trace metal mixtures was noted for other algae. The first success was the buffering of the extremely dilute media for Synura at pH 6.0 (Table 2). Buffering these media was particularly difficult yet imperative because solutions are so dilute, owing to the sensitivity of the organisms to concentarions of any solute. Histidine adequately solved the problem: it is well tolerated at 15-30 mg.%, just enough to buffer the medium effectively at pH 6.0-65. Much later, following the suggestion of Droop (personal communication), who had found glycylglycine less toxic than TRIS for Oxyrrhis marina, we tried this buffer for Volvox, Woloszynskia, and Phacus. Surprisingly, minor changes in pH no longer affect metal availability in Volvox

Table 2. Culture medium for Synura media

| Metal-buffered | | pH-buffered | |
|---|---|---|---|
| | mg.% | | mg.% |
| EDTA | 5 | $Na_3$ citrate.$H_2O$ | 2 |
| $NaNO_3$ | 2 | $(NH_4)_2SO_4$ | 6 |
| $KH_2PO_4$ | 1.4 | $Na_2$ glycerophosphate.$5H_2O$* | 5 |
| Mg (as $SO_4^=$) | 0.2 | Mg (as $Cl^-$) | 0.05 |
| Ca (as $Cl^-$) | 1.3 | Ca (as $Cl^-$) | 0.4 |
| K (as $Cl^-$) | 0.5 | K (as $Cl^-$) | 0.2 |
| Fe (as $Cl^-$) | 0.07 | Fe (as $Cl^-$) | 0.05 |
| Zn (as $Cl^-$) | 1.0 | $Na_2SiO_3.9H_2O$ | 3 |
| Mn (as $Cl^-$) | 0.2 | Mn (as $Cl^-$) | 0.001 |
| Mo (as Na salt) | 0.001 | L-histidine (free base) | 20 |
| Co (as $Cl^-$) | 0.003 | $B_{12}$ | 0.04µg |
| Cu (as $Cl^-$) | 0.0005 | pH 6.0 | |
| Na H glutamate | 10 | | |
| Na acetate.$3H_2O$ | 4 | | |
| pH 5.5 | | | |

* a mixture of $\alpha$ - and $\beta$ - glycerophosphates.

Table 3. Culture Medium for Volvox globator and V. tertius

| | | | |
|---|---|---|---|
| Ca (as $NO_3^-$) | 2 mg.% | $B_{12}$ | 0.01 µg.% |
| $MgSO_4.7H_2O$ | 4 mg.% | Biotin | 0.01 µg.% |
| $Na_2$ glycerophosphate.$5H_2O$ | 5 mg.% | P. IV Metals* | 0.3 ml./100 - 4 ml./100 |
| KCl | 5 mg.% | | |

| | | | V. globator, average optical density | V. tertius, average optical density |
|---|---|---|---|---|
| pH 6.0 | histidine | 20 mg.% | 0.13 | 0.16 |
| pH 6.4 | histidine | 20 mg.% | 0.23 | 0.24 |
| pH 6.6 | histidine | 20 mg.% | 0.26 | 0.23 |
| pH 7.2 | glycylglycine | 50 mg.% | 0.22 | 0.28 |
| pH 7.5 | glycylglycine | 50 mg.% | 0.29 | 0.24 |
| pH 8.0 | glycylglycine | 50 mg.% | 0.23 | 0.24 |

* 1 ml. of PIV metals contains:

| | | | |
|---|---|---|---|
| HOEDTA (1) | 1 mg. | | |
| Fe (as $Cl^-$) | 0.04 mg. | Co (as $Cl^-$) | 0.001 mg. |
| Mn (as $Cl^-$) | 0.01 mg. | Mo (as $Na^+$) | 0.005 mg. |
| Zn (as $Cl^-$) | 0.005 mg. | | |

(1) see note Table 5

media (Table 3) when glycylglycine is used as a pH buffer in place of TEA. Not only could good growth be obtained with the same trace metal mixture between pH 7 and pH 8, but we could also employ a wide range of concentrations (1-4 ml./100).

During our work with Phacus pyrum and Wolloszynskia limnetica, we designed suitable media for the alkaline and acid region, with the hope of eliciting more growth by changing the penetrability of the substrates. With TEA and succinic or malic acids as buffers, we had to vary the media and the chelation to suit the pH and the buffer system. The resultant acid and basic media gave less or no growth when the pH was changed. After suspecting that the choice of the pH buffer affected chelation, we tried both the acid and alkaline media at

Table 4. Culture Medium for Phacus pyrum

| | pH 5.5 Base mg.% | pH 7.6 Base mg.% | | pH 5.5 Base mg.% | pH 7.6 Base mg.% |
|---|---|---|---|---|---|
| Ca (as $Cl^-$) | 2 | 4 | Zn (as $Cl^-$) | 1 µg. | 5 µg. |
| $MgSO_4$-$7H_2O$ | 30 | 4 | Mn (as $Cl^-$) | 9 µg. | 0.04 |
| $Na_2$ glycerophos-phate.$5H_2O$ | 7 | 4 | Co (as $Cl^-$) | 0.3 µg. | 1 µg. |
| KCl | 3 | 15 | Cu (as $Cl^-$) | 0.03 µg. | --- |
| $(NH_4)_2SO_4$ | 50 | - | Mo (as $Na^+$) | 0.2 µg. | --- |
| $(NH_4)$ acetate | - | 50 | Boron | -- | 0.2 |
| $Na_2$ EDTA | - | 1 | Fe (as $NH_4$ citr.) | 0.1 | 0.07 |

| | | pH 5.5 Base Optical density | pH 7.6 Base Optical density |
|---|---|---|---|
| pH 5.8 malic acid | 30 mg.% | 0.37 | 0.64 |
| pH 5.8 succinic acid | 30 mg.% | 0.28 | 0.52 |
| pH 6.5 L-histidine | 30 mg.% | 0.23 | 0.52 |
| pH 8.0 triethanolamine | 30 mg.% | 0.24 | 0.40 |
| pH 8.1 TRIS | 30 mg.% | 0.25 | 0.09 |
| pH 8.2 glycylglycine | 80 mg.% | 0.30 | 0.50 |

NB. The low pH medium was buffered with amalic acid; the high pH medium with TEA.
* a mixture of $\alpha$- and $\beta$ - glycerophosphates.

different pH but with histidine and glycylglycine as buffers (Table 4 and 5). The pH 7.6 base for Phacus gives, with suitable buffers, good growth from pH 5.2 - 8.2; the toxicity of TRIS in the pH 7.8 medium is puzzling, since TRIS is not toxic for the pH 5.5 base. The pH 6.0 base for Woloszynskia limnetica is more versatile than the pH 8.0 base.

In any attempt to understand the reason for the successful use of histidine and glycylglycine as pH buffers, we have to consider:

a) that histidine is a metal chelator almost as strong as EDTA and that glycylglycine is much weaker; b) that in the zone pH 5.0-8.5, an increase in pH results in more chelated metal ions and less free metal ion. Therefore, if a chelated metal mixture is kept constant and the pH is raised, this rise will result in a metal deficiency (i.e. more metals should be added or the chelator should be reduced); decreasing the pH results in an excess of free metals (i.e. one should add more chelator or reduce the metals); c) how much the chelator is in excess of the quantity needed for the 1:1 chelation of the trace metals (and therefore how much Ca and Mg will be chelated); d) whether the chelator employed is a bulky molecule unable, to or slowly penetrating, the cell, or whether it is small enough

Table 5. Woloszynskia limnetica

| | pH 8.0 Base mg.% | pH 6.0 Base mg.% | | pH 8.0 Base mg.% | pH 6.0 Base mg.% |
|---|---|---|---|---|---|
| $CaCl_2$ | 21.6 | 10.8 | $Na_2$ glycerophosphate.$5H_2O$† | 4 | 2 |
| $MgSO_4.7H_2O$ | 15 | 20 | Mo (as $Na^+$) | 4 µg. | 0.02 |
| KCl | 1 | 1 | Co (as $Cl^-$) | 4 µg. | 0.03 |
| $NaNO_3$ | 20 | 20 | Cu (as $Cl^-$) | 0.4 µg. | 3 µg. |
| $B_{12}$ | 0.1 µg. | 0.1 µg. | Zn (as $Cl^-$) | 0.32 | 0.1 |
| HOEDTA (as $Na_3$)* | 7 | -- | Mn (as $Cl^-$) | 0.32 | 0.8 |
| $Na_2$ EDTA | -- | 6 | Fe (as $NH_4$ citr.) | 0.1 | 0.2 |

| | | pH 8.0 Base Optical density | pH 6.0 Base Optical density |
|---|---|---|---|
| pH 5.6 succinic acid | 30 mg.% | 0.02 | 0.14 |
| pH 6.3 succinic acid | 30 mg.% | 0.015 | 0.13 |
| pH 6.3 L-histidine | 30 mg.% | 0.07 | 0.85 |
| pH 7.8 TRIS | 80 mg.% | 0.54 | 0.52 |
| pH 7.8 glycylglycine | 80 mg.% | 0.13 | 0.54 |

NB. The pH 8 medium was buffered with TRIS and the pH 6 medium with succinic acid.
* hydroxyethyl ethylenediamine triacetic acid
† a mixture of $\alpha$- and $\beta$ - glycerophosphates.

to penetrate the cell.

In our case we have kept constant the trace metal/chelator mixture and added pH buffers, some of which are metal chelators. Therefore according to b) when one decreases the pH (e.g. when the pH 8.0 medium is lowered to pH 6.0 and 5.0), one should add more chelator and when one brings the acid medium to alkaline pH, one should decrease the amount of Chelator. TRIS does not control the availability of trace metals at pH 7 to 8 (Chenoweth, 1956) nor probably does, TEA. Therefore when TEA is substituted and the alkaline media of Volvox and Phacus are brought to pH 6.0-6.5, more chelator should be introduced. This is what we did with the addition of histidine as the buffer for the pH 6.0-6.5 zone. However we obtain good growth of Phacus at pH 5.8 with addition of succinic or malic acids which are poor chelators. The pH 6.0 base of Woloszynskia gives better growth when the pH is raised and succinic acid is replaced by histidine, TRIS, or glycylglycine. Since the succinic medium is under-chelated, it may give poor growth at pH 5.6 because it has too much metals; addition of a chelator like histidine could have adjusted the balance, but more growth is also obtained with TRIS, a non-chelator and by glycy-

glycine, a weak chelator. The behavior of these pH buffers in our media cannot therefore be explained as a pure chelating effect, nor can the two Synura media (Table 2). If the milliequivalents of all the metals, including Ca and Mg, are compared with the milliequivalents of the chelators, we find that the EDTA-glutamate medium is overchelated 1.3 : 1 and the histidine medium 5: 1; yet they give similar growth. The media for Oxyrrhis marina of Droop (1959a) present a similar puzzle: at the same pH and with the same amount of trace metals and major elements similar growth can be obtained by chelating with 0.6 mg.% EDTA or by the joint chelation of 20 mg.% histidine, 4 mg.% citric acid and 50 mg.% glycylglycine; 2-6 mg.% EDTA on the contrary allows far less growth. Again the level of chelation does not explain in the data: histidine alone chelates $>$60:1; 0.6 mg.% EDTA, 2:1 and 6 mg.% EDTA, 20:1.

These discrepancies can be explained satisfactorily if one considers the consequences of the different molecular size of the chelators used. As mentioned, EDTA was chosen because it was supposed that the bulkiness of its molecule would prevent penetration into the cells and that it would be photo-stable. It was later found that Fe-EDTA chelates decompose in light (Jones and Long, 1953) and that some EDTA or its breakdown products penetrate in the algal cell (Krauss and Specht, 1958). However, the majority of the iron apparently does not enter as intact iron chelate because, on a molecular basis, 15 to 50 times more iron was absorbed by the cells than EDTA. Tiffin and Brown (1959) employing the iron chelate of ethylenediamine di (o-hydroxyphenylacetic acid) (EDDHA) found that roots of decapitated sunflower plants absorbed only about 0.3% of the total EDDHA and large amounts of iron, leaving most of the EDDHA in the nutrient solution.

Therefore for practical purposes EDTA is a non-absorbable chelator and the cells depend almost exclusively: a) on the available free metal ions which are very low because of the high stability constants of EDTA chelates, though in the case of iron more free ions may be made available by the partial disintegration of EDTA in light and b) on the ability of the organisms to compete for the metals in the EDTA chelates. This transfer is uphill since EDDHA and EDTA accumulate in the medium. The data in fact show that the algae behave as if they depend mostly on free ions present in the medium because any conditions, like variations in pH, over- and under-chelation, which upset the ratio metal chelates: free metal ions favorable for an organism and a given pH, result in toxicities or deficiencies which inhibit or suppress growth.

Histidine and other chelating small molecules are readily absorbed. Since in this case the free chelator, the metal chelates, and the free metals presumably all can be absorbed, the effect of over- and under-chelation and pH changes should, and do, affect far less the availability of the trace metals. The transfer and the competition for trace metals by the different biological internal chelators now can proceed freely in the interior of the cells. Furthermore the absorbable chelators when employed in large quantities as pH buffers have the power to smooth out, perhaps by mass action, the inflexibilities caused by the presence in the media of weak, slightly over-chelated EDTA-trace metal mixtures.

This way to supply metals by employing penetrable chelators parallels what may happen frequently in nature. Lichens and other plants growing on rocks must be able to secrete organic compounds which dissolve and perhaps chelate the mineral elements. Various fungi and bacteria produce and release in their media extremely strong chelating substances which are specific for iron such as: coprogen, produced by bacteria, actinomycetes and fungi, (Hesseltine et al., 1953), "terregens factor", produced by Arthrobacter pascens (Lochhead and Burton, 1953) and ferrichrome, produced by Ustilago sphaerogena (Neilands, 1952). Ferrichrome, amazingly, has a stability constant ten times higher than EDTA (Neilands, 1957) for ferric iron yet is an effective way to supply iron to tomato plants grown hydroponically. Arthrobacter terregens and other soil bacteria (Burton, 1957), Microbacterium sp. (Demain and Hendlin, 1959), and the fungus Pilobolus kleinii (Hesseltine et al., 1953) have a growth factor requirement which is satisfied equally well by terregens factor, coprogen and ferrichrome. These substances, though apparently different chemically, provide an extremely effective way of supplying iron. Because of their special biological activities at very low concentrations (ug./ml.) they are considered by Demain and Hendlin (1959) as "iron-transport factors". Nielands (1957), in a thoughtful review, postulated that they may act as coenzymes for the intracellular transfer of iron.

Only a few of the great variety of molecules tried, many of which are known chelators, can replace them. Outstanding are the compounds formed upon heating sugars with amino acids, the ketose-amino acids. Glucosyl-glycine is active for Microbacterium sp., fructose-phenylalanine and the products derived from autoclaving glucose and glutamic acid are active for Micrococcus lysodeikticus. Other bacteria, like Lacto-bacillus gayoni and Proprionibacterium freundenreichii require glucosyl-glycine, suggesting that they may also need "iron transport factors". It is interesting to note that the fructose- amino acids stimulate haem synthesis and amino acid incorporation into globin. (Kruh and Borsook, 1956). Other compounds like citric acid and 8-hydroxyquinoline are active for M. lysodeikticus and aspergillic acid for Micro-

bacterium sp., while EDTA is unable to satisfy the requirement for both organisms. So far the active substances are all strong chelators able to penetrate through the cell membrane. But many other penetrable metal chelators are inactive indicating that penetrability and chelating properties are not the only prerequisites for activity. A strong specificity for binding iron and other properties seem essential. For instance, the effectiveness of the transport function may require an easy release of iron to the apoproteins of the iron containing enzymes and this may be achieved in a number of ways. The activity of the ketose- amino acids as iron transport substances may be related to their role in stimulating, and perhaps participating in, haem production and amino acid incorporation in proteins. These new developments suggest means of making better, more flexible media for algae.

Hutner et al. (1951) had briefly mentioned that it is preferable, for the sake of obtaining heavy growth, to have the complexing agent serve as an auxiliary substrate. There is no evidence that histidine nor glycylglycine are utilized to any extent as substrates by Synura, Woloszynskia, and Volvox because these organisms are unable to utilize exogenous carbon sources under our experimental conditions. Perhaps this is an advantage. If the chelator employed is utilized as the sole substrate or is a needed building block, metal toxicities may result because the chelator may be utilized more rapidly than the metals. This obstacle may however be circumvented by offering several substrates or other building blocks along the same pathway of synthesis, so as to balance the rate of uptake of the metabolizable chelator. Another procedure would be to employ several chelators; some marine algae seem to prefer media chelated jointly by EDTA and nitrilotriacetic acid (Provasoli et al., 1957).

Though far more experiments are needed to find more versatile fresh-water media, the use of penetrating pH buffers endowed with chelating properties like histidine and glycylglycine seem to offer great advantages for the most important pH range (pH 6.0-8.5). Tracer studies are required again to tell the extent to which these buffer-chelators penetrate.

The supply of trace metals in the acid range offers a different set of problems. At pH below 5 the heavy metals are quite soluble. A chelator is not needed to prevent precipitates, but may still be very useful as a metal buffer to prevent toxicities and to stabilize the metal pool. EDTA and other chelators having acidic coordinating groups offer little promise: their chelating power decreases with increasing acidity because the hydrogen ions compete more and more favorably with the heavy metal for the coordinating group. Very little has been done with highly soluble, penetrating but non-toxic chelators having a predominance of basic groups.

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