

marine biology

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Interdisciplinary Conference

Edited by

CARL H. OPPENHEIMER.

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INTRODUCTORY REMARKS

Ever growing numbers of scientists today are rapidly extending the frontiers of knowledge. From outposts of research, streams of new information are pouring into already overloaded channels of communication. New methods of investigation lead to increasing specialization and to esoteric vocabularies difficult to understand outside the specialty of their origin.

Yet many of the crucial problems in medicine and biology require for their solution a multi-discipline orientation and often multi-professional teamwork. Thus, in the search for the cause, treatment or prevention of disease, or in the effort to achieve a "breakthrough" in such basic fields as genetics, homeostasis, or growth and development, the research scientist may find the needed clue unexpectedly in a new advance in one of the branches of biology or medicine, or in a new method derived from bio-engineering or computer research, provided only that he has the breadth of knowledge to grasp its significance.

The Interdisciplinary Communications Program was organized to counteract the narrowing effect of excessive specialization and to make available systematic opportunities for cross-discipline communication by means of informal group discussion.

These conferences are organized to facilitate the advance of science. Within this broad frame of reference they have several interrelated purposes. A major one is to provide an opportunity for communication within a group of scientists who, while belonging to different disciplines, share a common field of interest. A second goal is to provide an opportunity to thresh out and discuss in depth, problems that arise out of difficulties of communication and, thus, to narrow and specify the areas of disagreement. A further goal is the development of lasting friendships and cooperative efforts among the participants.

I should like to state here my basic assumption that nature is all of one piece and that the branches of science are branches of *one science* and not separate sciences. Science is man's effort to understand the laws of nature. The different disciplines or university departments are fragments of science. These tend to be too isolated from one another. Usually they are built up around a method, technique, or a point of view. But each of these disciplines is only one window through which man looks at nature. Each window permits the passage of only certain rays of light and also, inevitably, has some distorting lenses. If one wants to see nature whole, one must look at it through many windows. This is why there is a great need for multi-disciplinary orientation for the optimum advancement of science.

We are in a kind of information crisis. We are being overwhelmed by so much information, so many data, that we are inclined to limit ourselves to the study of those fragments that fall within our own narrow discipline. Important new developments in science have repeatedly come through a combined operation of several disciplines in exploring an area which had been previously neglected, an area lying between sharply specialized lines of previous advance. This conference is an effort to facilitate interdisciplinary communication in the field of Marine Biology.

We would like to express warm appreciation to Dr. Carl H. Oppenheimer, Professor of Marine Microbiology, Oceanographic Institute, The Florida State University, Tallahassee, for undertaking the arduous task of Scientific Editor.

Frank Fremont-Smith, *Director*
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I. NATURAL PHYTOPLANKTON SOCIETIES

COMPOSITION

Discussion leader:

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STEEMANN NIELSEN When I started to work with phytoplankton in the sea, more than 30 years ago, taxonomy and the distribution of species were the main foci of this science. For the past several years more general aspects of phytoplankton production and general ecology have been the central themes. We now have to recognize that organic matter is produced by the single specimen and, therefore, we must consider the species composition of the plankton found in the sea.

The distribution of a species may be due to either ecological or geographical factors. If a species of plankton algae is not able to live under certain ecological conditions, it is obvious that it will not be found in a locality where such conditions prevail; on the other hand, a species may very well be able to grow in a certain area, yet we won't find the species at all. This could be due to the effect of geographical barriers, and I think perhaps a few words about the geographical barriers in the sea might be appropriate. Then our fresh water colleagues may wish to comment on the barriers and the distribution of fresh water species.

Years ago I worked with one of the common groups in the sea, the Dinoflagellate, genus, *Ceratium*.¹ Enough species of this genus are found in the sea to enable us to use their distribution for general considerations. If we consider the tropical seas, taking the oceanic and the coastal waters separately, we find practically the same species in all three oceans: a very few are not found in all oceans, but only in one or two.

Let us consider the distribution of the three neritic (coastal) species: *Ceratium humile*, *Ceratium schmidtii* and *Ceratium dens*. They are found only in the Indian and the Pacific Oceans, and even there, only

in very warm water. They are not found near the subtropical boundaries. The distribution of the true oceanic species, *Ceratium filicorne*, is exactly the same; found in the warm water of the Indian and Pacific Oceans, but not in the Atlantic Ocean. All four species are very easily recognizable.

It is rather obvious that these four strictly tropical species are unable to tolerate slightly colder areas. All of the tropical species—common to the three oceans—must have been able to cross through colder water either from the Atlantic into the Pacific (Indian) Ocean, or vice versa. They are not restricted to very warm water, and are generally found in subtropical areas.

Only a single species of this genus is found in the Arctic, *C. arcticum*, it is not present in the Antarctic. I don't think there is a real Antarctic species of the genus *Ceratium*. It is easy to imagine that an Arctic species cannot be transported through the warm water of the tropics to the Antarctic.

Most of the temperate *Ceratium* species are found either in the Northern or in the Southern Hemisphere, although, there are a few that are present in both. *Ceratium furca* and *Ceratium fusus*, are found in both hemispheres, and it is easy to see why they are present in subtropical and even in tropical waters, thus having one single area for their distribution, covering tropical, subtropical, and temperate waters on both sides of the Equator.

The above example shows a little about the geographical distribution and the barriers found in the sea. I am quite sure that in fresh water the geographical distribution may often be due to other factors. In the sea, I rather doubt that many species are transported by means other than the currents, but we know that fresh water species may be distributed by other means. Perhaps Doctor Lund could say something about the graphical distribution of the fresh water species.

LUND Every lake or drainage basin containing lakes may perhaps be considered as a separate world, certainly separated physically and geographically, from other fresh water worlds. Nevertheless, fresh water algae, by and large, are cosmopolitan. It is perfectly possible, for example, for me to look at plankton samples from lakes in New Zealand and to recognize the common species. They are the same ones, apparently, that I see at home (England).

This really is rather extraordinary. It is quite true that many fresh water algae are able to withstand being air-dried and can therefore be transported as dust. This may occur only when they are in special resting stages, but the fact remains that some of the very commonest fresh water plankton algae have no known resting stages; there are no known conditions under which they can withstand drying. A classical example is a very common diatom called *Asterionella*. This has a

world-wide distribution outside the tropics, yet nobody has ever seen it in a resting stage, and I have been unable to find any means of drying this alga without killing it. Outside the tropics, it is one of the most widespread and abundant of all plankton algae, and this is true of several other very common planktonic algae.

How, then, is it that single species are distributed throughout the world? The answer always comes back: by birds. But no one has proved that birds do carry them all over the world. We don't know the answer.

There is, however, a clear difference when one considers the floras of hot places: tropics, semitropics, warm continental areas, and of the temperate and Arctic regions. There are species in the tropics which are never seen in temperate lands, and there are species in temperate lands which are never found in the tropics. The main reason for this, I think, is the temperature tolerances of the various species. Again, take the diatom *Asterionella* as an example. There are records of its occurrence in tropical lakes. So far as I know, all of these records are suspect. Such cases as I have been able to check have always been based on wrong identification. In the laboratory, it will grow very well up to about 22C. At 25 to 26C, it dies. This is, in fact, about the temperature of tropical waters, so I think temperature plays a very big part in algal distribution.

There is another barrier, not so much to distribution as to actual growth; namely, that very dry areas of the world are often rather salty. Because of loss of moisture by evaporation, the salts tend to be concentrated. Consequently, in these high salinity waters floras are found which are not usual for warm temperate lands; even in cold dry temperate lands, where salts may concentrate, unusual floras may be found.

Lastly, there is another curious thing. I may look at plankton from New Zealand, which have been brought to England, and say, "Oh, here are all my old friends," but often my "old friends," when looked at very closely, are slightly different. I don't wish to go into the reasons for this, but they *are* different; and it is not surprising because, even if we accept the theory that birds have carried phytoplankton around the world, this takes some time providing for some adapting processes to take place.

HUTCHINSON: The botanical problems here seem to me inseparable from the zoological problems, and if we want to try to solve this sort of problem we have, I think, to look at both plants and animals. In the Crustacea, one has a very interesting set of cases. In the Cladocera, the story is essentially as you have indicated. There are a great many cosmopolitan species and there is a considerable tropical element, and there is also perhaps a somewhat greater set of species with single-

continent distribution. In the cyclopoid copepods the distribution is a little bit more localized. There are, however, half a dozen species which apparently have a world-wide distribution, even in the minds of people who are professionally concerned in splitting species to the finest possible limits.

In the diaptomid copepods, there is no single case of a world-wide distribution. In fact, there are not very many that have a complete palaearctic or nearctic distribution, and none that are panholarctic.

The paradoxical thing is that in the cyclopoid copepods, although there are now quite a number of known resting stages, they have never been found in some of the species that have the widest distribution, whereas in the diaptomid copepods there are a large number of species of which the eggs can be dried easily without injury, yet they are no more widely distributed than the others. I assume also that you have a similar picture in the Protozoa where there is seemingly no correlation between the ability to withstand desiccation and the wideness of the distribution.

In your diatoms you have one clue that is hardly available in the microcrustacea: fossil individuals. Can you tell us whether anything is known about the fossil history of *Asterionella*, for instance? One of the things we need to know is how long it takes to achieve this world-wide distribution.

LUND No, I don't think I can answer that. The earliest records I can think of, off-hand, are from interglacial deposits that contain *Asterionella*; they are European.

HUTCHINSON: I think there is obviously something for the paleobiologist to look into here in connection with this distributional problem.

MCLAUGHLIN There has been some work done on dinoflagellates. W. R. Evett from Jersey Production Research Co., Tulsa, Oklahoma, came to me about 5 years ago with some photographs of fossil dinoflagellates. He asked me if I had anything in culture that looked similar. I gave him several species from our culture collection and he was surprised at the close resemblance. His photographs were taken from cores in Pakistan, Jersey, and somewhere in the Near East. I don't know how many millions of years old these cores were, but the similarity of the organisms of which he had pictures and the *in vitro* thecated forms was very striking.

He also found in this deposit tremendous concentrations of fish bones, which he has assumed represented fish kills of that era. He asked if I knew of any dinoflagellates that would kill fish. I showed him some cultures of *Gymnodinium breve* and told him the red tide story.²

HUTCHINSON Were the fossil algae with the fishbones toxic species?

MCLAUGHLIN: Yes, I believe they represented species which have been implicated in mass fish mortality.

STRICKLAND: May I make one naive observation? Do we know very much about the possibility of fresh water *Asterionella* having modified from a common oceanic species at one time, or is that quite impossible?

LUND: I don't think we do. I have no views on the subject.

STRICKLAND: Some of the organisms have quite a wide salt tolerance, have they not?

RILEY: *Asterionella formosa* has quite a high tolerance to salinity and is found in coastal waters well beyond the influence of immediate land drainage. In salinities as high as 32 to 33 per mille, it has at least a limited ability to grow. It appears in the spring during the freshets, when it is washed out from the land. It grows for a time and disappears during the summer. It is a remarkably euryhaline species.

LUND: That is not so in our part of the world.

PROVASOLI: A nonsalt-tolerating *Asterionella* sent to me from Windermere by Doctor Lund would not grow in more than 300 to 500 ppm of total solids* *in vitro* and in aseptic culture.

LUND: One of the difficulties is a very old one; namely, taxonomy. I have to admit that nobody knows exactly what this name, *Asterionella formosa*, means. There is also another *Asterionella* called *gracillima*. It is not known whether these are two separate diatoms and, if so, what the difference is between them; so we always have to bear that thought in our minds: Are we talking about the same thing?

HUTCHINSON: This, of course, applies also to animals. Price (1958) has shown that in what is commonly called *Cyclops vernalis*, there are six sibling species practically without any distinguishing characters except that they are not interfertile. If this phenomenon is general throughout the small organisms of the hydrosphere, taxonomy is in a very bad way indeed.

STEEMANN NIELSEN: I hope we hear a little about taxonomy at this meeting. For instance, Doctor Ray has told me about *Noctiluca*, and I, too, have looked at the *Noctiluca* found in Friday Harbor on the Pacific coast. It is apparently similar to our North Atlantic *Noctiluca* in the daytime, but not at all during the night. It does not produce light, as does the species in north European waters. I am quite sure that very often algae we refer to as belonging to the same species are not exactly the same. But perhaps Doctor Braarud could comment on taxonomy and its importance.

BRAARUD: I would like to comment on temperature as an important factor for the distribution of species. Doctor Lund has given a good example of the experimental support for assuming that temperature is

*Provasoli, L. and I. J. Pintner. Unpublished observations

a decisive factor. But with the marine species, it is more difficult because so very few experiments have been made and, when we do have experimental observations, it is often difficult to reconcile them in the field and in the laboratory.

For instance, *Skeletonema*, one of the most common diatom species, is found in many different kinds of water. It is found in the subtropical areas, but in the northern area it is a characteristic species of winter, of the darkest part of the winter, and also spring. The experiments of Cure and McLeod³ show that its optimum growth occurs between 20 and 30C when the rate of growth is about 4 to 4.6 divisions per day, while at low temperatures it divides only once every other day. Would it be correct, then, to say that this is a eurythermal species, or should we call it a tropical species, according to its temperature response? It is, in any case, conceivable that the temperature response alone is not decisive for its distribution. In other species, such as *Thalassiosira nordenskioldii*, distribution suggests that it is a cold-water form, as it occurs in northern waters during the coldest season of the year. If we culture this species at, for instance, 10C or at 15C, it has a high productive rate. So, in that case it seems as if our conclusion, drawn on the basis of geographical distribution, is wrong; that temperature is not the decisive factor, at least not a temperature effect, per se;⁴ and perhaps other environmental factors, which in these areas are coupled with temperature, may be decisive. One has to be cautious in drawing conclusions from a distribution pattern about the relationship with certain environmental factors.

KOCZY In this connection, I believe we must distinguish between two different concepts. One is the growth rate at that temperature which results in the most dense population. The other is the maximum growth rate at an optimum temperature range. It is rather difficult to define a single optimum temperature for plankton when these two do not overlap.

BRAARUD I quite agree that the tolerance and the growth rate are two different things. But if species are found in northern waters, for instance, they may be there at different seasons, and their optimum reproduction rate may vary.

MCLAUGHLIN Has anyone taken a southern or tropical form, moved it to a northern environment, and tested it there *in vivo*?

BRAARUD I haven't any information on the subject.

MCLAUGHLIN Would a difference in the clarity of the water affect the total population size? That is, is it possible that, in northern waters, an organism produces a limited amount of chlorophyll because of the lack of light transmission through the water as compared to abundant transmission in the tropics? Could this difference in chlorophyll content affect growth rate in the two areas? If you don't

think temperature is the variable responsible for this, what do you assume to be the variable?

BRAARUD: In the case of *Thalassiosira nordenskioldii*, the nutrient salt concentration may be decisive, because, at the season when the species occurs regularly in numbers, the nutrient concentration is high and, as this concentration drops during the spring maximum, the species disappears. When the organism occurs at other seasons, it is usually in connection with some special hydrographic conditions which are apt to have increased the nutrient concentration. The whole pattern of growth may be due to many factors and I think we know very little about it.

JONES. In our studies on the growth of *Carteria* sp. and *Nitzschia closterium* in continuous culture, we have noticed that by increasing the nitrate and phosphate concentration of the medium, the temperature tolerance of the organisms is increased. For example, 15°C was the optimum temperature of growth for *Nitzschia closterium* with 125 µg/liter nitrate-N and 12.5 µg/liter phosphate-P. By increasing the concentration of these nutrients some thousand-fold, the optimum temperature of growth was found to be 23°C.

STRICKLAND: Can you reduce it to a lower temperature? Can you alter the lower temperature tolerance?

FREMONT-SMITH: Down to 12 or 10°C.

JONES. No. So far, the best we have obtained with the lower nutrient levels is 15°C.; below this the rate of growth falls off rapidly. I do not know whether there is any evidence from the natural environment to indicate that species of phytoplankton with higher temperature-tolerance occur where the nitrate and phosphate concentrations are also high.

DROOP. In cultures of *Monochrysis*, there is an enhancement of vitamin B₁₂ requirement at both the lower and upper limits of the temperature range. I don't know very much about nitrogen and phosphorus or sulphur, though at the lower limit more is also needed for a given yield than at normal temperatures. But the situation is complex, for these major nutrients nearly always give nonlinear regressions of yield on dosage. In general, it seems fairly clear that the balance of nutrient concentration becomes increasingly important as temperature becomes more extreme. One has the impression of machinery running so fast at the higher temperature that it trips up, unless very nicely adjusted, while at the other extreme, at the lower temperature limit, it's a case of merely "freezing up the works."^{5,6}

PROVASOLI: It seems to me that we should keep in mind what was said earlier and be cautious in extrapolating data on one species from one locality to another. Are we sure that organisms called by the same name by different investigators are really identical morphologically?

They could be morphological races or varieties, or even closely related species. Many of the type-species descriptions are so incomplete that the latter possibility is neither insulting nor far-fetched. An exact species identification is imperative because the algae seem to have more physiological races than we suspected. Once the morphological identity has been established, we should test the various strains to see whether the observed geographical and ecological distribution depends on the versatility of the species (i.e., is common to most strains), or is achieved by adaptation or mutation.

Regarding temperature as a nutritional factor, the data of Doctors Jones and Droop are in accord with the findings of Doctor Hutner and collaborators⁷ on the fresh water algae *Ochromonas* and *Euglena*. Increased levels of vitamins and trace metals permitted growth above the normal lethal temperature limit. Similarly, Doctor H. Isenberg (Long Island Jewish Hospital, New York; private communication) has been able to raise the temperature limit of a marine *Hymenomonas* (No. 156, isolated by Doctor Mary Parke in the English Sea) from 20-22C to 26-28C by increasing the trace-metal level.

GUILLARD * We should remember that it is possible for physiological races to originate in two different ways. In one, clones may arise by mutation, and become dominant in a given ecological situation because they are adapted to that situation. On the other hand, it may be that in a population there can be sexual selection during the course of a growing season. What gave me this idea were some observations of a clone of *Skeletonema costatum* derived from Long Island Sound. In 1959, John Ryther and I did a number of experiments on growth and respiration at different temperatures.⁸ When we first transferred *S. costatum* from the standard culture conditions, at about 20C to about 5C, the culture remained stationary for a long time, a matter of weeks, then grew rapidly. Some years later, I wanted to compare growth of *S. costatum* at several temperatures with growth of another small diatom that I think is a different species of *Skeletonema*, electron microscopy has since shown that it is quite different from *S. costatum*. For these experiments, I used a new culture apparatus which had very good temperature control but low-light intensity, and I found that the original clone of *S. costatum* would no longer adapt to the low temperature. I think the adaptation noted before may have had something to do with the *S. costatum* going through an auxospore cycle during the adaption period. Perhaps this also happens in nature. It may be that if one isolates clones of *S. costatum*, for example, from Long Island

*The contributions of Dr. Guillard were made possible largely by NSF grant 10693, and AEC contract AT(30-1)-1918

Sound in the summer and in the winter, the properties of the clones will be different. But perhaps one can get winter forms from the summer ones, and vice versa, by continued subculture. On the other hand, a culture derived from one cell may not contain enough genetic variation to give rise to a different physiological race.

PROVASOLI: In other words you don't have both mating types?

GUILLARD: Probably. Or perhaps it is simply lacking in enough genes.

HUTCHINSON: There is at least one animal case, namely Banta and Wood's work on the cladoceran *Daphnia laevis*, in which they obtained a mutant that had a very high thermal tolerance. Its temperature optimum was moved up from 18-20°C to 27-20°C, at which temperature the wild form hardly survives. Initially the thermal clone did very poorly below 20°C, but after about thirteen months' parthenogenetic reproduction it became a eurythermal organism able to live at 16°C.

STRICKLAND: The *Skeletonema* on the Pacific coast appears to be considerably larger than the *Skeletonema* we have obtained from Woods Hole. In fact, by growing the Atlantic *Skeletonema* under various conditions, the largest cell is about the size of the smallest one we ever see in the Pacific.

DROOP: What are the sizes?

STRICKLAND: The largest Atlantic cell size is about $500 \mu^3$ per cell. We have had *Skeletonema* up to $2000 \mu^3$ or more from the northeast Pacific coastal waters.

One observation that interests me is that, in an attempt to keep all of our stock algae on agar, I put *Skeletonema* on agar and found, a few weeks later, what appeared to be a *Melosira* growing. This turned out to be a simply enormous *Skeletonema*. After it was removed from agar and put back into liquid culture, the cells decreased in size again. So, there may be some odd conditions which cause large size variations even within what may be the same subspecies.

PROVASOLI: Was the determination done by electronmicroscope or light microscope?

STRICKLAND: Just an ordinary light microscope.

OPPENHEIMER: Reference to the large size of some diatoms brings out a rather interesting bit of philosophy; that is, the extrapolation of laboratory experiments to the natural environment with respect to energy-temperature relationships. For example, a heavy diatom, with large test and specific gravity greater than that of sea water, must stay in suspension at some expense of energy to overcome gravity (protoplasm, sp. gr. 0.9; silicate, sp. gr. 2.6). It makes no difference whether stored energy or available energy is used. If this organism is moved to a northern latitude by currents, one could easily see that the organism may reach a low temperature in which its metabolism would decrease

to the point where the energy would not be sufficient to keep the organism in suspension, and it would fall below the euphotic zone.

Thus, if a diatom is examined in the laboratory for temperature response, the organism could be maintained at a lower temperature than one would expect to find in the natural environment, because in the culture flask it is always artificially maintained in light, whereas in the natural environment the more dense organism may not have enough energy to keep it afloat within the euphotic zone. This might have a very interesting effect on the comparison of laboratory culture results and latitude distribution of some of the larger organisms with heavier specific gravity, especially the diatoms.

HUTCHINSON Do you have any evidence of how much energy is used to keep diatoms afloat?

OPPENHEIMER No.

GOLDMAN In some systems, wind action provides the mechanical energy for maintaining the organism in the euphotic zone. This is the case, for example, in shallow Antarctic ponds that are wind mixed continuously during their liquid phase.

BRAARUD I would like to comment on Doctor Jones' and Doctor Provasoli's remarks. First, about the effect of the nutrient concentration upon the optimum temperature for growth. I think the experiments Curl and McLeod made on *Skeletonema*³ indicated just what you found.

Secondly, as to the question of physiological races or geographical races; when I read the Curl and McLeod paper, I thought at once that this culture must have been derived from a habitat where the temperature is different from that of the northern waters. But then, looking up the plankton data from Long Island Sound and elsewhere, I found that *Skeletonema* has its midwinter maximum at very low temperatures in this area, just as on the Norwegian Coast. So, for that reason, I didn't find much support for assuming that such races exist in this species. Obviously, we have to do more experiments on this problem.

KOCZY Do you have any cultures of *Skeletonema* from the North Sea.

BRAARUD. There are very few cultured *Skeletonema*. We have cultured it, but haven't very much experience.

KOCZY. We could never grow it above 14C.

STEEMANN NIELSEN *Skeletonema* isolated from Danish waters can be grown up to 25C. We have had it growing extremely well for more than a year, mostly between 20C and 25C. But there is one thing you have to remember: To get *Skeletonema* to grow well it must have suitable light conditions.

PROVASOLI Doctor Braarud, did you find any evidence in your studies of Oslofjord and other polluted areas, that environments rich

in organics allow growth of phytoplankton species normally restricted to colder water or colder seasons—in other words, deviations in geographical distribution which seem correlated to abundance of nutrients?

BRAARUD: As far as *Thalassiosira nordenskiöldii* is concerned, which was the species I mentioned, it does not grow well in the Oslofjord for some reason or other. It may be competition because of a large initial population of, for instance, *Skeletonema*. In the Hardangerfjord, we have observed that *Skeletonema* grows together with many other species during spring and early summer, but then it disappears, while other diatoms continue to grow in the same water mass. This is very strange.

Might I bring up another subject also related to distribution, and one of a more general character? What is the background for the different distribution of the meroplanktonic forms, usually found only in coastal waters, and most of which have resting stages, and the holoplanktonic species which are able to live out in the ocean? This is a classical problem in phytoplankton ecology and, from the days of Gran,^{9,10} it has been suggested that nutritional factors are involved and that some species need special nutrients which they don't get in sufficient amounts at sea. This is a point of view which has been taken up recently, and new evidence has been produced which we will hear about later. But what I would like to discuss is that this different distribution must in some way be related to flotation: to the specific gravity.

The most characteristic feature of the meroplanktonic species is their irregular seasonal occurrence. When they disappear most of the species form resting spores which obviously are heavy and sink to the bottom. When they reappear it must be due to a change in specific gravity. The holoplanktonic species appear to have sufficient flotation capacity throughout the year to enable them to maintain populations in the euphotic zone.

PROVASOLI. Do they form resting stages?

BRAARUD: No. We have now learned¹¹ that *Ethmodiscus rex*, a grant diatom, is able to maintain low specific gravity through ionic control of the composition of the vacuole in the same way that *Noctiluca* does. The same is probably the case with *Halosphaera viridis*, another oceanic form with a large vacuole, which is known to be very buoyant. If one takes a net-haul sample into the laboratory, *Halosphaera* floats up like a rubber ball, but after a while it sinks.

What is the situation with the intermediate size group, for instance, *Thalassiosira*, *Chaetoceros*, etc., and what about the very small ones? This is a problem which is essential for understanding not only the general but also the seasonal distribution of the meroplanktonic

forms. My friend Doctor Riley and others like to assume that specific gravity is equal to or a little larger than that of the seawater, but I don't think we have much real evidence of it. Since we have plant physiologists here, we should try to get them interested in this important problem.

I forgot to mention the data of Gross and Zeuthen.¹² They found very good indications that *Ditylum* is able to attain low specific gravity by reducing the concentration of heavy ions within the vacuole, and this has since been proved more definitely by Beklemishev for *Ethmodiscus*.¹¹

OPPENHEIMER: In previously speaking of energy and temperature, I was thinking of the mechanism by which any organism has to stay afloat if it is more dense than water. For example, in considering oil globules as energy storage, one can assume that they are formed at the expense of energy, photosynthetic energy or some other rearrangement of molecules within a cell. If at some time in the year the available energy or nutrients in the water decrease, the organism will utilize the oil as an energy source to keep it alive, and it is presumed that its specific gravity will change. Therefore, in certain situations, the organism could essentially destroy itself by falling out of the photosynthetic zone. The organisms could rise to the surface again only if they act as a heterotroph which can obtain energy from organic material below the euphotic zone or through advective processes, or if they somehow decrease their density as suggested by Doctor Braarud.

HUTCHINSON: Is there any thermodynamic reason to suppose that it takes more energy to make a less dense cell inclusion than a more dense one?

STEEMANN NIELSEN: It is very important to remember that plankton algae are a little more dense than seawater.

MYERS: I don't know that there is an unequivocal answer to Doctor Hutchinson's question. Oppenheimer was thinking about lipid. The lighter materials are those which are, in fact, hydrocarbon in character. They have the highest energy per gram and per mol, and I think, in that limited sense, there is an answer to your question.

STRICKLAND: But I don't think there is any evidence that the diatom is necessarily frightfully oily. This is one of the great myths that is getting into the literature.

MYERS: There is one other variable in addition to the question of energy supply. This is very simply the one of nitrogen deficiency which goes back to Beijerinck's generalization about microorganisms.¹³ Under nitrogen deficiency they are likely to build extraordinary quantities of lipid; so, I would add that Doctor Oppenheimer poses not only the question of energy supply in producing or demanding use

of stored lipid, but also of the diversion of metabolism to lipid synthesis, which is, I think, a very general consequence of nitrogen deficiency.

GUILLARD: I think there is no question of this. But what is lurking in the back of our minds is the idea that if a diatom produces a lot of lipid, it makes itself lighter. I think the general experience is the contrary, and under the circumstance where a diatom synthesizes more lipid, it also happens to get heavier.

OPPENHEIMER: Vacuoles must be formed at the expense of energy. I only used lipid as an example to clarify a point, but any change of state must be coupled with an energy reaction.

HUTCHINSON: The question about the formation of the vacuole is significant in the present discussion only in the large marine diatoms. It can't be a general explanation because it just won't work in fresh water. Sometime ago, after Munk and Riley's paper was published,¹⁴ I looked through a set of measurements on one of the fresh water plankton and plotted a graph comparable to theirs for marine ones. There was no doubt that the fresh water ones, diatoms long recognized, tend to be smaller than the marine ones, because they can't have the special class of big ones that presumably make these vacuoles; but for a general explanation one can't use the vacuole-regulated cations alone.—

STRICKLAND: Doctor Hutchinson has more or less anticipated one remark, and that is the inability of an organism growing essentially in distilled water to do much about its ions. Am I right in saying that there are no really large centrics—100 μ and above—found in lakes? There shouldn't be.

LUND: Yes, 100 μ is pretty well above the maximum, I should think.

STRICKLAND: The other point is that, if a cell runs out of nitrogen it obviously cannot make protein. If it is going to make something it will be either fat or sugar, but it doesn't follow that the amount of fat per cell is necessarily increasing. We have a little evidence that this may not, in fact, be the case, and that the fat per cell may not change greatly unless the nitrogen starvation is very severe indeed. As Doctor Guillard said, nitrogen-starved diatoms, fat or not, proceed to get extremely dense.

BRAARUD: There is a question about how the specific weight or specific gravity is being changed. This is rather obscure. The production of nitrogen has been suggested but, in my experience, the cells accumulate fat when conditions for growth are poor and, at that time, I have had the feeling that these cells are heavy and are sinking. But I have no very good observations and this is a general impression.

The principle of flotation which pertains to these large cells with large vacuoles, is related to change of the ionic concentration of the

vacuole, so that it is maintained differently from that of the seawater. I am not a physiologist, but I thought that the maintenance of such difference requires energy. So, if the cells are in good condition they may be able to control vacuole salts to their advantage, but if metabolism is disturbed for some reason, they may not be able to maintain a salt balance and then they get heavy.

EPPLEY Doctor Braarud, it is possible to have maintenance of different ionic composition without a requirement for energy. Eric Denton¹⁵ has done some work with a squid which, as I understand it, maintains a high concentration of ammonium chloride in a certain portion of its body, some sort of a sac. He claims that simply a difference in pH of the sac and the body fluids prevents the ammonia from coming out.

BRAARUD That would be a very favorable situation for the cells.

KOCZY: How is the pH difference kept stable in that case?

EPPLEY. Denton didn't say.

KOCZY Then we are back to the same problem again.

STEEMANN NIELSEN I think we should come back again to the weight of the cells. I think it is very important for plankton, ordinarily at least, that they are a little bit heavier than the sea. They have to fall through the water. If the concentration of nutrients is low, the water around the cells has to be renewed. Diffusion processes alone are not enough.

With 10,000 cells/liter, the average distance between every cell is about 5mm. For diffusion, this is a rather long distance. There are two different ways for renewing the water around the algae. The algae may have flagellae, and thus be able to move. In the areas of the oceans where we find especially bad nutrient conditions, most of the species generally have flagellae.

But in localities where high concentrations of nutrient salts are found, as, for instance, Friday Harbor where I worked during the summer of 1962, generally, none of the species have flagellae. Practically only diatoms were found at Friday Harbor. But if a plankton alga has no means of active movement, it is able to contact new water by sinking passively.

Doctor Riley told us some years ago¹⁴ about the temperature of sinking, and perhaps he would say some few words about that now.

RILEY I think Doctor Steemann Nielsen has laid out the general problem very well. If the phytoplankton cell is motionless with respect to the water mass, it will quickly use up the nutrients within the water immediately surrounding the cell and establish a gradient in concentration. The limiting factor then will be the rate at which diffusion brings new nutrients toward the cell—a relatively slow process if it is a matter of simple molecular diffusion.

It is advantageous, then, for the cell to move with respect to the water and to enter another area of water which has not been so denuded of its nutrients. That may be accomplished either by active motion, in the case of flagellated forms, or by sinking, in the case of diatoms—or by rising, if Doctor Oppenheimer would like to have that included as an alternative.

We generally find if we look at diatom cells in the laboratory that they do have a finite sinking rate, although this can be altered considerably by physiological conditions. We know both from laboratory work and from observations in the sea that old and senescent diatoms increase their sinking rate, and perhaps in part this is an ecological adaptation or a consequence of the kind of growth which gives them a better opportunity to increase their rate of nutrient absorption and, hence, to utilize their stored substances to reproduce and thereby to lower their specific gravity.

One of the ecological implications of all this is that a certain amount of movement of the cells is needed. This implies, perhaps, an optimum sinking rate which will bring the plants into contact with new water, but yet will not be such a rapid sinking rate that they are lost from the surface.

PROVASOLI And from the photic zone.

RILEY Yes, or to make the matter a little more complicated, we must think in terms of an interaction of sinking rate with vertical turbulence, which tends to bring the plants up again. For any particular amount of vertical turbulence, then, perhaps there is a sinking rate which is optimal in order to maintain the population in the surface layer.

As Doctor Steemann Nielsen pointed out, this situation changes during the year. In the winter and spring, frequently turbulence is sufficient to keep diatoms with a positive sinking rate within the surface zone. As the summer comes on, with increased vertical water stability and a lowered nutrient concentration, conditions frequently are no longer very suitable, in our temperate waters, for a diatom population to be maintained in the surface layer, and flagellates tend to come in, since they can maintain a favorable position. Often, then, we find that the diatoms are existing at a deeper level where the water is more dense and the nutrient concentration may be slightly more abundant.

These ecological situations are terribly difficult to predict and they deserve far more attention than they have been given in the past, and I accept some blame for not having worked on them any more than I have. I feel that this is quite as important as studies of primary productivity, because we have to consider situations causing loss of our population by turbulence and sinking, as well as an increase in

population by photosynthesis. We will not understand the problem of phytoplankton existence in the water mass until we go into all of these various aspects that are important.

OPPENHEIMER With respect to the buoyancy problem, if one assumes that fixation of CO_2 into organic materials results in a change in buoyancy of a cell with a silicate test (protoplasm sp.gr.0.9/silicate sp.gr.2.6), then an increase in buoyancy would occur during photosynthesis, whereas nighttime respiration would reverse the reaction. Thus, there is a possibility that a cell in a discrete portion of water may, by this mechanism, be able to move up and down throughout each 24-hour period.

DROOP That is true if light materials are formed.

BRAARUD: I was very much impressed by the paper by Munk and Riley¹⁴ on sinking and its importance for nutrient uptake. But there is one point I can't quite get into my head, and that is that turbulence is not much more important than that paper assumes. When one looks at the sea, whether out in the ocean or near the coast, and observes a small floating stick, it is always moving, and I can't readily accept that one can disregard the turbulence in the sea as an important factor in renewing layers of molecules around the organism. This does not mean that sinking is not also of importance, since it is apparent that diatoms may sink. However, if we consider the holoplanktonic forms, it would be difficult to understand how the population is being maintained in northern waters through the winter and during the period of intensive mixing without assuming that the specific gravity may be changed or may be kept so low that they have a certain buoyancy. This applies especially to the larger species. Halldal's records¹⁶ from the Norwegian Sea of centric diatoms, *Coscinodiscus* spp., as the only fairly regularly occurring diatoms during the dark winter months, offer some support for this point of view, but admittedly the available observations are too few.

KRAUSS In this discussion of how plankton keep themselves afloat, I think there is one other alternative available to the cell—other than synthesis of compounds of low specific gravity—that we have not examined. It may be helpful to consider the way in which the large forms of algae kept themselves afloat simply by secreting gas into certain chambers which actually provide them with very nice floats. Is it possible that at the cellular level there is a capacity to produce or entrap a certain amount of gas to provide a degree of buoyancy? Very good demonstrations of the ability of the unicellular, fresh-water *Arcella* and the marine *Tretomphalus* to produce gas floats are discussed in the review on buoyancy by Denton.¹⁷ The detection of small gas bubbles in the cells of minute phytoplankton is, nevertheless, most difficult.

MCLAUGHLIN. I have two observations to make. When a *Melosira* is grown in a test tube in certain nutrient conditions, the chains are very short. They are found packed down in the bottom of the tube or flask. If it is grown under other nutritional conditions, the chains are long; the test tube growth assumes a very beautiful pattern on the glass, with the chains spiraling up along the sides of the walls. How much, Doctor Riley, does this have to do with floatability of short versus long chains? What effect does chain size and cell length have on buoyancy?

If many of the cells stick together, then some cells in the chain could have a negative buoyancy, where others would tend to hold the whole population up, while auxospore formation or something akin to it is going on.

The second point concerns *Gymnodinium halli*, that Doctor Kenneth Gold worked with for his thesis at New York University in 1962. He found that a spent culture, between a stationary and dead phase, tends to encyst in the death phase. If thiamine and biotin are added to the spent, encysted culture, motility is produced again.¹⁸

I think we must consider buoyancy in terms of chain length and individual cell growth as one goes along a chain. Every once in a while a cell will be seen with a big round chloroplast structure. I don't know how this should be interpreted in terms of adding to or decreasing buoyancy.

RILEY. With respect to that, I might say the Munk and Riley paper¹⁴ which has been mentioned was one in which Walter Munk, a physical oceanographer, provided the information on fluid dynamics that would help us to evaluate the problem of flotation; it was, in essence, an analysis of potential sinking rates in terms of sizes and shapes of cells and cell chains.

You certainly are quite right, Doctor McLaughlin, that the production of a long chain will help to slow the sinking rate. It is also quite true, I think, that in most bloom periods long chains have a minimal sinking rate because of their shape, regardless of what their internal physiology may be. This, however, does not completely solve the impasse that we are in at the moment as to whether diatoms do fundamentally sink or not, I think a lot more work needs to be done on the physiological problems that are involved in determining what their specific gravity is at any particular time.

DROOP. On this question of chain length, under the conditions you mentioned, when diatoms are in long straight chains that float, the chain length and friction will affect their rising as well as their sinking. Whether they go up or down is a question of specific gravity: what role does friction have in buoyancy?

HUTCHINSON: There are three points that I would like to make. One is to indicate that one method of reducing specific gravity hasn't been mentioned, and that is the production of gelatinous sheaths. If the sheath has a density that is less than the mean of the densities of the water and the cell, then there can always be a thickness of sheath which will reduce the density of the whole system. If the organism had a lead skeleton of great thickness—hardly any do but I gather this is a possibility in the future evolution of diatoms—then it would take an inconveniently large sheath that would pose problems of diffusion of nutrients; but with the kind of organisms we actually have, the sheath is certainly an entirely practical way to cause buoyancy and it is very important in fresh water. I don't know enough about it in the sea to know whether it is worth considering, but it is at least one possible method of adaptation.

The second point I wanted to make is that in all discussions of turbulence, one has to remember that turbulence has a spectrum, and if you start with the great gyrls of the Atlantic Ocean, you can go to smaller and smaller swirls until you get down to molecular proportions; but so far as I know almost nothing is known about turbulence in nature of the kind that we are really interested in—little swirls of 2 or 3 mm. diameter.

I have also looked at little floating specks, mainly in lakes, and one gets the impression that there is a good deal of damping. It may well be that this fits in with the Munk and Riley concept.¹⁴ While sinking through elements of water more or less at rest relative to the initial position of the organism in which it would remain if it were not falling, the element containing a number of organisms may be slowly being moved around as a swirl of moderate size, but with very little actual wobbling around of the organism relative to its immediately adjacent water.

The third point I want to make, simply in hopes that someone will one day do something about it, is that there is, so far as I know, virtually no information about the thermal expansion of living organisms, a matter of considerable importance if you are going from warm to cold water—the question, for example, of whether if a thing is less dense than water at, say 4C, it will still be less dense than water at 20C. I think there is some data relative to the posterior end of the eviscerated frog and possibly some for rabbit muscle, but aside from these, I don't know of any. I wish someone would produce good data for plankton organisms.

STEEMANN NIELSEN I think what Doctor Hutchinson has stressed is very important. Some years ago, I tried to start some work in the field of turbulence and diffusion, and I persuaded an able physicist to cooperate with me. But after only one month he refused to work with

the problems. He found them too complicated. How much is diffusion? How much is turbidity? He was absolutely confused. I hope that someone some day will try to attack the problems. They are so extremely important.

It is not enough just to consider that there is turbulence. We are aware, when we are making experiments on, for instance, photosynthesis and respiration in higher aquatic plants, that we must stir the water. But how much stirring is necessary for plankton algae?

RAY. I would like to come back to one of Doctor Hutchinson's remarks on the importance of the secretion of gelatinous or mucopolysaccharide sheaths. It is well-known, among many vertebrates, that this is an extraordinarily important process in keeping the eggs afloat. There is very little possibility, metabolically, of their doing anything else. The gelatinous coat, which sometimes is very thick and quite visible, is an important flotation mechanism. But flotation also occurs among mollusk eggs where there is no visible gelatinous coat. It can be shown that, nevertheless, there is the constant secretion of a very large amount of polysaccharide material, apparently a mucopolysaccharide, which very, very slowly diffuses into the water. It is not visible in an optical sense but can be demonstrated by chemical means, and it is quite clear that this is not only an important flotation mechanism, but also is important for keeping the eggs associated with each other—that it is one of the means of binding an aggregate together and this may also be important.

HUTCHINSON. May I ask a question at this point? Is there any possibility that they can secrete it in a polarized way and use the secretion as a sort of fire balloon, producing something less dense below them and cushioning them?

RAY. I don't know the answer to that, but I would not be surprised if it were so because in almost all cases where it has been studied carefully in a spherical cell, there are so-called active sites of secretion on the cell membrane. These active sites are (in those cases where they have been studied, either at what we call the animal or vegetable pole—the anterior or posterior end) identifiable in terms of the position of the cleavage plane or the position of the mitotic spindle.

A second thing I wanted to comment on was the question of a mechanism of producing gas as a flotation device. My understanding is that the secretion of discrete gas bubbles intracellularly is extraordinarily difficult and rare in nature. It is a problem quite different from the extracellular production of gas. In the case of the gas floats of the larger algae and the swim bladders of many fishes, cells secrete gas that accumulates in some sort of sac. This air sac, though the mechanisms are not well known, is not too difficult to visualize, but to accumulate gas bubbles within a cell is a problem of another magni-

tude. I know that Helge Larson in Trondheim has studied gas production, but I am not familiar with the recent results. Maybe someone else would know about this.

HUTCHINSON. There have been two instances of intracellular gas production mentioned in the literature, both of which I tend to believe. One of them was something I might have mentioned before, but with such distinguished algologists here I felt uncomfortable about it. It is commonly believed that the pseudo-vacuoles of blue-green algae, which can be destroyed by pressure, are gas vacuoles. The other is rather less well-known because it was published in such a very strange paper by E. J. Bles in the late 1920s and relates to *Arcella*. Bles claimed, and the work looks extremely good, that if you take *Arcella* and turn it upside down it produces an oxygen vacuole intracellularly, which in some circumstances allows the animal to right itself.

Some species, *Diffugia*, are good planktonic organisms; they generally have quite massive shells. One species has been described as *Diffugia hydrostatica*, which suggests that it must be a Cartesian diver. If you read the literature you can never find that any person actually saw a gas vacuole in it or in other comparable species, but I have a feeling they probably do have such vacuoles and they work something like those of *Arcella*. I know it must be difficult to make gas vacuoles but I don't think it is quite as rare as you suggest, Doctor Ray.

DROOP Is this vacuole actually within the protoplast?

RAY That is what I was going to ask. I think the *Arcella* case is believed, actually, to be extracellular.

HUTCHINSON. Both Bles' figures and earlier reports that he summarizes show the bubble actually arising with protoplasm around it and when stained with methylene blue there is a complete blue cloud around the bubble.

MCLAUGHLIN. I have a paper in preparation, Doctor Hutchinson, with Doctor J. Wittenberg of the Albert Einstein Medical School, New York,¹⁹ in which he has analyzed the bubble found in *Arcella*. We studied the physiology of the production of the bubble in agnathobiotic cultures. It appears that *Arcella* does form a bubble under certain physiological conditions.

FREMONT-SMITH Intracellular?

MCLAUGHLIN. That is the question I am waiting for Doctor Wittenberg to decide. It is possible to have the *Arcella* float and/or sink depending on many factors, most of which are still not clear.

HUTCHINSON What is the bubble?

MCLAUGHLIN. We believe it to be oxygen.

STEEMANN NIELSEN. I should like to say a few words about the distribution of size groups. In general, in the sea we find rather small cells in waters of lower concentrations of nutrients. In low concentra-

tion of nutrients, it is very likely of importance that the relative size of the cell surface is high in proportion to the cell volume: the smaller the cells, the larger is the surface to volume ratio. In this manner small cells would be more efficient in dilute nutrients.

PROVASOLI. Is this an actual correlation, or is it just based upon physiological principles?

STEEMANN NIELSEN. Oh, surely, yes. It will be observed, for instance, if a silk net (No. 25) is being used for collecting plankton in a very oligotrophic part of the ocean, it will catch very little phytoplankton; perhaps about five per cent of the actual standing stock.

VOLLENWEIDER. That is not true of lakes in the sense that we have examples of high nutrient content and low size groups.

STEEMANN NIELSEN. Oh, occasionally the same may be found in the sea, but at low concentrations of nutrients the large-sized plankton algae will not be found.

LUND. They will in fresh water. In Lake Windermere, there is a seasonal succession and, among the very largest of algae present in the plankton of this lake, desmids occur where the nutrients are lowest.

STEEMANN NIELSEN. That is very interesting. We must try to find out why there is this difference between lakes and the sea.

LUND. This is not peculiar to Windermere. It is a well-known phenomenon in fresh water, I am sure. The most oligotrophic lakes are characterized by wonderful and glorious and beautiful collections of these desmids, which are among the most delightful of all algae.

HUTCHINSON. One aspect of the beauty and glory of desmids, at least the big ones, is that they are divided up so that the ratio of surface to water is enormous—in *Micrasterias*, for example.

LUND. That is only partly true.

HUTCHINSON. I realize it is only partly true.

LUND. Some of the desmids in Windermere, particularly, have relatively less surface area than a desmid such as *Micrasterias* which, in Windermere, only occurs on deposits.

HUTCHINSON. Admittedly *Staurastrum* spoils it all by putting on a gelatinous sheath.

LUND. Yes, it does, indeed. [I should add that, although large desmids (and dinoflagellates) are characteristic of many oligotrophic lakes, the very small algae, aptly named μ -algae by Doctor W. Rodhe, of the Institute of Limnology, University of Uppsala, are probably the more important agents in primary production in such lakes and that, as in the oligotrophic seas, if one collects only with a net, one will be woefully misinformed about the plankton.]

STEEMANN NIELSEN. To return to taxonomy, it is very difficult to say why a taxonomical group is found only in a special area. Take, for instance, the blue-green algae, which are not found too often in the

Arctic waters. I can't see any reason why blue-green algae should not grow in the Arctic. As another example, the Coccolithophorides are rarely found in Arctic waters. Of course, that is perhaps due to historical causes. The group originated in warmer waters and, therefore, it has not yet developed Arctic species. I don't know if anybody is able to speak about the distribution of taxonomical groups of plankton algae other than to simply state what the distribution actually is.

PROVASOLI Could we have some general hints as to which taxonomical groups are more prominent in certain geographical areas than in others? Is there any difference between the different geographical areas?

STEEMANN NIELSEN Yes, at higher latitudes the diatoms are the dominating group during spring. Later, during the summer, it will generally be the Dinoflagellates. In the tropical regions, in places where there is enough silicon, there will be a lot of diatoms, too. But, very often, out in the open tropical sea the role of the diatoms is not very important. Silicon concentration in the tropical sea could support the growth of diatoms for some time, at least, but it is difficult to explain why so few diatoms are found out in the open ocean. Perhaps Doctor Braarud will comment.

BRAARUD Our impression of the occurrence of diatoms in the warmer waters may be based on too scarce evidence, because the observations which Mrs. Hasle²⁰ has made in the equatorial Pacific, using the sedimentation method, showed that diatoms were just as numerous as the coccolithophorids, and that these small diatoms may be more numerous than hitherto assumed in oceanic waters, at least, in areas where the nutrient supply is good.

STEEMANN NIELSEN Oh yes, it is well known that often many diatoms are present in coastal eutrophic, tropical waters, but I am thinking more about the oligotrophic waters. According to Lohmann's²¹ and Hentschel's²² investigation in the Atlantic, diatoms are rather scarce in the oligotrophic parts of the tropical ocean.

GUILLARD Perhaps some of our observations in the Sargasso Sea may be significant here, especially in connection with the relative abundance of different size groups. To oversimplify a little, I believe that in the Sargasso Sea during the course of the year there is, in general, a constant and relatively small number of dinoflagellates, coccolithophorids, and undetermined flagellates. Occasionally there occurs what is called the "annual bloom" in the Sargasso Sea, which is to say that a population of diatoms appears, occurring in conjunction with the flagellates. I have cultured more than a dozen species of diatoms from water samples taken from the sea near Bermuda during times when diatoms were present and have found at least five very small species. The one I wish to mention is a small *Chaetoceros*, varying

in size from about $1\mu^3$ to perhaps $2 \times 5\mu$. It is interesting that neither Doctor Edward M. Hulburt (of the Woods Hole Oceanographic Institution) nor I recognized this diatom in the water sample from which it was isolated, though it was present in relatively large numbers. I made dilution cultures by adding a large volume of sterile enriched sea water to a small volume of the seawater sample and dispensed the mixture in many culture tubes. On the basis of a cell count of the original water sample, I had calculated that the dilution employed should have resulted in the presence of only one or two cells in each culture tube. I expected to get cultures in only a few tubes. To my surprise, the small *Chaetoceros* appeared in almost every tube. Upon re-examining the preserved plankton sample, we observed a number of objects about 1μ in size, having an occasional bristle. I feel that these were resting spores of the *Chaetoceros*. They were present both at the surface and in samples from other depths within the euphotic zone. I wonder if this *Chaetoceros* doesn't survive in the form of these resting spores, which may sink during the winter to the thermocline and remain there without sinking further, either because the density of the water is greater, or because there are more nutrients in the deeper water, which would tend to make the cells more buoyant, as Steele and Yentsch²³ have found. At the time of the next water overturning, the spores may give rise to the vegetative population.

Collier and Murphy²⁴ have recently described a small *Chaetoceros* from Galveston Bay, and I have found clones in several other places, including an impoundment of the Mystic River near Boston. The water there was practically fresh, having only a few hundred milligrams per liter of salts, and had typical freshwater plankton, but it also had a large population of a *Chaetoceros* no larger than 5μ in size. I would like to know if anyone else has found small species of *Chaetoceros* in other places. I know that small species of *Cyclotella*, such as *C. nana*, are found in blooms.

STEEMANN NIELSEN: The *Chaetoceros* plankton was found near the Bermudas, where there is a seasonal cycle and mixing during the winter, and very good conditions for nutrients during a part of the year. But have you found real stocks of diatoms in the central part of the Sargasso Sea, Doctor Guillard?

GUILLARD: I haven't looked.

STEEMANN NIELSEN: I wouldn't expect it at all. According to Lohman²¹ and Hentschel,²² there shouldn't be many.

KOCZY: There are, but very, very few.

RILEY: I had some samples that were taken in the Sargasso Sea, about 500 miles east of Bermuda, where the seasonal cycle is much like it is in the Bermuda waters. There is a limited period during the winter and early spring when diatoms are dominant, during the one period

when the nutrient situation is a little better than it is at any other time of the year. I think the diatoms require a slightly better general nutrient situation than these small flagellates, and the Sargasso Sea certainly is marginal for their existence, but they can dominate the population occasionally.

KOCZY We have discussed standing crops of more or less permanent population; that is, permanent in their location. That is understandable when the water motions are slow, but what happens when we have a current of five to six knots? The population would be blown away in a very short time. In order to escape this eventuality, we have to assume a mechanism which keeps the population stationary. There are two mechanisms possible: diffusion, or formation of resting spores sinking to deeper water, either to the thermocline, or better, pycnocline, to stay there for some time. On the other hand, the plankton may sink to 3,000 meters or more, where they can be transported in a direction opposite to the surface current, and may finally again rise to the surface. We have found living diatoms at 3,000 meters depth by enrichment culture technique with deep water samples. We assumed that a resting spore was contained in the water sample. These spores may be distributed in deep water by turbulence and currents until, on some occasions, they are triggered and a mechanism makes them float. I believe strongly, therefore, in the mechanism outlined by Doctor Braarud. Not only are the resting spores responsible for the reappearance of a diatom species in the spring, but also for the maintenance of a stationary permanent distribution of plankton in a strong current system in the tropics. The vertical motion in the tropics is, on an average, about five meters per year upward, and the turbulence seems to be rather reduced in the thermocline.

PROVASOLI: Do you exclude oceanographic means for bringing back organisms which were in the depth?

KOCZY: The diffusion through the pycnocline is rather slow, and close to molecular diffusion.

PROVASOLI: So, the only possibility will be through flotation of the organism itself or permanent sinking and growing at the bottom?

KOCZY: Yes.

FOGG: Please, what is a pycnocline?

KOCZY: Pycnocline is the layer where density changes most rapidly.

HUTCHINSON: Returning to what I said earlier about thermal expansion. It is perfectly conceivable to design a spore that would sink in warm water but with an appropriate low thermal expansion, so that it would float when it hits the cold water, but so far as I know there is absolutely no relevant data.

PROVASOLI: Is there any difference in temperature that will permit this mechanism?

KOCZY: Yes, there is. The pycnocline is mainly produced by the temperature change.

RILEY: We speak very glibly of resting spores but I would like to introduce another possibility that is involved here. All of us, I am sure, have had the experience of growing diatoms in culture to a senescent state where they were no longer recognizable. We have also had the experience of adding nutrients to seawater and being able to grow species which were not recognizable in the water sample by microscopic techniques. In other words, these cells fade away to a point where there seems to be almost nothing in them, and yet they are still viable. I would expect that for every one of those found at 3,000 meters there would be hundreds in the surface water. They are there waiting.

KOCZY: But if the diatoms wait, they may be transported away from the area with the current. That is the question. They must wait in water that is not moving, or moving in the opposite direction. If they are only in the surface layer, where there is a strong current of up to six knots, they are transported, and the population would be replaced by another one dictated by the origin of the current, time and nutrients.

RILEY: Horizontal diffusion pretty well takes care of that problem. There is complete redistribution all the time.

KOCZY: Against a six-knot current the advection is larger by a factor of 100 or more.

RILEY: I remember Doctor Charles J. Fish at the Narragansett Marine Laboratory remarking that he had once found a specimen of *Calanus finmarchicus*, a temperate-water copepod, in the Florida straits. This is an example of the kind of thing I am talking about. A creature that would never normally be living within a 1,000 miles of that region can occasionally be found there.

KOCZY: That is like finding a salted herring south of the Galapagos Islands. I don't believe this observation. (Laughter)

HUTCHINSON: *Calanus finmarchicus* has been recorded from a lake that is about as far away from the sea as any lake in the world, in the Altai Mountains. I don't know whether to believe the record, either.

PROVASOLI: I think we should now consider the subject of light as one of the factors influencing phytoplankton growth and distribution.

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LIGHT AS A CONTROLLING FACTOR

Discussion Leader:

PER HALLDAL

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HALLDAL: First of all, we may state that light is a variable ecological factor, both in that the intensity varies during the day, and also in respect to spectral composition. There is more blue light when the sun is high in the sky, and more red light when it approaches the horizon. We know that spectral changes occur when the sun's rays penetrate the water. In ocean water, a deep penetration of blue-green light takes place, but near the shore the "yellow substance" absorbs this fraction of the sun's rays, causing a shift toward more red light in shallow water.

Changes in pigment composition of plankton organisms also occurs. For instance, the diatoms, dinoflagellates, and coccolithophorids contain pigments in addition to chlorophyll *a*, that can, at least partly, use light absorbed by them for photosynthesis. The excitation of these pigments are, however, not determined solely by the spectral composition of the light in the sea, which varies greatly with water type, depth, and sun altitude. The blue-green algae when exposed to low-light accumulate higher amounts of accessory pigments. It has even been demonstrated that quite new accessory pigment may show up at extreme low-light intensities in the blue-green alga, *Anabaena*, which I investigated for this effect a few years ago.²⁵

Light intensity affects photosynthesis of plankton organisms, and their distribution in the sea. Earlier, we discussed the buoyancy of nonmotile forms, and there was some uncertainty as to what makes these nonmotile forms remain in the upper layers. When it comes to the ability of the motile forms of the green algae (dinoflagellates and coccolithophorids) to stay afloat, we can make a somewhat more certain statement since they use phototaxis to seek out an environment favorable for growth and reproduction.

There have been several reports of phototaxis in surveys of the algae; for instance, those reported in the Oslofjord by Mrs. Hasle²⁶ showed that the organisms moved at different rates, and in different ways, up and down vertically in the fjord during 24 hours. What af-

fects these differences in movement? Practically all motile forms demonstrate both positive and negative phototaxis. If we expose these organisms to a light gradient, they will gather at some distinct intensity. What sort of apparatus is operating in directing and keeping these organisms at this particular intensity? We can only find out how light is involved in this process by doing action spectra analyses of the reaction. The action spectra data shows that swimming direction can be directed only by blue and shorter wavelengths of light; a careful determination of the light response at different wavelengths will allow us further to conclude that the light is absorbed by a carotenoprotein.²⁷

We know that light is decisive in determining whether a positive or negative phototactic response will take place, and an alternation between positive and negative response must direct these organisms to a certain environment for light. When the action spectrum for this balance was determined, it showed that photosynthesis was involved. So, by using the photosynthetic apparatus, these organisms will induce a certain positive or negative phototactic response. If the photosynthesis is too great, a negative response is induced. By using the photoreceptor which is involved in the direction-finding mechanism, that is, the carotenoprotein as the light absorber, a swimming away from the light source results, and *vice versa* for low photosynthetic rates.

Still another pigment is involved in the mechanism that directs the organisms to a certain intensity; a yellow-absorbing pigment with maximum absorption around 590μ . Light absorbed by this pigment antagonizes the effect of photosynthesis. To conclude, there are three different pigments involved in the balance of response of the algae for light: (a) the carotenoprotein which directs the swimming according to ray direction, (b) chlorophyll which induces positive response at low-light intensity and, presumably negative at high, and (c) a yellow-absorbing pigment which antagonizes the effect of photosynthesis, at least, at low-light intensity.²⁸

We who are working on phytoplankton, whether it is taxonomy, ecology, or physiology, wish to learn about the whole complexity as a unit. More than that, we have a desire to understand the overall distribution of life in the sea. To do this, we need more than ever to consult specialists in other fields. There are a lot of problems one ought to abandon because of lack of the necessary background or training. I, personally, feel that too little emphasis is being put on pure physiology, biochemistry, and biophysics. I am also certain that fundamental processes within these three fields cannot be by-passed by, so to say, "putting the ocean into bottles," moving them to the laboratory, placing them under different conditions, and seeing what happens. The interpretation of what is going on in such bottles must be based upon our knowledge of what happens to isolated biochemical and biophys-

ical reactions within the organisms present. Without such knowledge, it is impossible to evaluate environmental factors.

We will now attempt to concentrate upon some experiments on pure physiology, which may seem to be quite a way off from the problems in the sea. This is, however, not true. Every piece of information we can get about the biology of plankton organisms will ultimately help us to understand the general problem of distribution and behavior.

HUTCHINSON: May we have a definition of these organisms? I feel lost as to what things we are talking about.

HALLDAL: What I have been talking about are motile unicellular forms.

HUTCHINSON: Can you give us some generic names?

HALLDAL: Dinoflagellates (*Ceratium*, *Goniaulax*, *Peridinium*); green motile forms (*Stephanoptera*, *Platymonas*, *Dunaliella*); coccolithophorids (*Coccolithus*).

HUTCHINSON: And all of them have all three mechanisms?

HALLDAL: I have investigated the action spectrum only on one form, but all these forms gather in a light gradient. I suspect that this gathering is imposed through the same mechanism (photosynthesis), and I am also fairly sure that all forms have a light absorber of the mechanism that directs the swimming according to ray direction, which involves a carotenoprotein.

HUTCHINSON: Would the problems be different in *Peridinium* and the green algae?

HALLDAL: I suspect the action spectra would be somewhat different, but I assume that the pigment component would be the same. The attachment pigment-protein could vary. This is indicated in action spectra where distinct deviations may occur from species to species. The overall picture of action spectra of phototaxis is, however, very much alike from species to species.

ALLEN: I don't quite agree that the action spectrum of phototaxis is the same in all organisms. Bendix's experiments with desmids²⁹ indicate a very different spectrum.

HALLDAL: This is true. I am only talking about the motile forms (flagellates) which have a photosensitive spot, or a distinctly situated photoreceptor. The desmids respond phototactically by a mechanism which involves photosynthesis directly, not indirectly, as in the Flagellates. Doctor Bendix³⁰ separated two types of responses in desmids. The response which was restricted to the shorter wavelength, up to 570μ , could quite possibly involve a carotenoprotein. This has not been investigated. The diatoms and blue-green algae also differ from flagellates in their phototaxis.³¹⁻³³

PROVASOLI: On which particular species did you do the action studies?

HALLDAL: On *Platymonas subcordiforma*.

KOCZY: Are all able to move?

HALLDAL: Yes. They are all flagellates.

KRAUSS: We have been especially concerned so far with variability, and this is something, I think, that confronts anyone who works with intracellular algae. These organisms are variable and they are extremely versatile. I would like to expand on that theme, if I may.

FIGURE 1 shows a rather typical view of a group of *Chlorella* cells taken from a normal culture in the light. This is a random distribution. Some are cells of small size—those that have just recently been released from the mother cell wall. Others are large and mature and are about ready to begin division. The chloroplast in this particular species, which is *Chlorella candida*, is a bowl-shaped type of structure. This *Chlorella* is being supplied with inorganic nutrients only. FIGURE

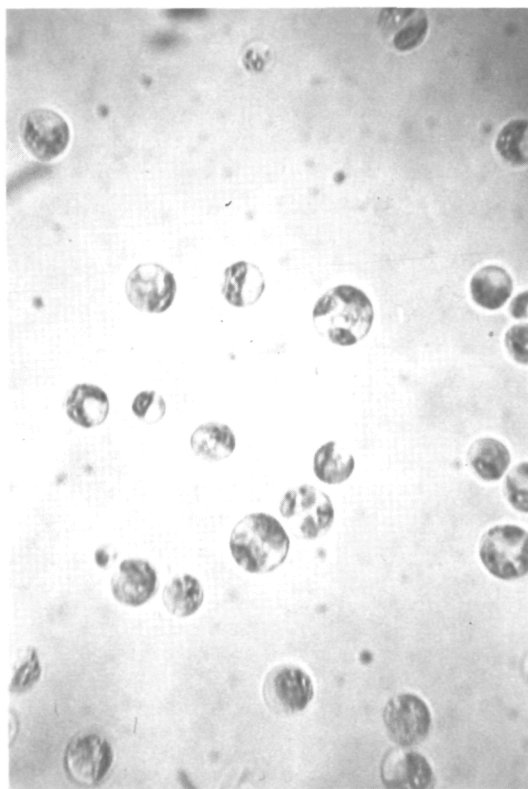


FIG. 1. *Chlorella candida*. Photographed in an actively growing liquid culture utilizing an inorganic medium. 410X.

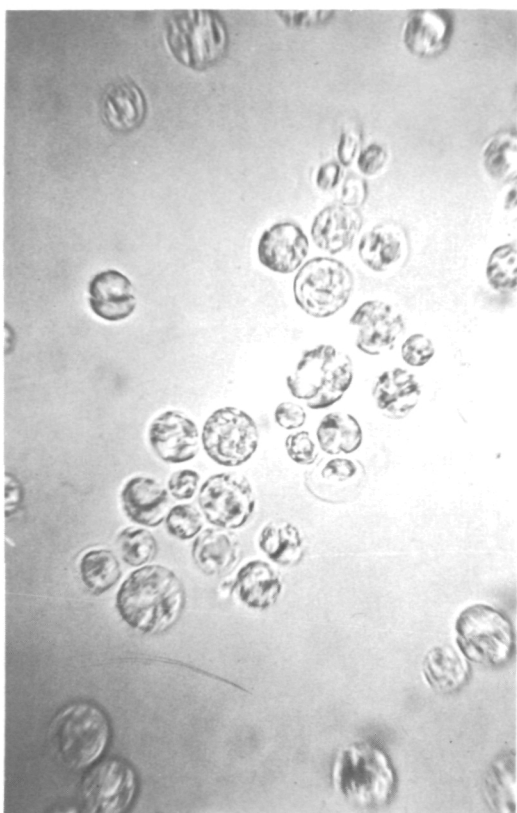


FIG. 2. *Chlorella candida*. Photographed in an actively growing liquid culture utilizing glucose as well as inorganic nutrients. 410X.

2 shows the same organism after it has been placed in the presence of the organic substrate glucose, also grown in the light. Very much like the effect seen in *Ochromonas*, there is a metabolic shift to the utilization of sugar and almost a complete disappearance of the chloroplast. Not only will shifts in organic substrates modify what the organism does, but shifts in light density may do the same.

FOGG: Is this like *Chlorella variegata*?

KRAUSS: It is an entirely different *Chlorella* but is closely related to *variegata*.

DROOP: This phenomenon does not occur with algae such as *Haematococcus pluvialis*, which will grow on acetic acid in the dark without any diminution of the amount of chlorophyll.³⁵

ALLEN: Chloroplasts don't disappear in the dark in other strains of *Chlorella*, either. This seems a very unusual thing for a *Chlorella* to do.

KRAUSS: It depends on which *Chlorella* one is working with. This is extremely critical. There also are organisms, similar to *C. candida*, that become absolutely white in darkness, as long as one supplies a sugar, but if one gives casein hydrolysate to the organism in the dark it maintains a beautiful rich green. In other words, chlorophyll synthesis is accelerated by the presence of amino acids. Why this should take place is very difficult to understand, but it is a very clear and dramatic picture.

FREMONT-SMITH: Is that absolute darkness?

KRAUSS: Absolute, yes.

GUILLARD: Does a small amount of light, an amount of light too small for photosynthesis, make the *Chlorella* stay green?

KRAUSS: That again depends on the organism to which we are referring. We have been working for some time with a *Chlorella* that tends to bleach somewhat in the dark but, when given minute amounts of

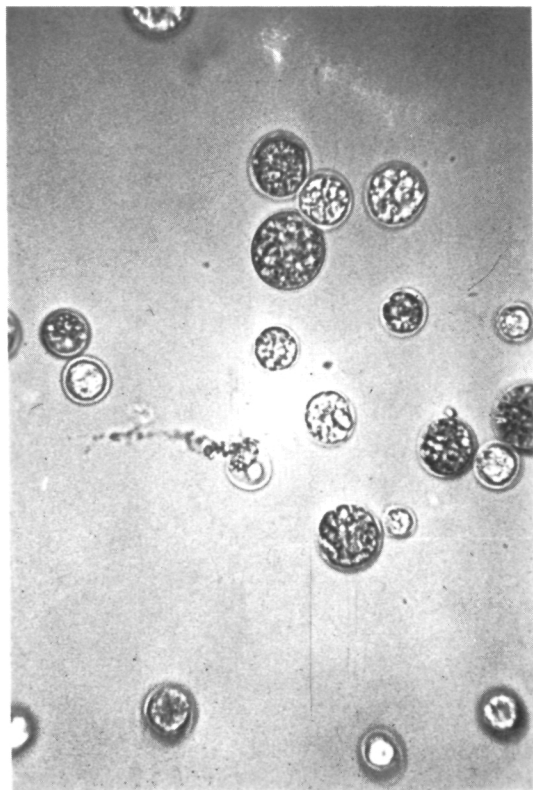


FIG. 3. *Chlorella protothecoides*. Photographed from a slowly growing, old culture grown under high illuminance in a medium deficient in nitrogen. 410X.

light, it does increase chlorophyll synthesis.³⁶ We have the action spectra for the wavelengths of light which bring about this increase in chlorophyll synthesis, and the major effect appears to be in the blue end of the spectrum. However, all wavelengths are somewhat involved.

If the right species of *Chlorella* is chosen, it can be made to perform in almost any way desired for the experiment one has in mind. This may be very disconcerting, however, if one is unaware before the experiments are started of the tricks that the organism can play.

FIGURE 3 shows *Chlorella protothecoides* grown under very high-light intensity. As expected, a heavy carotenoid pigment is present. The nitrogen level in the solution was also quite low. The same *Chlorella* grown for a long time in the dark on sugar has the appearance shown in FIGURE 4. The cells are much larger. There is scarcely a

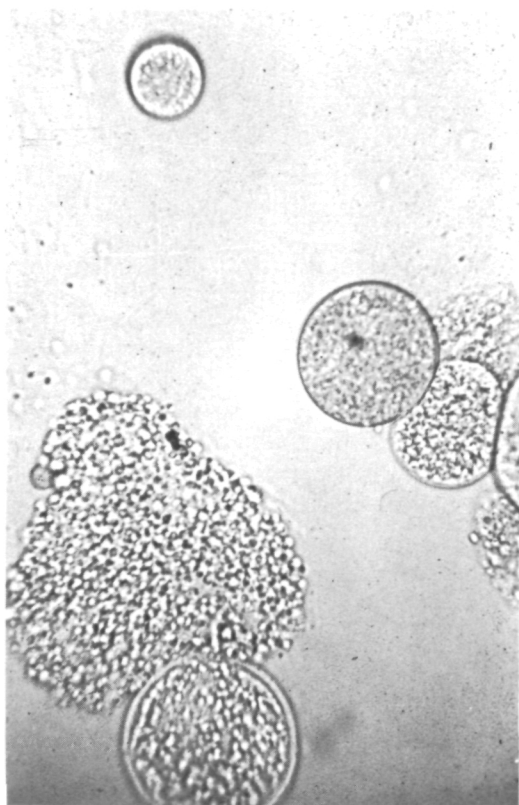


FIG. 4. *Chlorella protothecoides*. Photographed from a slowly growing old culture in the dark in a medium containing glucose. 410X.

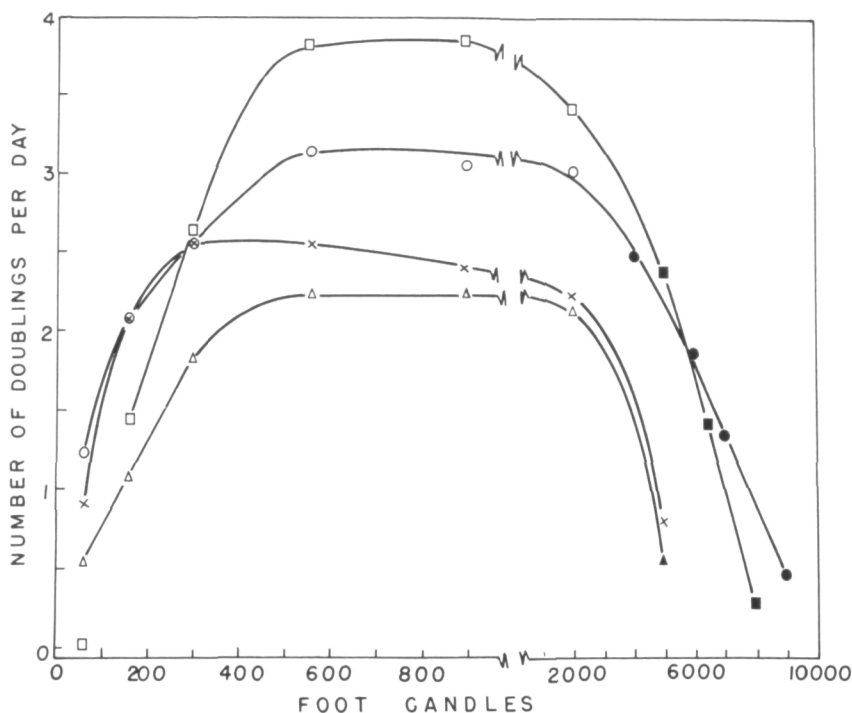


FIG. 5. The growth rates of four species of algae at 25°C. measured at limiting, saturating, and inhibiting light intensities. The symbols are as follows: *Chlorella pyrenoidosa* van Niel's strain, circles; *Chlorella vulgaris*, crosses; *Scenedesmus obliquus*, triangles; and *Chlamydomonas reinhardtii*, squares. Open symbols show growth under fluorescent light; closed symbols show growth under incandescent light (from Sorokin and Krauss, 1958.³⁷)

trace of the heavy carotenoid level, and the organism looks and acts quite differently in culture.

I have presented these figures to indicate that among the species within a single genus, and even within an individual, there is a dramatic capacity for modifying the pigmentation and the metabolic pathways that may be involved in the synthesis of pigments in the organism. There are certainly other aberrations in metabolism in cells grown under different conditions of nutrition, light, etc. that are less obvious to the eye.

In addition to the effects of nutrients and light on the appearance of the cell, I should like to present some metabolic data to illustrate another type of variation that all of us have been forced to meet. FIGURE 5 shows the growth response curves of algae to light intensity. These represent four species of algae—*Scenedesmus obliquus*, *Chlamy-*

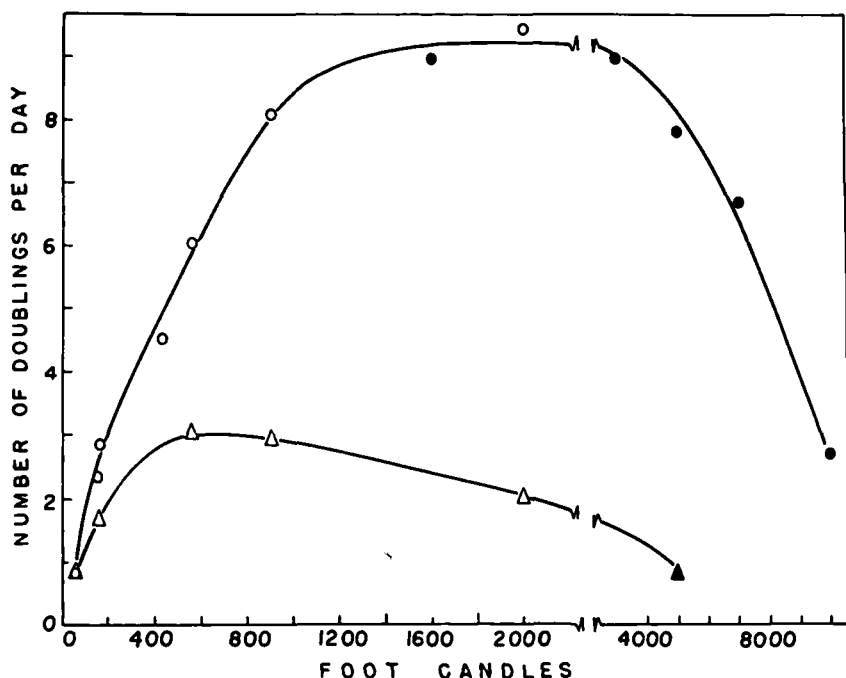


FIG 6. The growth rates of *Chlorella pyrenoidosa* strain 7-11-05 measured at limiting, saturating, and inhibiting light intensities. Circles plot rates at 39C., triangles plot rates at 25C. Open symbols show growth under fluorescent light; closed symbols show growth under incandescent light (from Sorokin and Krauss, 1958.³⁷)

domonas reinhardtii, *Chlorella emersonii*, and *Chlorella van niellii* By carefully studying the growth rates of these organisms in relatively dilute solutions, one can establish curves, such as the ones shown, which indicate where the light saturation level begins, the length of the light saturation plateau, and also where light intensities become high enough to be damaging.

This particular set of experiments was run at 25C. Let us look at another organism, also a *Chlorella*, where the temperature is varied with light intensity (FIGURE 6). It is a high-temperature species, and the curves demonstrate what happens to the response of the cell to the various light intensities imposed. You see that the growth rate is much higher in the upper curve, which was run at 39C. This is an organism which Doctors Sorokin and Myers³⁶ isolated some years ago in Texas. At 25C, the temperature at which we normally culture the algae, the rate is low and light saturation is achieved much sooner. There is a rather dramatic decay in the response of the organism to higher light intensities and a much quicker influence of solarization.

STRICKLAND: The experiment was conducted over what period of time?

KRAUSS: Rather long periods of time—a number of days. I don't recall at the moment exactly how many. What one does, you see, is to achieve a given growth rate at a given light intensity and then, by serial transfer, continue the growth at this rate for several days, until it is quite certain that the growth rate is constant at a given light intensity, at a constant temperature.

STRICKLAND: So, these points represent conditioned algae that have been exposed to this light and temperature for some considerable period of time?

KRAUSS: Yes. They have become stabilized at that level.

JONES: Is this in continuous light, or in a cycle?

KRAUSS: These particular experiments are in test tubes. One permits the concentration of the algae to reach a point somewhere below the optical density of 0.8, where there is no effect of mutual shading on the response of the organism to light intensity. As soon as one gets a little beyond this, the cultures get too dense and there is significant mutual shading. These are very dilute cultures, in other words.

STRICKLAND: Is this a light-dark cycle?

KRAUSS: No, this is in light the whole time. The only point I am making is that we have here an example of how cells change in growth rate, and probably metabolically as well, in response to light at different temperatures.

FIGURE 7 gives the chlorophyll content of a group of cells grown in a continuous culture apparatus, essentially a chemostat, where the optical density has been held constant and the light intensity has been changed. The data, again, are out of context, but they are the result of a series of experiments where we were looking at the effect of very high light intensities.* You will notice that there are two conditions: cells grown with two lamps and cells grown with four. Each of these lamps is a quartz-line, iodine-vapor lamp that delivers 7,800 ft.-c. per lamp at the surface of the culture. With two lamps, 7,800 ft.-c. are being delivered to each side of the culture chamber where the cells are being grown. When four lamps are used, 7,800 ft.-c. are being delivered from each of four sides—something equivalent to 31,200 if a direct conversion could be made. One culture is at an intensity that is twice that of the other. Both, however, are growing fairly well.

The point I want to make here is that there is a change in total chlorophyll percent-dry-weight with the change in illumination. As

*Krauss, R. W., January 15, 1962: Limiting Factors in the Culture of Unicellular Organisms, Annual Progress Report, Office of Naval Research, Contract Nonr 556 (00), Project NR 104-101.

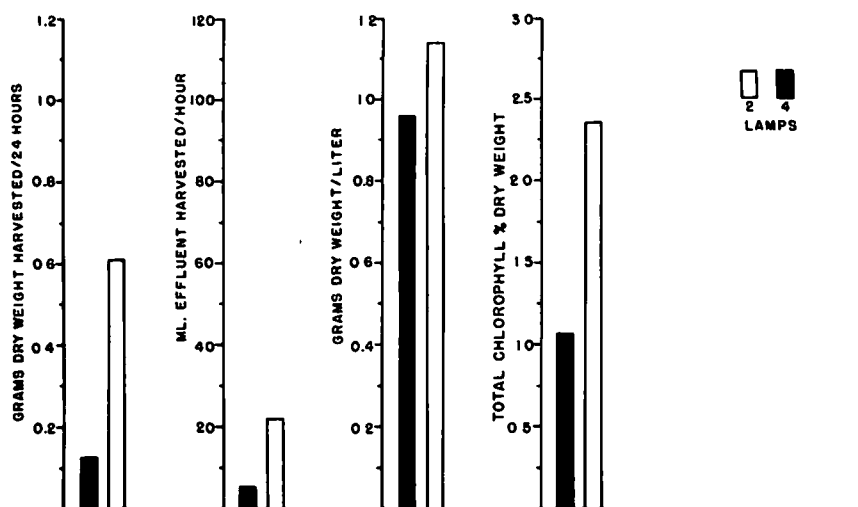


FIG 7. The effect of increasing light intensity on the yield, dry weight, and chlorophyll concentration of *Chlorella pyrenoidosa* growing in a continuous culture apparatus at 25C. The culture was illuminated with either 2 (clear bars) or 4 (solid bars) quartz-line lamps giving an illuminance of 7,800 foot candles for each lamp. The optical density of the cultures was held at 3.0 as read in a spectrophotometer at 550 $m\mu$ in an 18 mm cuvette.

the light intensity increases, the amount of chlorophyll in the cell dramatically decreases. The growth rate also is less at the very high light intensities.

This is simply another example of how the chlorophyll content, the growth rate, and ultimately the rate of photosynthesis are affected by light intensities that may be high. I should say that one cannot achieve growth with such extremely high light intensities until after a rather long period of adaptation has taken place. I have some other data indicating that there is a great deal of fluctuation in the types of rates that one gets in cultures until one gets them adapted to a given light intensity. Some experimental results that have been reported indicate that the algae should not be growing at all at this light intensity, but, indeed, they do grow very well even though the chlorophyll content has dropped. Again the versatility and adaptability of the cell shows itself.

STEEMANN NIELSEN: What light source do you use?

KRAUSS: We were using quartz-line iodine-vapor lamps which are new General Electric products giving high light intensities.

STEEMANN NIELSEN: Is there some ultraviolet in this light source?

KRAUSS: No. The light intensity was measured at the level of the cells after it had been screened through very large water filters which

will take out a good portion of the infrared. It goes through a number of layers of glass, so there is no UV.

STRICKLAND: What wavelength of UV?

KRAUSS: That's a good question. I do not have the actual absorbance characteristics of the filter system that we used, but in the normal sense, coming through the amount of glass involved here, the shorter wavelengths are very strongly screened. I cannot give you the exact spectrum.

ALLEN: The lamp surely doesn't emit very much UV. It is a tungsten filament lamp.

STRICKLAND: I am not splitting a hair here. There is UV technically below 4000 Å, and there is UV at really short wavelengths, say below 3500 Å.

HALLDAL: This UV is probably down to about 3200 Å.

KRAUSS: These lamps, as Doctor Allen has pointed out, are tungsten.

HALLDAL: It doesn't matter; there is still UV.

KRAUSS: There isn't an appreciable amount coming through.

HALLDAL: No, it is not appreciable, but it is there. It doesn't harm anything.

OPPENHEIMER: E. J. Ferguson Wood and I, during routine microscopic examination of sediments, found that UV at 4000 Å will degrade chlorophyll in blue-green algae, but will not affect the chlorophyll in some flagellates.³⁹ About five minutes' exposure would convert the normal red fluorescence of the blue-greens to a bright yellow fluorescence.

MYERS: I think it unlikely that the phenomena in question can be attributed to UV. The effects of high visible light intensity in causing inhibition or "solarization" of photosynthesis are well known.⁴⁰ Furthermore, it is clear that there is less UV in Doctor Krauss' system than there is in sunlight.

STRICKLAND: These are pretty bright lights.

MYERS: But both the fraction of total energy and the absolute energy of UV in this system are less than one would see in sunlight.

STRICKLAND: But the absolute amount is not necessarily negligible.

MYERS: That's true for the long UV.

VOLLENWEIDER: I have made a number of experiments with UV of 3600 to 4000 Å. Experiments on photosynthesis in the water, in the natural situation, normally show inhibition at the surface and an optimum some meters below. If a normal light source is added (and this may be a filamentous light source, such as Philips lamps, stronger than the lamps that were used here) and if a little UV is added (say less than one per cent, or even 0.5 per cent of the total light intensity)

the photosynthetic rate will be depressed very markedly to about 50 per cent of the previous level or less.

This depression varies from species to species, and it is also different at various stages of growth. A young culture will respond differently from an old one. We often find that old cultures do not respond at all. It is enough to add a small amount of UV. The experiments were conducted in glass which absorbs—surely below 3600 Å—so that essentially there is no UV passing. The depression is more or less reversible. In a two hour experiment a depression may be observed. If the UV is interrupted, the original state of photosynthetic activity will be restored, and this may be reversed several times during the day. In other experiments with a duration of perhaps 10 to 12 hours, there is a certain adaptation to UV. First there is a certain depression, and later the rates increase. But still at the end of about 12 hours there will be a depression, as compared with the control.

HALLDAL: This contradicts, somewhat, experiments I have been doing on algae. I have been irradiating at quite another UV region, down to about 220m μ , for a long time, and it does not harm *Chlorella* at all. It even increases the photosynthetic capacity a certain amount. So, I am very surprised to hear that there is evidence of harm at around 400m μ , or between 300 to 400m μ . It could be a question of intensity rather than wavelength.

VOLLENWEIDER: In your experiments did you grow natural cultures in UV?

HALLDAL: No, they were grown in normal light. They were under fluorescent tubes and then removed and irradiated for two hours, and then the photosynthetic capacity was observed, using C¹⁴ as a tracer. After that, I irradiated with UV to see if any harm had been done, and I got an increase of 25 per cent in photosynthetic capacity.

HUTCHINSON: Your UV was given separately, Doctor Vollenweider?

VOLLENWEIDER: Yes.

PROVASOLI: While in your case, Doctor Halldal, it was given together?

HALLDAL: Yes.

ALLEN: Several different people have used UV as a selective inhibitor of photosynthesis. With it, one can eliminate photosynthesis before respiration is affected. I find this a little hard to reconcile with your observation.

OPPENHEIMER: Bacterial cells inactivated with UV at 2537 Å can be reactivated with other wavelengths of visible light from blue to red, depending on the organism.⁴¹

HALLDAL: It is a question of what is meant by UV. There are so many different regions in that part of the spectrum.

OPPENHEIMER: I refer to 2537 Å, which is lethal to bacteria.

HALLDAL: Talking about UV is very difficult if the spectral region is not stated. In the visible, we are very careful to state whether the light is blue, green, or red. In the UV, the whole region is put in a big sack, though the effects of radiation there differ much more than in visible light. McLeod and Kanwisher⁴² have investigated action spectra in the UV and found photosynthesis occurring down to a wavelength of 3000 Å, or something quite remarkably low; but of course the damage done to the cell is rapid, so that the time scale is very important. UV quantum may be useful in photosynthesis, but the concurrent damage that occurs may soon offset any beneficial effects, so that the time scale is important.

MYERS: I think it is not surprising that there are divergent results here. There are, for example, a number of reports that fluorescent lamps are not desirable for growth of algae. Algeus⁴³ made such a statement about daylight fluorescent lamps, and other laboratories also believe they are undesirable.

The only UV one anticipates with fluorescent light is the 3650 mercury line which does come through the phosphor and glass envelope. We have tried to see if, by adding 3650 from a mercury lamp to tungsten illumination, we could obtain symptoms with respect to growth. We were never able to observe adverse effects. So we use fluorescent and tungsten lights almost interchangeably as light sources for growth of algae. However, one cannot ignore the fact that other laboratories have had difficulties. All we can properly say now is that there are uncertainties in effects of the long end of the UV spectrum, say beyond 3200. In the lower UV, of course, there are severe effects.

If anyone wishes to check the longer wave UV, he has an easy tool in the 3650 mercury line. There are lots of pigments that absorb here. Absorption by the carotenoids, chlorophylls, flavins, or pteridines might cause difficulty, but no one has yet demonstrated a clearcut effect. At shorter wavelengths, there are other difficulties because one is faced by absorption by the media as well as by the glass of a container. For example, iron citrate in the culture media will largely screen out 2537 and protect algae against damaging effects.

KOCZY: Have any of the people who are measuring light in these experiments measured the light intensity in terms of energy per square centimeter for different wavelengths?

HALLDAL: Yes, we did that.

KOCZY: We have to know, really, the energy per square centimeter for different wavelengths.

PROVASOLI: And inside the test tube.

KOCZY: Yes. Otherwise, I think we are talking about things that cannot be compared.

ALLEN: In the average experimental design, measurement of the distribution of energy at different wavelengths may be an almost insuperable problem. Even getting the energy distribution of a fluorescent light is a major undertaking.

GOLDBERG: One thing that has worried me in these light experiments is the possibility of photochemically induced reactions in the inorganic medium itself. I think we have some evidence that they are occurring. Recently, Miyake and Tsunogai⁴⁴ pointed out that with light in the near UV region there is a significant production of free iodine in sea water by the photochemical oxidation of iodine. Such photochemically-induced reaction products may have some inhibiting or enhancement effects upon the phenomena that are observed in nature or in the laboratory.

HALLDAL: I used an absolutely organic-free medium during the UV irradiation. I centrifuged and resuspended in 2 media containing only inorganic ions, and measured the incident quanta within the cuvette by measuring the outside energy and correcting or checking the absorption within the vessel, using a photomultiplier tube with a known sensitivity curve. But Doctor Goldberg's point about decomposition in the inorganic medium and natural sea water should be checked.

PROVASOLI: Your organisms were marine organisms?

HALLDAL: Yes.

PROVASOLI: Therefore, you employed an artificial solution simulating sea water. The impurities brought in by the "chemically pure" salts, as stated on the bottles, are not small; for iodine it is about 40 $\mu\text{g}/100\text{ ml}$ of medium.

HALLDAL: But I centrifuged the organisms down and then suspended them.

GOLDBERG: The levels of iodide plus iodate which Miyake and Tsunogai⁴⁴ used in their experiments were natural seawater values, about 50 $\mu\text{g}/\text{liter}$. Iodate was not affected in these experiments. The iodide was in part oxidized to free iodine.

PROVASOLI: The label on the chemicals does not say whether the iodine impurity is iodide, iodate, or a mixture.

There is another effect of light we must consider when we employ artificial media containing EDTA as a metal binder. Jones and Long⁴⁵ found that the EDTA-Fe chelate is photosensitive and decomposes. In stagnant cultures, therefore, the medium changes in time depending on the kinetics of decomposition and trace metal utilization.

GOLDMAN: Iron is very light sensitive, too. I would like to emphasize the ecological importance of high-light intensity, particularly at high latitudes. My colleagues and I have been working on light inhibition in Antarctic waters, and we find that the organisms just

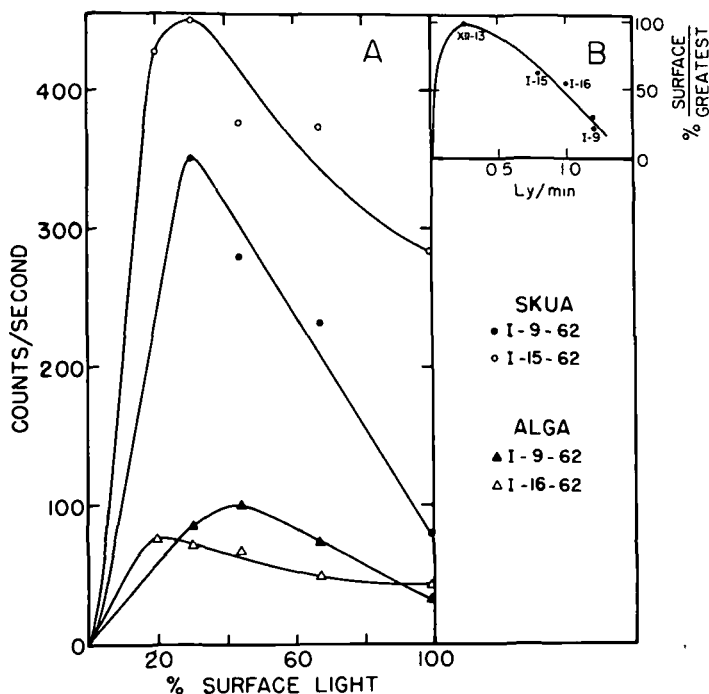


FIG. 8. (A) Photosynthesis (C^{14} uptake) *in situ* as a function of total incident light intensity in Skua and Alga lakes on 9 and 15 January 1962; four hour incubation time, reduction of incident daylight achieved by neutral density screens. Insert (B) shows the degree of realization of photosynthetic potential through the season's experiments as a function of the average total incident light during each experiment.

aren't adapted to the high intensities they encounter.⁴⁶ One gets an inverse photosynthetic relationship to light intensity over a 24-hour period, even in the deeper water of shallow lakes (FIGURE 8).*

These experiments were run simply by placing neutral density filters over the natural phytoplankton population and reducing the incident-light intensity in a graded series to 20 per cent of full daylight. A great increase in photosynthesis is achieved by removing incident-light with the neutral density filters. Notice that the maximum rate of carbon fixation occurs at only 20 per cent of full daylight. The upper curve shows the relationship of a maximum rate achieved by neutral density filters to the rate of photosynthesis at the surface. Maximum

*Doctor Goldman wishes to acknowledge the support of this work by the U.S. Antarctic Research Program, NSF grants G18020 and G23868.

photosynthetic rates are at about 0.2 Langlie [at 15C, g cal ($\text{cm}^2 \times \text{min.}$)].

Here we have an ecological paradox. Algae which are dependent upon light energy to provide a favorable environment are, at the same time, extremely inhibited by this light energy.

STRICKLAND: This is in sunlight?

GOLDMAN: Yes, with a natural plankton population.

STRICKLAND: Were the plankton suspended in the medium?

GOLDMAN: They were suspended in the medium and also placed under neutral density filters.

STRICKLAND: But under neutral density filters there is a very small amount of water, so any UV in the sunlight is still getting through.

GOLDMAN: Right.

STRICKLAND: Whereas, in the natural water conditions it isn't.

GOLDMAN: These were under about one centimeter of water.

STRICKLAND: So, if UV is causing inhibition, any work done with a neutral filter in a trough with sunlight can produce inhibition.

GOLDMAN: Yes.

STRICKLAND: Whereas, if you hang bottles down in the water and filter out the UV, while providing the same percentage of light, do the results agree?

GOLDMAN: Both the deepest water, which is only one meter below the surface, and the surface water show an inverse relationship to light intensity. Maximum carbon fixation is at midnight.

HUTCHINSON: What is the angular height of the sun?

GOLDMAN: This occurred about midsummer in the Antarctic. I don't know the exact angle.

KRAUSS: What is the light intensity?

GOLDMAN: Over 10,000 ft-c.

MYERS: Measured normal to the sunlight?

GOLDMAN: Yes.

MYERS: But not normal to the surface?

GOLDMAN: No.

HUTCHINSON: The proportion of UV would be much lower than at moderate latitudes.

GOLDMAN: I should think so.

STRICKLAND: It depends very much on the cloud cover, of course. The slightest haze will affect it.

STEEMANN NIELSEN: We did some experiments in Friday Harbor, during the summer of 1962, to see if the UV in sunshine would do any damage after it had passed through the glass of the experimental bottles. We found, for the surface plankton, that there was no damage at all; the walls of the bottles were able to filter off the UV sufficiently. But, if we adapted the surface plankton, to a relatively low-light

intensity for two days, even 20 per cent of the UV in ordinary sunshine reaching the surface of the bottles was enough to damage the algae inside. Therefore, the prehistory of the algae is of real importance.

HALLDAL: It is a question of intensity.

MCLAUGHLIN: May I pose two questions which I feel bear on this problem of light and chlorophyll production? If we grow phytoplankton in low-light intensities, and then move the cells to a higher-light intensity, does one get hyperchlorophyllous cells as one does with *Euglena gracilis*?* Would the experiment terminate with cells having more chlorophyll than if they had been grown continuously under "normal" light intensities? Another question: Do we know how the ratio of chloroplast structure to cell volume influences C^{14} uptake?

KRAUSS: There is a difference in the ability of the cells to photosynthesize. I cannot answer your first question. We know that the chlorophyll level changes. As light intensity increases to damaging proportions, generally the chlorophyll is reduced. Whether this means that the whole chloroplast structure is reduced in size or not, I do not know, so I cannot answer that question.

MCLAUGHLIN: Suppose they form 10 chloroplast structures under normal light conditions, but in low-light conditions form 16; then the low-light condition *Chlorella* is moved up to a high-light condition—one would expect them to photosynthesize at a greater rate with 16 chloroplasts than at 10.

KRAUSS: I suspect they do photosynthesize at a greater rate, but there is a time factor to consider. Some of that excess photosynthesis probably causes photo-oxidation and, in turn, the cells are damaged. The chlorophyll level will finally stabilize at a lower level, but one can expect a great deal of fluctuation over a period of days while the cells are achieving this new chlorophyll level and this new growth rate.

I cannot tell what is happening in terms of how much chloroplast there is. There could be the same amount, the same bulk of chloroplasts with fewer lamellae contained therein, or with a much lower number of chlorophyll molecules per unit of chloroplast surface. This, I cannot answer. It certainly is an attractive problem. We can, however, demonstrate some differences in the nature of the chloroplast in different species.

FIGURE 9a, no. 1 shows a drawing of the chloroplast structure of *Chlorella regularis* which has a mantle-shaped chloroplast. The drawing on the left is a cross section of the mantle; the drawing on the right is an exterior view. This type of chloroplast is bulky and large. The other figures represent differing types of structure in other

*J. A. Schiff, Brandeis University: Personal communication.

species. Of special interest is the net-like chloroplast found in *Chlorella ellipsoidea* (FIGURE 9b, no. 7).

In young cells, and in cells that are under high-light intensities, this mantle-shaped chloroplast is somewhat reduced, but in another species of *Chlorella*, such as *Chlorella vulgaris* var. *luteoviridis*, the chloroplast is normally a small disc-shaped structure and the photosynthetic capacity of this species is enormously less—about one-third or less at optimal conditions—than the maximum photosynthesis in *Chlorella regularis*. So there is little doubt that the photosynthetic rate of the organism can be conditioned by the size of the chloroplast. I think this is fairly obvious.

GUILLARD: I think this may bear on the question you asked about chloroplast numbers, Doctor Krauss. I was working with *Cyclotella nana*, a diatom 5 μ or less in diameter. Ordinarily, it looks as if it has only one parietal chromatophore. When a culture is put into dim light, however, it gets much darker in color and the individual cells look darker. I found that if I crushed a cell under a cover slip, what appeared to be several small chromatophores came out of the cell. But if I crushed a cell from a young culture growing in strong light, there seemed at most to be two larger chromatophores. Consequently, when I added to the description of the species,⁴⁷ I said that there can be one to several chromatophores. I think there really are several, not just one large one breaking into pieces. (This judgement is based on comparisons with other species having several chromatophores.) In short, possibly when this particular species is grown in dim light there are more chromatophores, rather than just more chlorophyll in the same number of chromatophores.

PROVASOLI: Or both.

GUILLARD: Probably both.

MCCLAUGHLIN: We have grown cultures under what we call normal light conditions, and with varying phosphate concentrations. When they are harvested, the planchets can actually be separated by their color as a function of phosphate concentration, but cell numbers are fairly uniform, particularly in short experiments.

Sometimes, when water samples from the ocean are examined, they evidence populations of cells with very small chloroplast structures. When they are sampled a few days later, the cells are loaded with chloroplasts. Could it be that these plants produce more chloroplasts under cloudy-dull skies to take advantage of the decrease in light?

In some unpublished experiments using light as a variable, we put cultures under very low light after having previously been in high light, and within a short time they developed a high chlorophyll content. If, after exposure to low-light intensity, they are moved to high light, they die. It appears to be a conditioning cycle; if they are

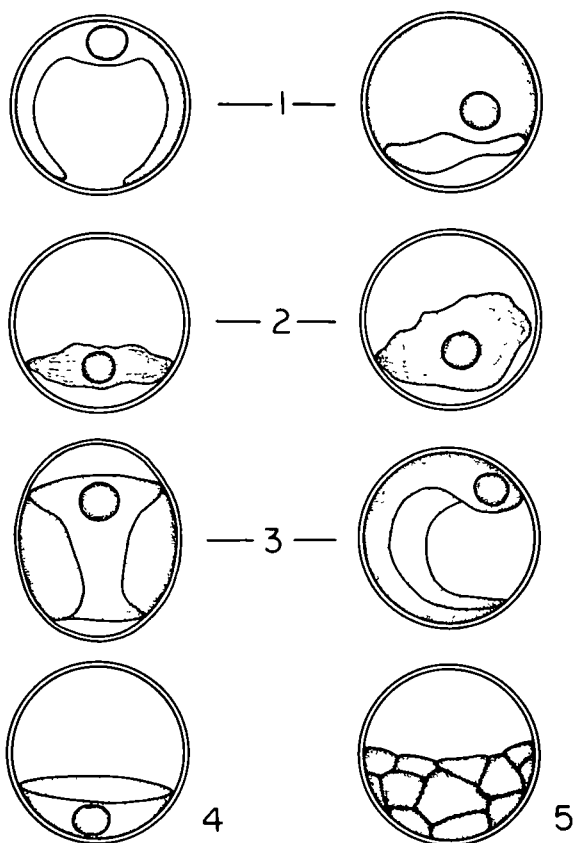
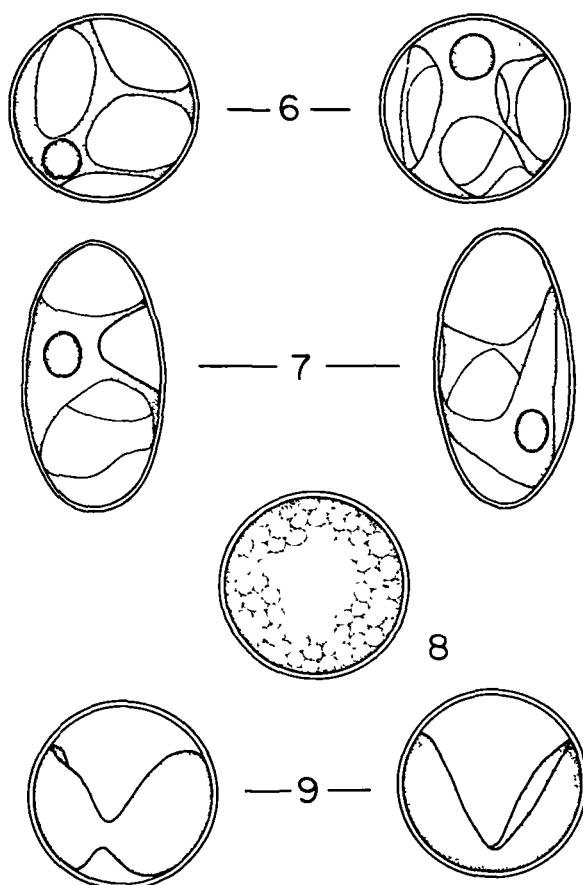


FIG 9a (above) and b (opposite page) Diagrams of three typical chloroplast types in species of *Chlorella*. a. Net-like chromatophore. b. Disc-shaped chromatophore. c. Mantle-shaped chromatophore (from Shihira and Krauss, 1963³⁴).

growing in low light, our laboratory axiom is: Don't move them quickly into very high light, because the culture may well be lost.

MYERS: I would like to comment on the effects of light intensity on the growth and photosynthesis of phytoplankton. FIGURE 10 presents two curves drawn diagrammatically and purposely without actual values. Curve A describes steady-state growth as a function of light intensity. It is based upon experimental curves obtained with a number of algae,⁴⁸ including the lower-light intensity portions of the several curves presented by Doctor Krauss (FIGURES 5, 6 and 7). Rate of growth is measured as the simple interest rate, or first-order



reaction rate constant, or specific growth rate, $k = dN/Ndt$, with essential dimensions of reciprocal time.

Curve B is the classical light intensity curve of photosynthesis, again based upon experimental curves often obtained. Curves A and B seem very much alike in character and, since growth depends upon photosynthesis, it is natural to suppose that they are identical and superimposable. The point of this discussion will be that the curves are not identical, and I reflect here my own bitter experience because I once was quite confident that they were.

In testing for identity, we first need to put the abscissas in the same dimensions. Photosynthesis is often expressed in convenient but mixed-up units, such as $\mu\text{l O}_2/\text{mg cells/hr.}$ or $\mu\text{l CO}_2/\text{mg. cells/hr.}$ However, these units can be converted to homogeneous units, such as

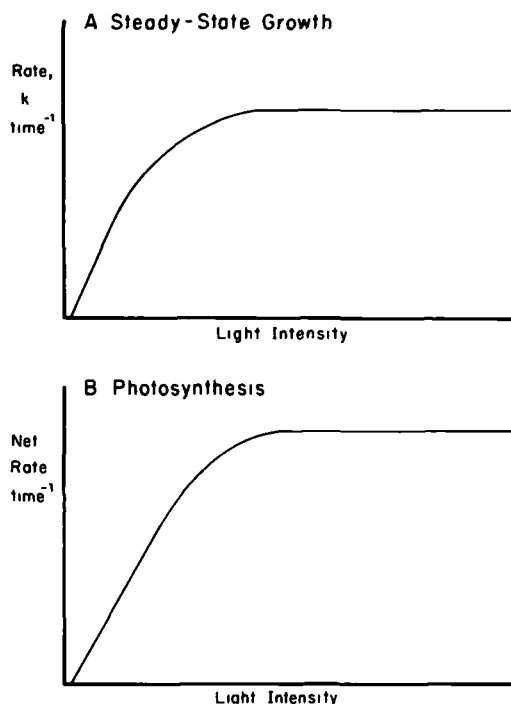


FIG 10 Generalized comparison of growth and photosynthesis as a function of light intensity.

carbon atoms assimilated per cell carbon/hr., or reciprocal time. In short, we can express the information of each curve in precisely the same dimensions as is done in FIGURE 10.

Now that photosynthesis and growth have been made expressable in the same dimensions, one can test for identity of the two curves. A simple partial test of identity is to ask whether the light-saturated rates are identical. Wherever we have made this test, on *Chlorella*, on *Ochromonas*, on *Euglena*, or on blue-green algae, the answer has been negative. Often, the rate of photosynthesis, measured in a short-time manometric experiment, was something like twice as great as the rate of growth observable under steady-state conditions.

There is the anomaly that, over a short time period, algal cells can photosynthesize faster than they can grow. The anomaly is a consequence of great variability in composition in terms of storage products and, as has already been discussed, in terms of pigment content. Let us examine unspecified parameters of the curves which explain why the curves are not alike.

In determining the light intensity curve of photosynthesis, one uses a single batch of cells (usually grown in the laboratory under effective low-light intensity). Measurements of photosynthesis, as by manometry, are then made on replicate samples of this one batch of cells, using a different light intensity for each sample. The results, as in Curve B, are homogeneous, in the sense that all points on the curve were obtained on exactly the same cellular material and determined within a time period that was short compared to the life cycle of the cells.

In determining the light-intensity curve for growth, one must proceed in quite a different way. If the curve is to have meaning, each point on it must be obtained under steady-state conditions maintained over time periods that are long compared to the life cycle. The curve is not homogeneous, in the sense that it does not describe uniform material; it describes cells which were both *grown* and *measured* at each specified light intensity. Each point on the curve describes two effects: (a) the effect of a specified light intensity in regulating the character of cell machinery, notably pigment content, and (b) the performance of that machinery under the specified incident-light intensity.

From these considerations, the two curves may also be viewed in another way. The light intensity curve for growth is an intrinsic character of the alga used and the temperature. In contrast, the light intensity curve for photosynthesis is not an intrinsic character of an alga, as has been rather thoroughly documented.⁴⁹⁻⁵¹

Up to this point, we have been discussing optically-thin cultures in which all cells receive essentially the same light intensity. Incident-light intensity on each cell has been virtually identical to the incident-light intensity on the whole culture. How can we now extend our ideas to the more practical case of an optically-thick culture in which there is a large gradient of light intensity within the culture? This problem was first tackled when a number of us concerned with algal physiology were trying to understand mass culture of algae under sunlight illumination. How could we predict the production rate of a dense culture which absorbs most of the incident sunlight illumination?

Clearly, such a prediction requires knowledge of the light intensity curve. At this point, a number of workers, myself included⁵² made a conceptual error. We assumed an invariant light-intensity curve, such as the curve for growth. Our error since has become clear. In an optically-dense culture, whatever the incident-light intensity, the cells adjust their machinery and pigmentation as a slow response to average-light intensity per cell. But if they move around in the culture at any rate which is rapid compared to their life cycle, they move alternately through regions of high and low intensity. What we

really need is the relation of "instantaneous" growth rate versus light intensity, and this is the real meaning of the lower Curve B of FIGURE 10. But we have already seen that this lower curve, short-period photosynthesis versus light intensity, is not an invariant character of an alga. We have had occasion to test and at least crudely confirm these ideas,⁵² but unfortunately under limited conditions far removed from ecological conditions.

One consequence of this line of discussion is that an estimation of the production rate of a dense algal culture is faced with a special difficulty. Some of the features of this same difficulty also apply to estimates of productivity of photoplankton under natural conditions, as has come to be recognized.^{51,53} However, I think that a more important consequence follows from the very nature of the phenomenon. The differences in the two curves of FIGURE 10 could not arise if it were not for the very great possible variation in composition and working behavior of the algal cell.

As with other microorganisms, the algae are essentially protein-synthesizing organisms. But they have great potential variability in synthesis of storage materials and in synthesis of even essential machinery, such as their photosynthetic pigments. This variability is easily brought into play by light intensity (as well as by nutrient deficiencies, which have not entered this discussion at all). I think that we introduce a rather serious error whenever we think of an algal cell as an invariant unit of machinery.

JONES: In connection with this discussion, FIGURE 11 illustrates an interrelationship between light intensity and nutrient levels. Cultures of *Carteria* sp. and *Nitzschia closterium* were grown in continuous culture in an artificial seawater medium with 125 $\mu\text{g/liter}$ nitrate-N and 12.5 $\mu\text{g/liter}$ phosphate-P at 16C, at light intensities varying from 80 to 390 ft-c. The organisms were also grown in a medium containing 140 mg/liter nitrate-N and 15.5 mg/liter phosphate-P at the same temperature and over the same range of light intensities. It was noted that by increasing the nutrient level, not only was there an increase in the rate of division of the cells, but the organisms can grow at much higher light intensities. This is particularly marked for the *Carteria* sp., where it was seen that maximum growth in the lower concentration of nutrients occurs at about 120 ft-c, but, at the increased nutrient concentration, maximum growth rate occurs at about 300 ft-c.⁵⁴

I have already spoken of the role of temperature in this process, and I would like to say that if we are to study the ecology of phytoplankton, we must realize that we are dealing with a number of variables which interact with one another. In attempting to study physiological

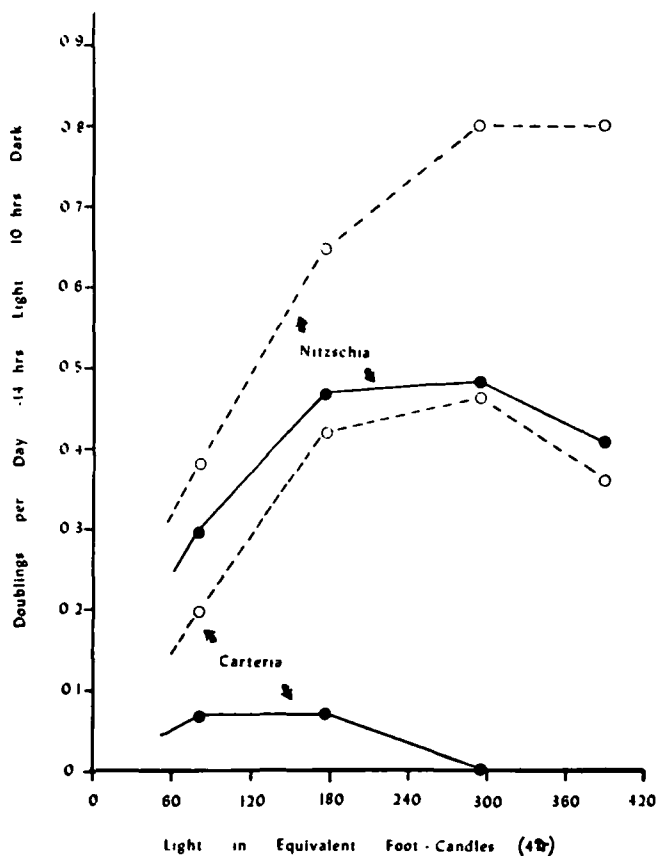


FIG 11. Effect of light intensity on the growth rate of *Nitzschia closterium* and *Carteria* sp. at two nutrient levels
At 140 mg N/liter and 15.5 mg P/liter (dashed line) and 125 ug N/liter and 12.5 ug P/liter (solid line) Temperature constant at 16C.

problems in ecology, therefore, we must attempt to get away from trying to study the effect of one factor at a time.

STRICKLAND: Do you have anything between a factor of one and 1000? As I understand it, the level in one experiment was as found in the sea and the others had 1000 times more?

JONES: That's right. No, we haven't. We wanted to start with a concentration of nutrients which we thought was at least applicable to the normal environment, and another which is frequently used in typical batch cultures to obtain good growth of cells. I might add that the population of cells in these cultures is maintained at about 38

cells/ml, so they are not dense cultures. We would like to work with slightly lower cell populations, which would be more applicable to that found in the open ocean, but the sensitivity of the machine makes it a little less desirable at present.

In summary, then, I would like only to mention that in comparing each other's growth data at various light intensities, we should also consider the level of nutrients used.

DROOP: The converse is equally true with regard to the study of nutrients. So much depends on physical conditions.

JONES: Yes.

HALLDAL: In a green alga the main pigments are chlorophyll and carotenoids. When the alga grows in the upper part of the sea, chlorophylls *a* and *b* will mainly determine the shape of the absorption curve, with high peaks at 435 and 675m μ . In this situation, the alga has plenty of light. It doesn't have any difficulty in keeping the photosynthetic apparatus running.

PROVASOLI: This is for green organisms, green algae?

HALLDAL: Yes, and also blue-green alga grown at high-light intensity will have the same two peaks.

VOLLENWEIDER: What is high intensity?

HALLDAL: In my experiments, it is 10,000 lux of white light. In the blue-green alga, phycobilins are accessory pigments; in the species I used, it was mainly phycocyanin with a maximum absorption at 625m μ (FIGURE 12). The species was *Anabaena* from Doctor Allen's laboratory, an *Anabaena* not classified as to species. If this alga is grown at high-light intensity, phycocyanin will be recorded with maximum at 625m μ . If the intensity is reduced, changes in pigment composition occur. This is shown up in the absorption spectrum of the alga by an increase in the relative amount of phycocyanin to chlorophyll. It will enable the alga to catch more light at wavelengths where chlorophyll absorbs poorly.

If the intensity is reduced still further, the absorption curve will reveal an increase at about 625m μ , indicating that more phycocyanin has been formed, but there is no trace of phycoerythrin at 6,000 lux. At a still lower intensity, 800 lux in FIGURE 12, a peak is originating around 570m μ . I am not quite certain about the wavelength here. The chlorophyll and phycocyanin maxima are correct, 570m μ probably is not. The last peak shows up in the spectral region where chlorophylls and phycocyanin absorb poorly. So phycoerythrin may be covering this gap.

PROVASOLI: Can you give us an idea of what the intensity is where the phycoerythrin is being produced?

HALLDAL: This showed up at between 200 and 800 lux. I had a light

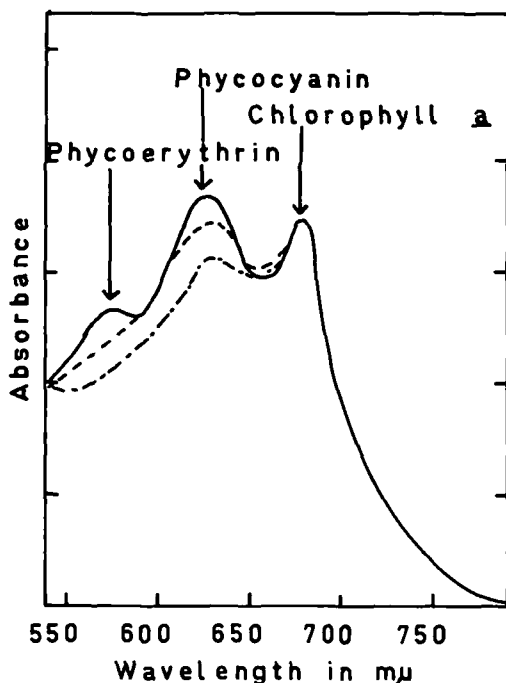


FIG 12. Absorption curves of *Anabaena* sp. grown at different light intensities. 800 lux. —; 6000 lux - - - -; 10 000 lux - · - · - ·.

gradient in my experiments from 10,000 to 200 lux. Samples were picked out, put in a spectrophotometer, and these curves came out on a sheet, showing the differences in pigment composition, with a moderate amount of phycocyanin at high intensities, increased amounts at lower, and, finally, the accumulation of the additional accessory pigment phycoerythrin at very low intensities.

The accumulation of phycocyanin and phycoerythrin can also be demonstrated by the use of colored light. By shining blue, green, and yellow light on blue-green algae, phycobilins will be formed and accumulated. The causes of these accumulations are not clear. Low-intensity white light is also very effective in producing the same pigments.

PROVASOLI: How long was the exposure to monochromatic light?

HALLDAL: I did not do the experiments with monochromatic light. Such analyses have been performed by Hattori and Fujita⁵⁵ among others. They obtained distinct differences after 40 hours at 1.5×10^5 erg/cm⁻² sec⁻¹.

STRICKLAND: Did you ever extend this work to an organism which didn't have the phycobilin pigments, to see if the chlorophyll *c* increased with low-light intensity?

HALLDAL: No, chlorophyll *c* won't be picked by means of spectrophotometry with these algae *in vivo*.

STRICKLAND: But have you looked to see if this low-light effect is found with any other organism?

HALLDAL: No, I have not.

PROVASOLI: Can you say something about red algae also?

HALLDAL: I did not do experiments on red algae, but others have shown⁵⁶ that the accessory pigments of red algae, which distinctly cover the spectral region where chlorophyll is a poor light absorber, are photosynthetically very effective. These observations lead us back to the problem of complementary chromatic adaptation, and light intensity adaptation. We know that weak white light causes increased amounts of accessory pigments, but we know that colored lights of equal intensity do so also, and more effectively when the pigments are excited.

HUTCHINSON: Tom Goreau told me earlier this week that in Jamaica, red algae give out a much higher point than *Halimeda* does, which is the benthic alga that goes down farthest and looks perfectly green. You have, therefore, got the opposite of the ordinary picture, and he felt that there is probably a good deal of complexity in this story.

PROVASOLI: Do I understand you correctly, that *Halimeda* was growing at greater depths?

HUTCHINSON: Much deeper than any of the red algae.

HALLDAL: There is a similar example to this in Norwegian waters, namely *Chaetomorpha*. It is beautifully green and it grows even farther down than the red algae in the Oslofjord. This algae is capable of photosynthesizing, thus keeping up with respiration at much lower intensities than most of the other greens.

FOGG: Isn't the point here that a very high concentration of chlorophyll will absorb a lot of light, even in the green where the relative absorption is least?

HALLDAL: Right.

BRAARUD: There are also some very deep green algae.

STEEMANN NIELSEN: In very clear water at greater depths, there will only be blue light. In this case, the red algae won't have much better light conditions than the green algae, which absorb the blue light very well.

HALLDAL: Yes, that is true. It is a question of the photosynthetic capacity of the algae. If they contain the accessory pigments, the amounts increase at low-light intensity. Those which do not contain

phycobilins may, in some cases, compensate for this lack by increasing the absolute amount of chlorophyll.

JONES: One can change the pigment ratio in red algae by other means than light intensity. *Porphyridium*, for instance, when grown under constant light conditions, but with limited nitrogen, will produce considerably less phycobilin pigment and appear green rather than red. The same effect is produced by limiting the amount of sulfate.

In the synthesis of chlorophyll and phycobilins there are common precursors. It would be interesting to know if light in any way influences the "switch" which regulates the amount, or rate of synthesis, of these two pigments. Is there any suggestive evidence that something like this may occur?

STEEMANN NIELSEN: There are several causes for light inhibition. It may be due to UV light, and we have already discussed this. Also strong light of all wavelengths may inhibit photosynthesis. Finally, there is the color composition of light. For instance. Algeus⁴³ couldn't grow green algae in fluorescent light, and he used Osram natural lamps. The algae couldn't grow because the proportion between red and blue light wasn't correct. We have had exactly the same experience growing *Chlorella* in our laboratory with light from Philips T L 20 W/33. But if we give some red light at the same time we have no difficulty. Thus, there are several reasons for inhibition, both of photosynthesis and of growth; and we don't know too much about this.

PROVASOLI: This brings up the topic of the simulation of natural light under laboratory conditions. Most of us employ cool white fluorescent lamps with or without an incandescent booster. Are we right?

STRICKLAND: I have spent the last 12 months wrestling with incubators in order to get some kinetic work done. In view of the fact that there is an infinitely variable spectral composition of sunlight in the sea, according to the water characteristics and the depth, and in view of what we have just heard, it is obviously of some importance to be cognizant of the spectral distribution.

One really has to start making a decision as to what sort of light one should use. I think there are three difficulties. One is to measure the light in a way which will be reproducible between one laboratory and another. Another is to decide on a proper spectral distribution. The third, which involves some technical difficulty, is to get an extremely high-light intensity of a spectral distribution which resembles sublight in the sea. There is no difficulty now in measuring the total energy of light by radiometers which are readily available. These are thermocouples, one set of junctions along an absorbing surface and

another in some sort of a heat-sink. A small gadget of this sort, with a reasonable sensitivity, can be made and put in bottles or in incubators.

PROVASOLI: Are these units available commercially, or does one have to build them oneself?

STRICKLAND: The essential unit can be purchased from the Eppley Co., Ltd. for about \$180. It has thermistor compensation, and one has to mount it oneself. The radiometer measures total energy. It is entirely blind to wavelengths, and, in order to get "photosynthetically active energy," whatever that is, a little bit of filtering has to be done. In a crude way, we have been putting a piece of Corning Infrared absorbing IR 69 glass over the unit.

The curve in FIGURE 13 is that of the IR 69 glass. When this transmission is combined with ordinary sunlight in a blue sky, the radiometer sees rather more light than it should beyond the photosynthetic range, and a little less in the photosynthetic range. The two together cancel out, roughly, so that when this glass is put on a radiometer, it records, in most daylight conditions, pretty nearly the total energy between about 4000 and 7000 Å. It is slightly crude, but not too bad, and we have constructed deck units and underwater units so that we can now get the transmission of "photosynthetic" light in sea water, which is useful. We can also use these units in any incubator we construct so as to get the light intensity in energy units.

It is quite a job to obtain a high-light intensity artificially—I mean something equivalent to bright summer sunlight that has passed through only a few meters of seawater. However, it is possible, using the new tungsten filament lamps which are heated in the presence of iodine vapour. They give something like 3,000 K light with a lamp life

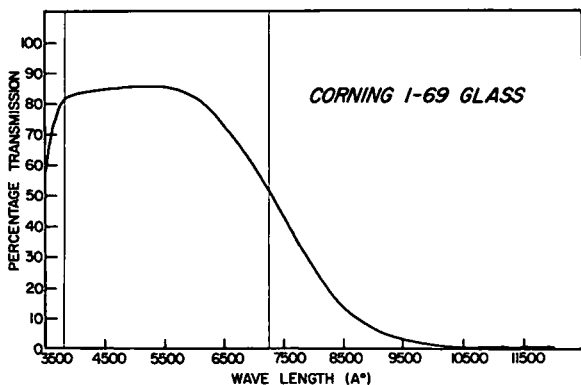


FIG 13 Transmission of 2mm of Corning I-69 Glass. (Permission, Limnology and Oceanography)

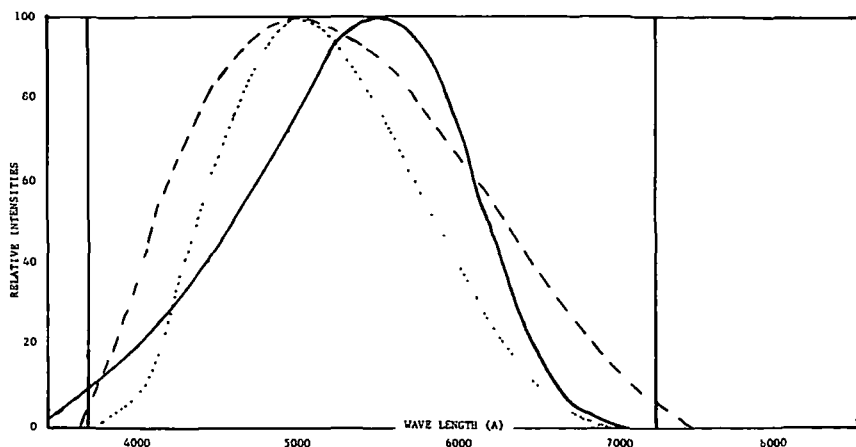


FIG 14. Spectral distribution of sunlight in sea water and of tungsten light through a chemical filter.

of 1,000 or 2,000 hours. By putting these lamps near the algae and providing adequate cooling, practically full sunlight conditions can be simulated.

Another problem is to get the light to look like light in the sea, which is, of course, technically insoluble because of the infinite variability. One has to decide on some light condition which resembles light in the sea and say: "I am going to standardize on this." We are going to standardize on the light shown in FIGURE 14, which is obtained by using 2cm. of a solution of 20g. ferrous ammonium sulfate, 4g. copper sulfate, 5ml. of syrupy phosphoric acid, and 100ml. of water. Much better light could probably be obtained if a combination of the right glasses were used, but by doing so a lot of energy is lost, which makes it almost impossible to get very high light intensities. There is also the expense, especially if large areas are to be covered. This is pretty prohibitive at \$30 or \$40 for six square inches of colored glass.

The dotted curve in FIGURE 14 is the spectral distribution of light in five of Jerlov's coastal (No. 2) seawater. This is simply a common type of seawater, the spectral characteristics of which have been obtained by Doctor Jerlov.⁵⁸ The big dashes are for two meters of water. The other curve is the spectral distribution of filtered 3000 K tungsten light.

Such a distribution is admittedly not that of sunlight in the sea. The worst thing is the displacement of the peak by about 500 Å from most peak transmission wavelengths, but it certainly is better, I think, than fluorescent light, and much better than unfiltered tungsten

light, which has a great packet of red light. It also has the virtue of having reasonably small amounts of UV and no red light. The distribution is as "typical" as one can hope for unless one goes to great trouble and expense. We are studying photosynthesis and cell division kinetics with this source of light, expressed in absolute energy units. The great difficulty of course with this kind of chemical filter is to get the peak at shorter wavelengths. If anything is added to cut out more long wavelength light, a quite drastic loss of energy begins to occur.

KRAUSS: Are the salts mixed in one solution, or in separate chambers?

STRICKLAND: In one solution. We put in phosphoric acid, which complexes the ferric iron, which is always present and gives undesirable short wavelength absorption.

KRAUSS: Doesn't it get quite hot? Is there a cooling coil in the filter?

STRICKLAND: This must be backed up initially with a lot of cold running water to take the massive infrared out and act as a coolant for the filter. It is cheap, dirty, convenient, and roughly standard.

PROVASOLI: Has anyone done experiments with different fluorescent lights? We have grown various flagellates in "cool white," "deluxe white," "daylight," (FIGURE 15) and "natural white" (FIGURE 16). We did not find any substantial difference. The organisms seem to adapt to and reach similar densities in all the lights tested. We could not detect by visual inspection any evident change in coloration, except that some dinoflagellates seemed darker when grown in some lights.

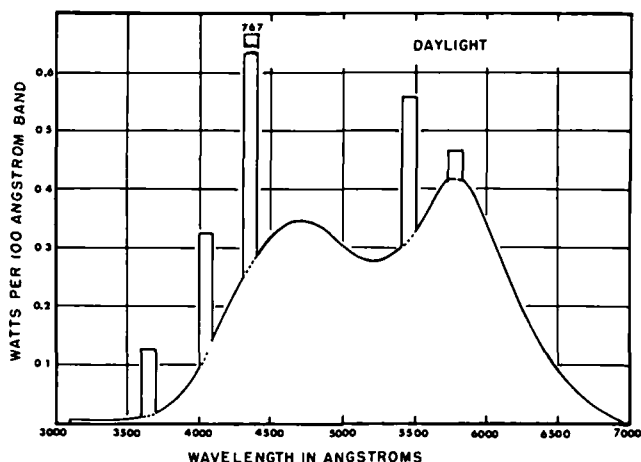


FIG 15 Spectral response of fluorescent daylight lamps

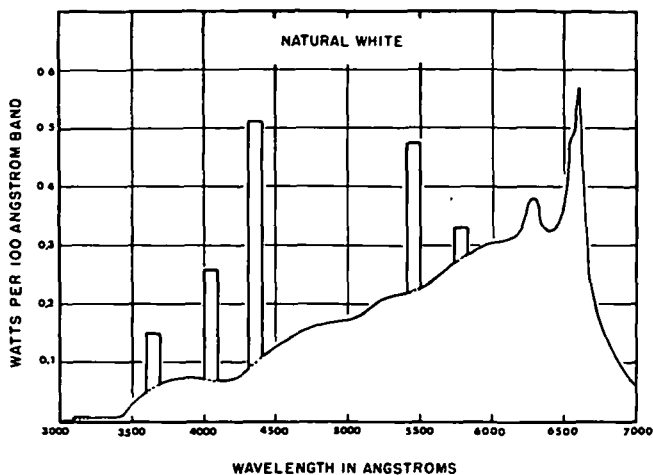


FIG 16 Spectral response of fluorescent natural white lamps.

We had, however, an interesting result with the newly-developed Gro-lux lamps (FIGURE 17): These Sylvania fluorescents are designed to give light that is almost exclusively within the spectral range required for chlorophyll absorption. It was surprising to me that *Antithamnion sarniense*, a red seaweed, grows as well under this light as in the other lights which are richer in the bands absorbed by

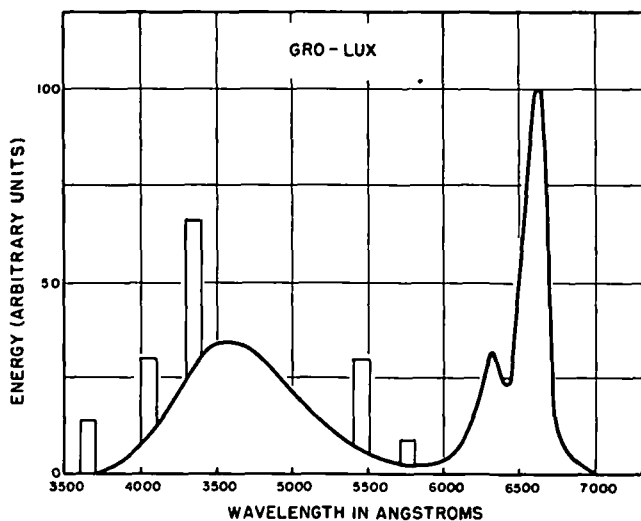


FIG 17. Spectral response of fluorescent Gro-lux lamps.

phycoerythrin, and that it maintains the typical red-bluish color of this alga in nature. Photosynthesis is apparently going on with light directly absorbed by the chlorophyll, and the accessory pigment is still synthesized although not used to absorb light energy. On the other hand, the filamentous "*Conchocelis*" phase of *Porphyra tenera* prefers a mixture of "daylight" and "natural" which gives more light in the 500-575 region than other fluorescent lights. In this light, the filamentous tuft-like colonies grow well and are pigmented dark brown (almost black); i.e. the color of the *Conchocelis* growing naturally inside the calcium layers of empty oyster shells. In Gro-lux light, the *Conchocelis* colonies grow poorly and are purple pigmented like *Antithamnion*. I ask if anybody else has done similar work because I wonder how "dirt-cheap" Doctor Strickland's system is. I know how expensive it is to cool lamps, and I could not employ his system in a freezer-incubator.

STRICKLAND: I think this is probably necessary only if quite precise kinetics are required. I don't think it matters a hoot for growing cultures reasonably well.

GUILLARD: Many organisms are light-saturated at a few thousand foot candles, which can be obtained with fluorescent light. Consequently, unless the purpose is to compare the experiments with natural conditions, are such high intensities necessary?

STRICKLAND: I think studies with supraoptimal light may turn out to be terribly unimportant, but I think it worth doing some work on it. It has turned out that we cannot get supraoptimal effects in many experiments, but I did not know this when I started to make the apparatus.

ALLEN: Doctor Provasoli, were your statements based on equal intensities of light, or equal absorbed energy, or equal what?

PROVASOLI: No, they were not based on equal light intensities. We selected a few species of dinoflagellates, chrysomonads, and cryptomonads which could grow between 24-26°C. Each species was inoculated simultaneously in several tubes of the same medium; the tubes were placed in racks screened with black paper around the sides and placed on a glass. Below the glass, at about 10cm., two 20-watt fluorescent lamps were placed side by side.

KOCZY: Didn't Jerlov⁵⁹ devise a filter combination for a filament light that simulated the light composition at, say 25m depth?

STRICKLAND: Yes, he did, actually, but it is terribly expensive to cover a large area.

ALLEN: If one is willing to take a little loss of intensity, there are several colors of blue Plexiglas which are rather cheap, so that one could construct a whole bath of these to approximate seawater.

STRICKLAND: I'll bet they are not stable to strong light, are they?

ALLEN: I don't know. [We are now testing this. I have arranged to have samples exposed to Hawaiian sunlight for several weeks, after which the change in transmission will be measured.]

STRICKLAND: Any organic dye is just hopeless. It bleaches almost immediately.

GOLDMAN: At the tenth Pacific Science Congress, Honolulu, 1961, Doctor H. R. Jitts* presented some light curves for Belgian glass that had, he felt, almost the same spectral absorption as sea water?

STRICKLAND: We bought some of that Belgian glass, but it is not really very satisfactory, in my opinion.

VOLLENWEIDER: I would like to say that, in my opinion, it is rather difficult to build an experimental device for simulation of a natural light field. It may be possible for certain kinds of sea water, but it becomes extremely difficult for coastal or fresh water. We have to consider the fact that the light field is changing along a vertical axis.

PROVASOLI: You mean from the angle of the sun?

VOLLENWEIDER: Yes, and not only from the angle of the sun. In lakes, and I can imagine it is the same in coastal waters, there are quite big fluctuations during a year.

STRICKLAND: I thoroughly appreciate this. I realize it is impossible to get such a thing as marine light. It is a question of whether one tries something a little better than raw daylight or tungsten lamps.

PROVASOLI: We can't neglect this new problem. We know that some algae are sensitive to photoperiodicity.⁶⁰ We cannot exclude the possibility that they might also be sensitive to periodic variations in light composition. We should try at least a few experiments even if it means setting up a terrifically complex apparatus to simulate the daily variations in light intensity and composition.

STRICKLAND: That is appalling.

PROVASOLI: Exactly, but that still doesn't have to block our thinking about it.

STRICKLAND: No, I think we should try to find out my stage first.

HUTCHINSON: Is there experimental evidence yet that there are algae that actually react differently to light of different or spectral composition, comparable to those that are found over a diurnal or annual cycle in a natural water?

HALLDAL: I don't think I have any; there is no experimental evidence, so far as I know, that these small variations that are found in the ocean would do anything to change the photosynthesis, and so forth.

*Jitts, H. R., Commonwealth Scientific and Industrial Research Organization, Division of Fisheries and Oceanography.

ALLEN: How large are these variations?

HUTCHINSON. If one went to sleep at midday and woke up at five o'clock, a white wall would still look like a white wall. We adjust for this very easily, so that one's biological system is arranged so that this doesn't happen.

STRICKLAND: I think, Doctor Provasoli, it can at least be said that there should not be a lot of IR, and perhaps not a lot of UV in the system I described.

VOLLENWEIDER: If we consider the fact that the composition of different plants is important, it is then important to understand what is going on in nature for succession of populations. We have such big changes between daylight, red light and fluorescent lamps.

HALLDAL: With the fluorescent light, the energy cut-off is at longer wavelengths in the spectral region where the red chlorophyll peak is situated. So a small difference from one fluorescent tube to another may cause a great difference in the fraction of the emitted energy that will be absorbed by chlorophyll, while in the ocean, the major variations are in the blue-green and green part of the spectrum where a conflict with chlorophyll absorption peaks does not take place to any significant degree. Changes in this spectral region (blue and green) do not interfere too much with photosynthesis, while differences in fluorescent tubes which mainly for the white types occur at 600 to 650 m μ , are most important in this respect.

BRAARUD: I wonder if it is a little too early to raise a question about the light factor from the point of view of field ecology. If the seasonal variation in the composition of the plankton is studied, we find that at certain seasons, especially during the dark season, there are certain species which predominate; for example, *Skeletonema*. Do such species react differently with respect to the light factor than species which occur mainly at other seasons? It may be too early to ask this question because the methods for studying the light effect are being discussed so much here, but there are several such questions which the ecologist may ask.

It would be very interesting to learn whether, for instance, diatoms and dinoflagellates behave in the same way with respect to changes in light intensity and light composition. This problem has been brought out very clearly in some Norwegian fjords, where the water is stratified with regard to salinity and light, because glacial waters are discharged into the fjords.* There is very turbid water on top which strongly affects the spectral composition of the submarine light. In these waters we find that dinoflagellates predominate during most of the summer season, while the diatoms are very scarce indeed. Of

*Unpublished observations.

course, phototaxis may be involved but the conspicuous distribution pattern raises the question of light response of the two groups.

STEEMANN NIELSEN: In Copenhagen during the past year we investigated how different species of plankton algae are able to adapt to different light intensities. We started with *Chlorella*, as a culture; this species adapts very easily either to a low-light intensity or to a high-light intensity. As the next species we chose *Skeletonema costatum*, as this diatom is easily adapted to different light intensities. However, we found that *Skeletonema* is very little able to adapt itself to low-light intensities. I would never have expected it. We have investigated other diatoms which easily become shade-adapted, but *Skeletonema* is always, I would say, adapted to high-light intensities.

BRAARUD: That is very surprising, because in the Hardangerfjord *Skeletonema* reaches its maximum population during November and January at 60° north.

PROVASOLI: At what temperature, Doctor Steemann Nielsen?

STEEMANN NIELSEN: We varied the temperatures from 20C down to 5C, but couldn't get any shade adaptation for *Skeletonema*. I can't explain it.

MCLAUGHLIN: Did you change the nitrate-phosphate ratio?

STEEMANN NIELSEN: We have also done that. Doctor E. Jørgensen from my laboratory has made some experiments limiting the culture by nitrate, as well as by phosphate. Only in the latter case was it possible to get something which may be shade adaptation.

BRAARUD: Were the experiments in the fjord with cultures?

STEEMANN NIELSEN: No, in Friday Harbor last summer, we took diatoms from the surface of the sea. There, as you know, the water is vertically mixed and we didn't find shade-adapted algae, but if the plankton were submerged for two days at 10 or 15m, very nice shade-adapted plankton resulted.

BRAARUD: Then it may be a question of difference of life cycle.

STEEMANN NIELSEN: Perhaps. We know that *Skeletonema* is very difficult. One can never repeat experiments absolutely.

PROVASOLI: Everybody corroborates you on that point.

HALLDAL: Talking about the spectral changes and so forth may, I think, lead us into something that Doctor Allen would like to add.

ALLEN. I would like to bring up for discussion another way in which different wavelengths of light can affect photosynthetic organisms. Whether we know enough about this at present to suggest any ecological consequences, I am not really prepared to say. One of the great advances in our knowledge of photosynthesis during the past few years has been the discovery that, for effective utilization of light energy, we require not one but two light reactions, and that these light reactions have different wavelength dependence. The general

picture which has emerged from a very large body of data is that we must activate not one but two pigments in the organism, so that the particular wavelength dependence of the two reactions will depend upon the pigment system of the organism.

There is also some small evidence available that these two reactions have different light saturation characteristics. It seems unlikely that, under natural conditions, light is always sufficiently monochromatic to activate only one pigment, but I have been wondering if the necessity for these two reactions might not have something to do with the high incidence of chlorophyll *c* in many samples of phytoplankton. Looked at from the point of view of a light absorber, chlorophyll *c* appears to be a very poor choice. A tiny peak in the red is rather completely overlapped by the chlorophyll *a* peak; as Doctor Halldal said, *c* can't be seen in the living cell. In the blue absorption region, it is competing for light with the other chlorophylls and, usually, with a large body of carotenoids and organisms containing chlorophyll *c*. It just might be that because *c* is an efficient catalyst of the second light reaction, it will aid in the utilization of light energy by these organisms without having any marked effect on the absorption spectrum.

So far, this is merely a speculation. In another year or so we hope to have some data and maybe know more.

JOHNSTON: Can you elucidate the products of this second light reaction?

ALLEN: Maybe I should explain a little more about how these things have been observed. The late Robert Emerson⁶¹ observed that at the red end of the spectrum the efficiency of photosynthesis fell off faster than the absorption spectrum of chlorophyll. This was with the green alga *Chlorella*. A few years later, Haxo and Blinks,⁵⁶ working with red and blue-green algae, found more dramatic lack of activity in the red. Light absorbed by chlorophyll itself appeared to be used scarcely at all, but light absorbed by the biliprotein was effective. This was very puzzling, because there was also evidence that light energy was transferred from these pigments to chlorophyll.

It was found, however, that if one added auxiliary wavelengths of light, absorbed by biliprotein, at the same time that the effect of the red light was being measured, then the light absorbed by chlorophyll became effective. Most experiments carried out in this field have measured what has come to be called the enhancing effect of one wavelength of light upon another.

Such experiments cannot give information on products. However, a few years ago Doctors Myers and Stacy French⁶² did an experiment in which they found that the two wavelengths of light could be applied, not simultaneously, but one after the other within a few seconds and

still show the enhancing effect. This suggested formation of some product that would last for several seconds.

This experiment led me to look into the subject by the electron spin-resonance technique. It is not certain that the signals we observe with this method correspond to the products responsible for the long-lived enhancement effect, but one does obtain from illuminated photosynthetic organisms two different types of spin-resonance signals that have different decay times, different light saturation intensities, and, most important, different wavelengths of activation. We would like to feel that in this way one is able to look at products of the two different light reactions. This is a field which is actively under investigation at present.

MYERS: Could I add a remark which I think you all might enjoy. Consider the fact that we are talking about a photosynthetic mechanism which evolved to an efficient use of the very broad band of the visible spectrum. Now consider the temerity of your colleagues in physiology who proceeded to study photosynthesis with the belief that any and every wavelength within the visible region would be just as effective as the whole region. So many of the most critical and careful measurements were made in monochromatic light. Looking back, it is of interest that far-reaching errors were introduced by this rationale.

GOLDMAN: I would like to add a cautionary note concerning field activity in this particular area. In working under the ice, as in the laboratory, we are concerned with the importance of previous light history. When working with photosynthetic organisms from this twilight zone, beneath an ice and snow cover, they are very apt to have been injured if they are exposed to surface light intensity and then replaced below the ice again, and they are incapable of carrying on photosynthetic rates comparable to a control series that had been shielded during the exposure at the surface.

DROOP: Is that a de-adaptation to light?

GOLDMAN: It may simply reflect a destruction of sensitive pigments mobilized to utilize the extremely low-light intensity. I think we have enough information to show that in natural waters, the lower limit for photosynthesis is below one per cent and when weak light-adapted organisms are exposed to high surface-light intensity, they are in some way injured by the intense ultraviolet, infrared, or a combination of these. I should add, however, that the light harmed are able to recover from the injury. In the Antarctic we have exposed organisms which live in very-high-light intensity under natural conditions to the injuring effect of direct sunlight, and then have placed them in the water at a lower light intensity to measure their recovery

rate. It takes about four hours for them to return to 98 per cent of the photosynthetic rate of controls. So, this is real injury. It isn't just inhibition. I think this distinction should be made.

This is one of the limitations of these field experiments, since we were using a cosmopolitan population of microplankton and diatoms and were measuring the total effect on the phytoplankton community.

BRAARUD: Were these greens, blue-greens?

GOLDMAN: There are a lot of blue-greens, by the way, in the Antarctic. The Family Oscillatoriaceae, *Oscillatoria* and *Phormidium*, are particularly well represented.

STEEMANN NIELSEN: May I ask if the algae were in a bottle or without any cover when they were put in the full sunlight?

GOLDMAN: They were in a Pyrex glass bottle and, of course, Pyrex has a rather high ultraviolet absorption.

STEEMANN NIELSEN: If surface plankton from the temperate zone is exposed without a glass cover, rather strong inhibition will be obtained due to UV light.

GOLDMAN: It would be very nice if we could measure the UV light, with more precision in nature to determine how important a factor it is where the phytoplankton is actually occurring.

JONES: I would like to ask Doctor Allen or Doctor Myers a question with regard to the enhancement effect in photosynthesis. Although there is an enhancement of O_2 evolution with two wavelengths of light, is there any evidence of enhancement of CO_2 fixation?

ALLEN: I don't know of anyone having done this experiment.

MYERS: A very clean answer. The experiment has not been done. There are some suggestions, none of them published, that there are some anomalies with respect to carbon dioxide. The measurements, however, are made over sufficiently long-time periods that if CO_2 is not being reduced one has to ask the question: What is being reduced? I think your question could be restated: Is there some anomaly here peculiar to oxygen evolution? All I can say is that people in the field are proceeding at least in the belief that it is not an anomaly peculiar to oxygen. Is that really an answer to your question?

JONES: This is the sort of thing I was after.

KRAUSS: Are you quite sure there aren't three light reactions?

ALLEN: In a recent paper, Witt⁶³ claims just that. On the basis of absorption spectral changes, he is proposing three light reactions.

RILEY: I would like to second and reemphasize what Doctor Braarud said about the importance of dinoflagellate phototaxis in heavily stratified waters. I think all of us recognize the fact that it exists, but probably not to the degree to which it frequently does exist.

I will recount an incident which is perhaps trivial in itself but certainly illustrates this problem. We have a Japanese research associate

in our laboratory, Prof. Satoshi Nishizawa, who is studying the transparency of seawater with an *in situ* meter which makes an automatic record of transparency versus depth as it is lowered in the water. In stratified water it shows the most tremendous layering and vertical variations in transparency, and my part in this, as a biologist, has been to sample these different levels which appear to be significant and to analyze the biological conditions for light extinction in terms of, well, sometimes detritus, sometimes phytoplankton, or whatever it may be that causes it.

On one occasion, early last summer, we found a tremendous peak in turbidity which was narrowly limited within a depth of half a meter and about three meters below the surface. On sampling this, we found a swarm of *Peridinium trochoideum*, which was two orders of magnitude larger than the number anywhere above or below. This illustrates, first, the fact that phototaxis can limit these organisms very narrowly under optimal conditions and, second, the fact that our ordinary sampling procedures at a series of specified depths generally miss them completely.

I think if we really want to know what our phytoplankton population looks like under these circumstances, we have to change our sampling methods considerably.

MCLAUGHLIN: While in Puerto Rico, looking for the luminescent *Pyrodinium bahamense*, our approach was to observe the bay waters at night. We put a net down and dragged at different levels. Wherever the net luminesced, we found dense concentrations of the dinoflagellate. We worked round the clock, every hour, and found that early in the morning the *Pyrodinium* concentrated at three fathoms. As the sun rose, they moved up and would stay at one to one-and-a-half fathoms from about 1100 until 1300 hours. As the sun set, they would move to three to seven fathoms.

At night, at about 2100 hours, when dusk had just set in, they seemed to rise again to three fathoms. There was an ichthyologist with us and, sitting there, as a fish went by, he would say, "That's a so-and-so." He could identify it by the light produced when it passed through this very dense layer of *Pyrodinium*.

While fishing, as soon as the bait got down to the *Pyrodinium* layer, the bait would become visible. The predatory bone fish would streak from the bank, and the whole action was visible as long as the bait and catch remained in the luminescent layer.

In *Peridinium trochoideum* cultures, grown in a five gallon cylindrical culture vessel illuminated on the side wall, we noted during various parts of the day heavy concentrations of cells at the surface of the liquid. Some time later, usually at noon, for unknown reasons these cells will seem to start moving down the side of the vessel much like a

swarm of fish. They will reach a certain depth and then turn around like a column of soldiers and go back up towards the surface. They may do this for two or three hours, after which most will go to the bottom. Sometime during the night or early morning, 80 per cent of the population will be found once again at the surface.

PROVASOLI: Is this in constant light?

MCLAUGHLIN: No, we turn off the light at night so it is alternately light and dark. In constant light, the up and down vascillation goes on constantly. I thought for a while that convection currents were present, but dye marker technique eliminated convection as an explanation for the movement.

With some blooms in Long Island Sound a similar phenomenon is found. In fact, we found a thermocline in the Sound which presented a two centigrade difference between a cooler bottom layer and the warmer top layer. The organisms were just below the warmer layer—stratified almost at the junction.

BRAARUD: We find a rather striking stratification in dinoflagellates, for instance, *Ceratium*, in small fjords where there is brackish water on top. They may accumulate in layers one or two meters thick, and one may find populations of 16,000/liter, which is quite dense. One may ask: How is phototaxis affected in stratified waters like these, where there is a very pronounced salinity gradient? Observations in the Dramsfjord⁶⁴ made me think that the *Ceratium* and other dinoflagellates migrated upwards and were caught in the outflowing fresh and brackish water, but when I looked at two other fjords there was no indication of such a trapping, so my first interpretation may have been wrong. It would be very interesting to see some experimental work on this question.

HALLDAL: I think we have such experimental data.⁶⁵ I am thinking of experiments of ion effect on phototactic movement, and also on movement in relation to light intensity, and so on. We have so many, many different factors acting on phototaxis that I think it is absolutely impossible to do an analysis in the sea. It is clear that there is variation in so many things.

BRAARUD: It would be very interesting to know if the salinity factor is involved.

MCLAUGHLIN: *In vitro*, in five gallon jugs, or even in longer columns, I doubt if there is a salinity gradient. The organisms still seem to seek a preferential location in the vessel. What effect pressure has on this migration we don't know, as very little is known about the effects of pressure on these organisms. It seems possible that some diatoms, which are dragged up from the depths, are heterotrophic at great depths and phototrophic up near the surface. There may be a change in the membrane permeability which permits them, if they have the

enzyme system, to utilize the detritus and other organic material below the euphotic zone. I know of no experiments on algae in terms of interpreting their movement, nutrition, or their other physiological parameters in terms of pressure. I believe such work might give us some fascinating data.

GUILLARD: Doctor McLaughlin's remarks on the gathering of *Peridinium trochoideum* in swarms in large cultures brings to mind some interesting theoretical work done by Mr. Chaës Rooth* at the Woods Hole Oceanographic Institution on the subject of "bioconvection"—the establishment of small-scale convection patterns in water by organisms. He was led to study this by observing the beautiful convection patterns in still cultures of the flagellate *Olisthodiscus*. Mr. Rooth finds that to have convection patterns established it is necessary first that the organisms concentrate in some layer, say the surface, because of phototaxis or some other response. The existence and size of the "bioconvection" pattern established then depends upon the relationship between values of the swimming speed of the organisms and of their density excess over that of the water. I have never seen *P. trochoideum* establish convection patterns in small still cultures. It isn't clear, of course, to what extent the treatment developed by Mr. Rooth applies to events in nature.

OPPENHEIMER: The stratification of organisms within sedimentary environs is much more striking than that in water. In siliceous or clay environments in shallow water, where tides cover the sediments with one or two inches of water for parts of the day and allowing exposure during other parts of the day, one will find at the surface of the sediment a discrete layer of diatoms which are brown in color; directly underneath will be a layer of blue-green algae and flagellates. Underneath that, when sulfate-reducing bacteria are present at a depth of one centimeter, photosynthetic bacteria will be present. Each of these three types of photosynthetic organisms assimilates different quanta and different spectra of light. For example, the photosynthetic bacteria use the infrared, and the diatoms are apparently protecting the flagellates.

The same environment examined at night will show the flagellates mixed up with the diatoms, resulting in a more uniform condition as compared to daytime. It is a rather fascinating place to study light-energy relationships. The diatoms are exposed to direct sunlight and, in Texas sediments in summer where I have measured surface sand temperatures as high as 50C, the energy relationships must be rather important.

*Material as yet unpublished. Mr. Rooth's present address is: Chaës G. H. Rooth, International Meteorological Institute, Lindhagensgatan 124, Stockholm, Sweden.

PROVASOLI: Is there any possibility that a difference in redox potential causes stratification?

OPPENHEIMER: Yes, redox potential may be influential, especially for the photosynthetic bacteria which are active in anaerobic conditions. This brings up another rather interesting situation in that we find *Navicula* quite often in the anaerobic sediments underlying aerobic sediments. In summertime, for example, respiration rate is so great that bacteria will consume oxygen faster than it can be replenished, and an anaerobic zone is produced only one-half centimeter below the mud water or mud air interface. If the sediment is carefully dissected, large numbers of diatoms are found which apparently are growing in the reduced sediment heterotrophically, or are using energy that they have stored from the photosynthetic zone and have moved down into the anaerobic environment for, perhaps, some specific growth factor.⁶⁶

PROVASOLI: Doctor E. T. Moul also found diatoms moving up and down in the first few centimeters of the mud.^{67,68*}

OPPENHEIMER: We measured the total number of photosynthetic organisms during night and day, and we did get an indication of a diurnal migration within the sediment layers.

KRAUSS: How did you measure the migration of these organisms?

OPPENHEIMER: By direct fluorescent microscope counts. Core samples were dissected very carefully and examined under 4000 Å light. It is rather difficult to separate layers in a soupy sediment, so we relied on the trick of freezing. A core was gently removed in a plastic tube, frozen in dry ice, dissected, and examined immediately, within a matter of 10 minutes after it was put in the dry ice. In this way, we dissected discrete parts of the sedimentary column to see how the algal distribution was affected. The autofluorescence of chlorophyll was used as an indication of abundance. The diatoms were treated and classified.

Sediments provide an entirely different environment than that of the open sea, and it would be very interesting for those people working on light-energy phenomena to compare the two environments.

KOCZY: That brings up a problem. How does a diatom, or whatever the microorganism is, migrate in a small gradient? It must be able to sense this gradient; otherwise, how can it know that there is one? Light response I can understand, because light-sensing is directional, but response to changes in the chemical environment does not seem to be directional. F. Gross and I conducted an experiment nearly 25 years ago.† We put copepods in a salt gradient ranging from 35 per cent to

*Also, personal communication with Doctor E. T. Moul.

†Unpublished observations.

0 per cent. The copepods were taken from the Skagerak and put in the gradient at the normal salinity of 28 per mille to 30 per mille. Then we put a light on top of it and 80 per cent of the copepods were positive phototactic and moved toward the light source and into fresh water. There they turned crazy. Their speed was increased by a factor of three to four, but the motion was at random in all directions. They were not seeking the higher salinity. If they reached the deeper and more saline water, they slowed down, but immediately started to move toward the light again. Now the same dance started again; they jumped around until by chance they reached higher salinity again. So there is the question of how a dinoflagellate can go up in the gradient.

STRICKLAND: I have been told by Doctor H. W. Harvey of the Marine Biological Association at Plymouth, England, that *Dunaliella*, in culture, will cluster around a knitting needle because it likes the iron.*

KOCZY: The population is being measured, but it does not mean they are moving in the gradient. Only that they found a better condition and move around less at random. Optimum condition could then mean less motion and, therefore, larger population. The only possibility for motion in gradient would be that the organisms know in what direction they have moved, remember the conditions at the former place, and then make an evaluation; that is, a comparison of the two situations, whereupon they decide to stay, move back, or move farther away. That means they must have a memory and a judgment of values.

ALLEN: The response to oxygen gradients is well documented. The reaction of photosynthetic purple bacteria to oxygen gradients was shown by Clayton⁶⁹ in a series of papers on chemotaxis and phototaxis, based on microscopic observation. These were short term experiments, so it can not be a matter of selective reproduction.

I was wondering during your earlier discussion, Doctor McLaughlin, if the response of the dinoflagellate going down just so far and no farther is an oxygen gradient response.

MCLAUGHLIN: I don't think so. Down in the bottom there is another population. Only part of the population goes up to the top and starts this gyration, but a good part of the bottom will be covered with very rapidly moving, pulsating cells, whipping their flagellae around—beautiful. In a tube I always find a certain percentage of the cells up at the top. Whatever the reason is, they are up there, but a good number of them will be down in the bottom and, if the temperature is dropped, this tends to force most of them down to the bottom of the test tube.

*Personal communications.

I have a strong suspicion that we should be doing something with pressure. How does pressure effect movement? What is the pressure of water on top of a mud flat? It is 1 atm. pressure when the tide is out, and then suddenly when the tide returns the organisms have six to eight inches, or perhaps several feet, of water on top of them. Does this affect their destruction? I don't know, but I think this is an important ecological factor that we must work on.

I obtained greater motility of some dinoflagellates by putting cultures in a pressure cooker and applying pressure by taking off the blow-off valve and attaching a pump. If it is opened up very quickly, the cells will be observed moving around rapidly at first, and then gradually slowing down to what is considered normal speed at room pressure.

KOCZY: My question is: How can the organisms move in the gradient?

DROOP: Movement in a chemical gradient is a statistical phenomenon surely; strictly speaking, a kinesis.

KOCZY: There I agree. If the cells can reach a region where they are happy, they stay, and consequently there is a higher concentration. But that is different from moving in a gradient and knowing it is done purposely. The apparent result is, in any case, a movement in the gradient. One is caused by changes in speed of the random movement, and the other is deterministic.

JONES: These are mixed populations, in a sense, that are not synchronized? They are at different developmental stages. Is there any evidence that, say in Doctor Strickland's case, the more mature cells sink, and the young, developing cells stay up, or vice versa? If they are at different developmental stages, they are probably going through different forms of metabolism.

I don't want to get back to oil droplets and things like that, but, because of their different biochemistry, they may be naturally sorting themselves out into a density gradient.

MCLAUGHLIN: It is true, that the physiological state affects the movement, but the large old fellows, just before they are ready to divide, at some time during a 24-hour period, will be up at the top, and their movement will be slowed down. They don't respond as quickly to whatever factors they are reacting to, whether it be a difference in gas pressure, external or internal, photosynthetic rates, phototaxis, etc. There is apparently a difference in the function as determined by the size of the cells that are moving. If there is a gas pressure factor or some other pressure factor, then the volume, the amount of gas in that volume, and, in general, the size and physiological state, do much to determine movement.

FREMONT-SMITH: Are you doubting memory in single cell organisms, Doctor Koczy?

KOCZY: No, I am not, but I would be happy if someone would show it to me.

MCLAUGHLIN: We can show something of the sort in diurnal migration, and how cells can be taken out of natural phase. The work of Sweeney and Hastings^{70,71} on the bioluminescence of *G. polyedra*, and our own work⁷² on *Pyrodinium bahamense*, have demonstrated a type of internal clock or "memory" system which operates in these dinoflagellates.*

*The work by McLaughlin and Zahl was supported by N.I.H. Grant No. R.G. 7022.

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DROOP: The particular concern in these discussions is organism *and* environment; that is, the organism in the context of its surroundings and neighbors. If nutrition is in question, we are interested in it because nutrition is one aspect of the interaction of the organism with its environment. However, to obtain data of sufficient precision one must usually extract the organism from its environment and subject it to a set of entirely artificial conditions. Because of this, the more precise one makes one's experiments the more difficult interpretation becomes. In general, one achieves precision at the expense of reality, or, in other words, the price of remaining "in touch" with nature is the inclusion of all the imponderables and the lack of experimental control which goes with them. This question of interpretation of laboratory results will probably come up for discussion later, but I mention it here because I expect that much of our discussion on nutrition will concern itself with the behavior of algae in cultures.

The study of algal nutrition is concerned with the conversion of materials from the environment into algal protoplasm. It can be studied from various points of view—at various levels, if you like: *a)* nutrient requirements, *b)* mechanism of uptake and growth kinetics, *c)* internal biochemistry, and *d)* ecological effects of assimilation, excretion, the availability of the required nutrients, and the interaction of organisms with each other. From an ecological point of view, one is particularly interested in the effects of nutrient cycling—depletion and regeneration by the algae and their cohabitants and competitors—on the occurrence and succession of phytoplankton. Nutrient requirements will be our first concern, and we will want to dwell on the ecological aspects, but I hope we will be able to discuss also some of the more fundamental questions (such as growth kinetics in relation to nutrient concentration) which are becoming more and more necessary to an understanding of ecological situations.

Perhaps it is underlining the obvious to say that it is the nutritional behavior of the ecologically important species that most concerns us.

In this connection, Doctor Provasoli has pointed out that this does not necessarily mean that we should confine ourselves to the most dominant organisms.⁷³ The zooplanktologists have shown how useful "indicator species" are in drawing attention to environmental variations too subtle for chemical analysis.⁷⁴ Again, much nutritional work is done also with species not of marine importance—usually littoral or supralittoral species—because they are generally more easily handled. They are not to be despised; they are most necessary, for we would get nowhere without these "stepping stones." A direct frontal attack on pelagic species has seldom if ever been rewarding.

Nor are all essential nutrients equally interesting from an ecological standpoint. Ecologically interesting nutrients are those whose concentration is at times liable to drop, by one process or another, below the physiological level of requirement, so that they become limiting. Furthermore, one must remember that this level of requirement is not an absolute thing, but may vary according to such factors as the physiological state of the organism, the nutritional climate, and external physical conditions (temperature, for instance, as was mentioned earlier). I expect we will want to discuss vitamin B₁₂ and phosphorus and silicon in this context. One has to distinguish between nutrients of general requirement, such as the major inorganic nutrients—though here again the response may differ from species to species—and those, like vitamins, which are needed only by a proportion of the species. The first may be expected to determine the "envelope" of the plankton bloom; the last, the details.

In the past, it has been assumed that algae were entirely autotrophic. This misconception arose because most of the earliest algae studied had been obtained by selection on purely mineral media; and, in the marine field, it was strengthened by the fact that the one amenable diatom (which was to become the "*Chlorella*" of the marine biologist), *Phaeodactylum tricornutum*, is indeed autotrophic. The result was that emphasis was placed on the major inorganic nutrient elements, carbon, nitrogen, phosphorus and silicon. And although the economics of these elements could often account for the distribution of phytoplankton in a general sort of way, it was naturally seldom successful in explaining the details of the successions of individual species. Then, first the introduction of soil extracts and the like^{75,76} widened the range of algae that could be cultivated in a bacteria-free state, and later its replacement by "metal buffering" agents and pH buffers⁷⁷ provided fairly satisfactorily defined media for many of these algae. This has led to the recognition, first, that a great many algae (including planktonic forms) are not entirely autotrophic but need to be supplied with vitamins—notably vitamin B₁₂ and thiamine, and, to a lesser extent, biotin; second, that the physicochemical condition of

the milieu largely determines the availability of trace metals, such as iron; and, third, that organic matter has an importance quite unconnected with any direct nutritional value. Apropos of the vitamins, Doctor Provasoli⁷⁸ has said: "It is a reasonable assumption that if an organism requires a growth factor *in vitro*, then that metabolite or its physiological equivalent should be found in the environment." This has been the case, and as we shall see, work has been, and is being, done on this aspect. Vitamin requirements of many algae have been mapped (though pelagic species are still poorly represented in our culture collections), and some of the more diligent of us have been bioassaying thiamine and vitamin B₁₂ and obtaining interesting, sometimes controversial, results. One could reply to Doctor Provasoli, apropos of organic growth promoters, whose virtue is due to their physicochemical properties, that their physiological equivalents are likely to be ill-defined and complex and, in some cases, no doubt, only of consequence in artificial cultures.⁷⁹ All this is grist to C. E. Lucas' mill,⁸⁰ though we are concerned here with nutrition, which forms only one facet of "nonpredatory interrelationships" between planktonic organisms. The ecological problems posed by this class of compound—the physicochemically active—are of a different order of difficulty, just because of the lack of specificity and the liability to interference from causes inherent in our methodology; though, as you will agree, the vitamins are also causing us a bit of trouble. Cultures pose as many problems as they solve. But this brings me back to my opening remarks.

I thought we would start by discussing the vitamin requirements of algae and the controversial question of B₁₂. To outline the sort of things we do for those who aren't familiar with the technology: Organisms are brought into the laboratory, isolated into bacteria-free culture and grown in very complex media in test tubes containing natural concoctions. Then, in order to find out what nutrients they require from that medium, one tries to replace the complex materials with progressively simpler ones until, finally (one hopes), one has a medium prepared entirely of materials from the chemical shelf. When an organism can be cultivated indefinitely in such a medium, it is possible to say one knows all its absolute requirements. TABLE 1 is the sum of our knowledge up to two years ago of the vitamin requirements among algae at that time and is still, I believe, later than any published. It includes freshwater species.

So, the question to be asked, first of all, is: What gaps have been filled in since then and what remain? Another question is: What is the meaning of these tables of vitamin requirements? It will be seen that some 70 per cent of all algae grown *in vitro* had requirements for one or more vitamins—B₁₂ being the most commonly required. Auxo-

trophs, that is vitamin-requiring, and nonauxotrophs are in roughly equal numbers in the Chlorophyta and Bacillariophyta. The Cyanophyta are, by and large, nonauxotrophic, while both the Chrysophyta and Pyrrophyta (Dinophyceae and Cryptophyceae) are almost entirely auxotrophic. There are gaps. Most of the diatoms are littoral; I think there is only one pelagic diatom in TABLE 1. One is interested in vitamins and, therefore, there is a tendency to isolate organisms with vitamin requirements, so the number of vitamin requirers shown in tables like this may be an over-estimate and not reflect the true situation—that is one factor.

PROVASOLI: I don't agree with that remark. Usually the isolation media contain vitamins; therefore, we are not preselecting. Besides, I think that you, like us, determine the vitamin requirements of whatever organisms can be cultured. Isn't that so?

DROOP: I, myself, do; but, in general, how many of the negative findings, for instance, get into the literature?

PROVASOLI: Yes, but the literature is limited mostly to persons like yourself, our group at the Haskins Laboratories, Doctor Guillard, and the Lewins. I think that the Lewins, too, tested all their isolates for vitamin requirements, so I don't feel that the printed results are very much biased since the species not requiring vitamins are reported as well as the requiring ones. What I feel we are missing is information on the vitamin requirements of 300 to 400 species of fresh-water green algae which are deposited in the culture collections. These algae are supposed to be autotrophic, but they have never been tested critically for vitamin requirements. Doctor Krauss and his colleague, Miss Ikuko Shihira, have recently done a survey on all the available *Chlorella* strains and it turns out that several *Chlorella* need vitamins!

ALLEN: I assume I am the person being quoted for 24 autotrophic blue-greens in TABLE 1. This, I am not quite convinced, is an improvement of the medium, by chelation, because having developed better media since then, those algae have been kept in culture for quite a few years without any vitamins added.

DROOP: Good! So TABLE 1 is right after all. That was the main point, to invite correction and show the gaps. I presume the same is true of those species of yours in TABLE 3 (page 94), Doctor Provasoli.

PROVASOLI: Yes. There is no calculation of numbers, but the data are the same, only brought up to date since the last publication came out 15 days ago.

DROOP: Would you summarize, Doctor Guillard, some of your work; have you any more data on pelagic diatoms or Dinophyceae? I happen to know that TABLE 1 has only one pelagic diatom in it. All the other diatoms are from the Lewin's⁸³ and are littoral or pennate forms.

TABLE 1
VITAMIN REQUIREMENTS OF ALGAE AS REPORTED IN THE LITERATURE*

Division	Total	Auxotrophs	Non- auxotrophs	Strains requiring—			
				only vitamin B ₁₂	only thiamine	vitamin B ₁₂ and thiamine	thiamine and biotin
							vitamin B ₁₂ , thiamine, and biotin
Chlorophyta	47	22	24	10	7	5	0
Euglenophyta	10	10	0	0	1	9	0
Cryptophyta	11	11	0	2	1	8	0
Pyrrophyta	17	17	0	12	0	0	1
Chrysophyta	13	12	1	2	1	6	1
Bacillariophyta	54	21	33	11	6	4	0
Phaeophyta	1	0	1	0	0	0	0
Rhodophyta	1	1	0	1	0	0	0
Cyanophyta	25	1	24†	1	0	0	0
Totals	179	95	84	39	16	32	2
							6

*Represented from Droop (1963).^{4,1}†From Allen (1952).^{4,2}

GUILLARD: All of the pelagic diatoms I have studied require B_{12} and only B_{12} . The only species I have found that do not need B_{12} are three clones of *Melosira nummuloides*, and *Detonula confervacea*; these are diatoms that live in an environment where there is plenty of B_{12} .

STRICKLAND: Doctor Guillard, have you studied *Thalassiosira nordenskiöldii*?

GUILLARD: Yes, that species and *Rhizosolenia setigera* gave me a great deal of trouble because they didn't respond in a manner I considered proper. Ordinarily, a diatom that needs B_{12} will die outright in the absence of B_{12} and will grow nicely in its presence; the growth will be more or less proportional to the amount of B_{12} added. These two species didn't respond in either way. As I recall, in the last experiment, the *Thalassiosira* without B_{12} grew well and then died sooner than the control culture with B_{12} , while *Rhizosolenia* without B_{12} didn't grow for a long time then finally produced some growth. I don't yet know what this means. In the case of the *Rhizosolenia*, the experiments might have been upset by an auxospore stage. I hate to blame all unexpected results on auxospore formation.

DROOP: I think you have to. I agree with you entirely. Doctor

MCLAUGHLIN: Yes, we have a *Peridinium trochoideum* that required B_{12} and also a *Gyrodinium* sp.⁸⁴ and *Glenodinium* sp.⁸⁵ We also found that *Glenodinium halli* has an absolute requirement for B_{12} and will not grow without it, but as a function of growing it in B_{12} and adding thiamin 264 hours after inoculation, the thiamin gives an increase in the growth; but it is not an absolute requirement.⁸⁶⁻⁸⁸ Furthermore, if the culture is allowed to go to senescence and thus encyst, and, subsequently, thiamin and biotin are added to the culture, once again excystment occurs with increased motility of most cells.

PROVASOLI: Doctor McLaughlin, I would like to know if *Symbiodinium microadriaticum* really does not require vitamins.

MCLAUGHLIN: No, *Symbiodinium microadriaticum*, at least the way it is grown under our cultural conditions, which is principally a vegetative stage, does not. For those who don't know, this symbiotic dinoflagellate is found in most marine invertebrates, and we have isolated it in culture from something like 12 different marine invertebrates, everything from the large *Cassiopeia*, *Anemones*, and soft corals. It grows vegetatively in an artificial marine medium⁸⁹⁻⁹¹ and has a gymnodinioid form which is motile.⁹² The gymnodinioid form was identified by Schmada in 1848⁹³ as *Gymnodinium adriaticum*, and we believe it is the *Gymnodinium* form of the vegetative cell found symbiotically in the algae. The only way we were able to discern this was to isolate the algae from the invertebrate tissue and grow it *in vitro* and confirm that it does have a form which Schmada

reported as being a normal free-living *Gymnodinium*. So far, under our culture conditions, we haven't been able to get it to show any absolute B₁₂, thiamin, and/or biotin requirements.

STRICKLAND: Doctor Droop, could we have an authoritative definition of absolute requirement and growth enhancement, and some comments on these?

DROOP: A substance which is required absolutely cannot be manufactured by the organism at a rate sufficient to support life. The test is that the organism cannot be maintained in a medium indefinitely without that substance.

PROVASOLI: Or its physiological equivalent.

FREMONT-SMITH: And how long is indefinitely? You have to put an arbitrary limit on it, don't you?

DROOP: I would be satisfied with 10 transfers. It depends very much on the inoculum size, on the dilution ratio at each transfer, as to how quickly they can deplete. These requirements aren't of a very big order.

PROVASOLI: And are only evident after depletion.

DROOP: Some vitamins are easier to deplete than others. Thiamine is less easy to deplete than vitamin B₁₂. I don't know why. It often appears to be stimulatory and one can't establish an absolute requirement for it. But my experience has taught me that here, "stimulatory" can usually be replaced by "absolute requirement" after much more work.

FREMONT-SMITH: Could one say in biology that absolute is always relative?

DROOP: Yes. One has the same problem with defining bacteria-free cultures. There is no such thing. Somebody, sooner or later, is going to show that there is some cryptic-organism in it, one that doesn't show up in ordinary exhaustive sterility testing. Everyone who plays around with bacteria-free cultures has to admit that fact to himself.

I think we might pass on to B₁₂ specificity. TABLE 2 shows specificity. There are a number of naturally-occurring variants of vitamin B₁₂, not all of which can be used by all species with a B₁₂ requirement. The vitamin molecule proper contains a benzimidazole base in its nucleotide. All B₁₂-requiring organisms respond to this form. The variants containing a nucleotide with an adenine-like base (Factors A, G, H and pseudovitamin B₁₂) can only be used by a certain number of species, and even the variant lacking a nucleotide altogether (Factor B) can be used by some. In general, it has been established that most algae have a specificity pattern like that of mammals—they need the vitamin proper, the one with the benzimidazole base; the other variants, Factors A, B, pseudo-B₁₂ etc., are no good to them. That

TABLE 2
SPECIFICITY OF B₁₂-REQUIRING ALGAE TOWARD
VITAMIN B₁₂-VARIANTS

Division	Specificity pattern similar to that of—			Total
	mammals ^b	<i>Lactobacillus leichmannii</i> ^c	<i>Escherichia coli</i> ^d	
Chlorophyta	9	0	0	9
Euglenophyta	1	3	0	4
Cryptophyta	2	0	1	3
Pyrrophyta	4	3 ^e	0	7
Chrysophyta	7	1	0	8
Bacillariophyta	0	0	2	2
Cyanophyta	0	0	1	1
Totals	23	7	4	34

Notes (a) Data from Droop (1959),⁹⁴ (b) responding only when the vitamin contains a benzimidazole base in the nucleotide (e.g. vitamin B₁₂, factor I); (c), the nucleotide contains the base adenine or near analog (e.g. pseudo-vitamin B₁₂, factors A, G, and H), (d), responding also to the planar group lacking nucleotide (i.e. factor B), (e) these three dinoflagellates respond to factor A but not to pseudovitamin B₁₂ (i.e. when the nucleotide contains 6, hydroxy-, not 6, amino-, purine)

applied to all Chlorophyceae so far tested, and to most of the Chrysophyceae, but not to the one diatom, *Skeletonema*, or to the one cyanophyte represented in TABLE 2, both of which can utilize all forms of the vitamin tested.

The tentative conclusion is that vitamin B₁₂ proper is the form that is likely to be of greatest ecological interest to phytoplanktonologists, though, of course, it may be that differences in ecological behavior between diatoms and dinoflagellates, for instance, result to some extent from their differing responses to vitamin B₁₂. But the figures are too small, and I would like to know whether anyone has any more data on specificity of the diatoms that we have here.^{81,94}

GUILLARD: Just a little additional information. Some students from the Marine Ecology course given at the Marine Biological Laboratory, Woods Hole, this past summer, made a rough bioassay comparing cyanocobalamin and pseudo-B₁₂. As I recall, *Skeletonema costatum* responded by growing to some extent, as you had previously reported,⁹⁴ but *Cyclotella nana*, a small estuarine diatom in which I am interested, didn't grow at all when supplied with pseudo-B₁₂.

PROVASOLI. What about Factor B? I think that is more important.

GUILLARD: That is without a nucleotide?

PROVASOLI. Yes.

GUILLARD: I don't know the final results.

PROVASOLI: We now have data on the specificity of more species, including Mrs. Lewin's diatoms.

DROOP: What is the general conclusion?

PROVASOLI: Five of Mrs. Lewin's diatoms utilize all the analogs, including Factor B. They are *Amphora coffaeiformis* strains 47-M, 45-M, and 56-M; *Nitzschia frustulum*; *Nitzschia punctata*. *Nitzschia ovalis* uses all except Factor B. *Nitzschia closterium* and *Achnanthes brevipes* have a narrow specificity and do not utilize Factors A, B and pseudovitamin B₁₂.

Perhaps we should summarize the vitamin data briefly before considering more specialized topics. A broad trend emerges if we lump together the data on marine and fresh-water algae under the main taxonomic groups. As Doctor Droop said, some algal groups—the green algae, the diatoms and the blue-greens—differ from the other taxonomic groups in having a large proportion of species which do not require vitamins. On the contrary, almost all the species so far studied of the dinoflagellates and chrysomonads require one or more vitamins. Table 3 is a summary of vitamin requirements by various organisms as to species (4, 12, 13, 15, 17, 18, 20, 21, 23, 27).

If we analyze the order of incidence we find that vitamin B₁₂ is needed by the largest number of species (115), followed closely by thiamine (91 species).

If we now return to the specificity of cobalamin, we find that the majority of the species requiring vitamin B₁₂ can utilize only the cobalamins containing a benzimidazole nucleotide like vitamin B₁₂. Other B₁₂ requirers, especially the diatoms, are quite versatile and can utilize most of the B₁₂ analogs, even factor B, which lacks the nucleotide moiety. Since the B₁₂ analogs are present in seawater and marine muds and, depending on localities, may be preponderant over true vitamin B₁₂, it is conceivable that the less specific requirers, like the diatoms, may have an advantage over the requirers of narrow specificity, which can utilize only true B₁₂ and the benzimidazole-containing analogs.

So many phytoplankton organisms need vitamin B₁₂ and thiamine that we cannot afford to neglect these vitamins as ecological variables. The yearly cycle of B₁₂ in seawater has been followed so far only in Long Island Sound⁹⁵ and in the Sargasso Sea.⁹⁶ In both cases, B₁₂ varies seasonally; it is higher in fall and winter and it decreases to a minimum during the spring diatom bloom; in summer, the quantity of B₁₂ is lowest. Other data in scattered localities indicate: (a) that a similar winter-spring-summer trend exists in the North Sea;⁹⁷ (b) that coastal water, especially shallow bays, are far richer in B₁₂ than oceanic waters.⁹⁸ Vitamin B₁₂ seems, then, to fluctuate with a pattern similar

TABLE 3
VITAMIN REQUIREMENTS OF SPECIES OF THE CHLOROPHYCEAE, DINOPHYCEAE,
CRYPTOPHYCEAE, BACILLARIOPHYCEAE, EUGLININAE, AND CYANOPHYCEAE

KEY R = required, O = not required, S = stimulatory

CHLOROPHYCEAE	Species	B ₁₂	Thiamine	# of Species
<i>Chlamydomonas agloaeformis</i> , <i>C. moewusii</i> , <i>C. reinhardi</i> , <i>Chlorella vulgaris</i> , <i>Chlorogonium elongatum</i> , <i>C. euchlorum</i> , <i>Dunaliella salina</i> , <i>D. primolecia</i> , <i>D. euchlora</i> , <i>D. viridis</i> , <i>Haematococcus pluviialis</i> , <i>Lobomonas pyriformis</i> , <i>Nannochloris atomus</i> , <i>N. oculata</i> , <i>Plinia</i> sp., <i>Platymonas</i> sp., <i>Polytoma obtusum</i> , <i>P. uella</i> , <i>Prasiola stipitata</i> , <i>Selenastrum minutum</i> , <i>Stephanoptera gracilis</i> , <i>Scenedesmus obliquus</i> , <i>Stichococcus cylindricus</i> , <i>S.</i> sp.		O	O	24
<i>Chlamydomonas chlamydogama</i> , <i>C. aff. zeinhardi</i> , <i>C. aff. snowii</i> , <i>Chlorosphaera consociata</i> , <i>Gonium pectorale</i> , <i>Lobomonas rostrata</i> , <i>Stichococcus</i> sp., <i>Volvox globator</i> , <i>V. tertius</i> , <i>Platymonas tetrathele</i> , <i>Chlamydomonas pulsatilla</i> , <i>Balticola buetschlii</i> , <i>B. droebakensis</i>		R	O	13
<i>Astrephoneme gubernaculifera</i> , <i>Chlamydomonas zeinhardi</i> (mut. thi-1 and 3, 4), <i>C. moewusii</i> (mut. 336), <i>Coelastrum morus</i> , <i>Gloeocystis gigas</i> , <i>Polytoma caudatum</i> , <i>P. ocellatum</i> , <i>Polytomella coeca</i> , <i>Prototheca zopfii</i> , <i>Selenastrum minutum</i> , <i>Volbulina steini</i>		O	R	13
<i>Astrephoneme gubernaculifera</i> , <i>Brachiomonas submarina</i> , <i>Chlamydobolus</i> sp., <i>Gonium multicoccum</i> , <i>G. octonarium</i> , <i>G. pectorale</i> , <i>G. quadratum</i> , <i>G. sacculiferum</i> , <i>G. sociale</i> , <i>Eudorina char-kouwenis</i> , <i>E. elegans</i> , <i>Pandorina morum</i> , <i>Pascheriella tetras</i> , <i>Pleodorina illinoensis</i> , <i>Pyramimonas inconstans</i> , <i>Stephanoptera pluviialis</i> , <i>Volvox aureus</i> , <i>V. globator</i> , <i>V. tertius</i> , <i>Volbulina steini</i>		R	R	20

DINOPHYCEAE	Species	B ₁₂	Thiamine	Biotin	# of Species
	<i>Exuviaella cassubica</i> , <i>Glenodinium foliaceum</i> , <i>Gonyaulax polyhedra</i> , <i>Gymnodinium splendens</i> , <i>Gyrodinium californicum</i> , <i>G. resplendens</i> , <i>G. uncatenum</i> , <i>Peridinium balticum</i> , <i>P. chattoni</i> , <i>P. trochoideum</i> , <i>Prorocentrum micans</i> , <i>Woloszynskia limnetica</i>	R	O	O	12
	<i>Symbiodinium microadriaticum</i>	O	O	O	1
	<i>Amphidinium klebsii</i> , <i>A. rhynchocephalum</i> , <i>Gymnodinium breve</i> , <i>Oxyrrhis marina</i>	R	R	R	4
	<i>Gyrodinium cohnii</i>	O	S-R	R	1
	<i>Glenodinium halli</i>	R	S	S	1
CRYPTOPHYCEAE	Species				
	<i>Cryptomonas ovata</i> , <i>Cyanophora paradoxa</i>	R	O		2
	<i>Chilomonas paramecium</i>	O	R		2
	<i>Hemiselmis virescens</i> , <i>Rhodomonas</i> sp. (5 species), <i>Chroomonas</i> sp.	R	R		7
	<i>Rhodomonas lens</i>	S	R		
BACILLARIOPHYCEAE	Species	B ₁₂	Thiamine		# of Species
	<i>Amphora coffaeiformis</i> , <i>Asterionella formosa</i> , <i>Detonula confervacea</i> , <i>Fragilaria capucina</i> , <i>Melosira nummuloides</i> , <i>Navicula pelliculosa</i> , <i>N. menisculus</i> , <i>Navicula</i> sp., <i>N. incerta</i> , <i>Nitzschia putrida</i> , <i>N. angularis</i> var. <i>affinis</i> , <i>N. curvilineata</i> , <i>N. filiformis</i> , <i>N. frustulum</i> , <i>N. hybridaeformis</i> , <i>N. laevis</i> , <i>N. marginata</i> , <i>N. obtusa</i> var. <i>scalpelliformis</i> , <i>Nitzschia</i> sp. (ovalis), <i>Phaeodactylum tricornutum</i> , <i>Stauroneis amphorooides</i> , <i>Tabellaria flocculosa</i>	O	O		23

TABLE 3
VITAMIN REQUIREMENTS (continued)

CRYSTOPHYCEAE	Species	B ₁₂	Thiamine	Biotin	# of Species
<i>Achnanthes brevipus</i> , <i>Amphora perpusilla</i> , <i>A. coffaeiformis</i> , <i>A. lineolata</i> , <i>Chaetoceros</i> sp., <i>Cyclotella</i> sp. <i>C. nana</i> , <i>Fragilaria brevisirata</i> , <i>Lichmophora hyalina</i> , <i>Nitzschia frustulum</i> , <i>N. punctata</i> , <i>N. ovalis</i> , <i>Skeletonema costatum</i> , <i>Skeletonema</i> sp., <i>Stephanopyxis turris</i> , <i>Synedra affinis</i> , <i>Thalassiosira fluviatilis</i>		R	O		16
<i>Amphipleura rutilans</i> , <i>Amphora coffaeiformis</i> , <i>Nitzschia closterium</i> ,		R	R		3
<i>Amphiprora paludosa</i> , var. <i>duplex</i> , <i>Amphora coffaeiformis</i> , <i>Nitzschia closterium</i>		O	R		3
<i>Stichochrysis immobilis</i>		O	O	O	1
<i>Syracosphaera carterae</i>		R	O	O	1
<i>Apistomena aestuarii</i> — <i>Pontosphaera roscoffensis</i> , <i>Coccolithus huxleyi</i> , <i>C. sp.</i> , <i>Dicranteria inornata</i> , <i>Hymenomonas</i> sp., <i>Pleurochrysis scherffellii</i> , <i>Syracosphaera</i> sp		O	R	O	7
<i>Isochrysis galbana</i> , <i>Microglona arenicola</i> , <i>Monochrysis lutheri</i> , <i>Ochromonas minuta</i> , <i>Pauloua gyraus</i> , <i>Prymnesium parvum</i> , <i>Synochromonas korschikoffi</i> , <i>Synura sphagnicola</i> , <i>Syracosphaera elongata</i>		R	R	O	9
<i>Ochromonas danica</i>		O	R	R	1
<i>Synura caroliniana</i> , <i>S. petersenii</i>		R	O	R	2
<i>Ochromonas malhamensis</i> , <i>Potriochromonas stipitata</i>		R	R	R	2

EUGLENINAE		Species							
<i>Euglena pisciformis</i>				O		R		O	1
<i>Astasia longa</i> (= <i>A. klebsii</i> , <i>A. chattoni</i>), <i>Euglena gracilis</i> , <i>E. mutabilis</i> , <i>E. stellata</i> , <i>E. viridis</i> , <i>Peranema trichophorum</i> , <i>Phacus pyrum</i>				R		R		O	7
<i>Trachelomonas abrupta</i> , <i>T. pertyi</i>				R		S		O	2
<i>Eutreptia</i> sp				R		R		R	1
CYANOPHYCEAE		Species		B ₁₂		Thiamine		# of Species	
<i>Agmenellum quadruplicatum</i> , <i>Anacystis marina</i> , <i>Lyngbya aestuarii</i> , <i>L. lagerheimii</i> , <i>Microcoleus chthonoplastes</i> , <i>M. tenerimus</i> , <i>Plectonema terebrans</i> , <i>Oscillatoria subtilissima</i> , <i>O. amphibia</i>				O		O			9
<i>Agmenellum quadruplicatum</i> , <i>Coccochloris elabens</i> , <i>Lyngbya lagerheimii</i> , <i>Oscillatoria amphibia</i> , <i>O. subtilissima</i> , <i>Phormidium persicinum</i> , <i>Synechocystis</i> sp.				+		O			7
PHAEOPHYCEAE		Species							
<i>Waerniella lucifuga</i> , <i>Sphacelaria</i> sp., <i>Ectocarpus confervoides</i>				O		O			3
RHODOPHYCEAE		Species		B ₁₂		Others			
<i>Antithamion</i> sp., <i>A. glanduliferum</i> , <i>A. sarniense</i> , <i>Bangia fuscopurpurea</i> , <i>Goniotrichum elegans</i> , <i>Nemalion multifidum</i> ,				+		O			6
<i>Porphyridium cruentum</i>				O		O			1

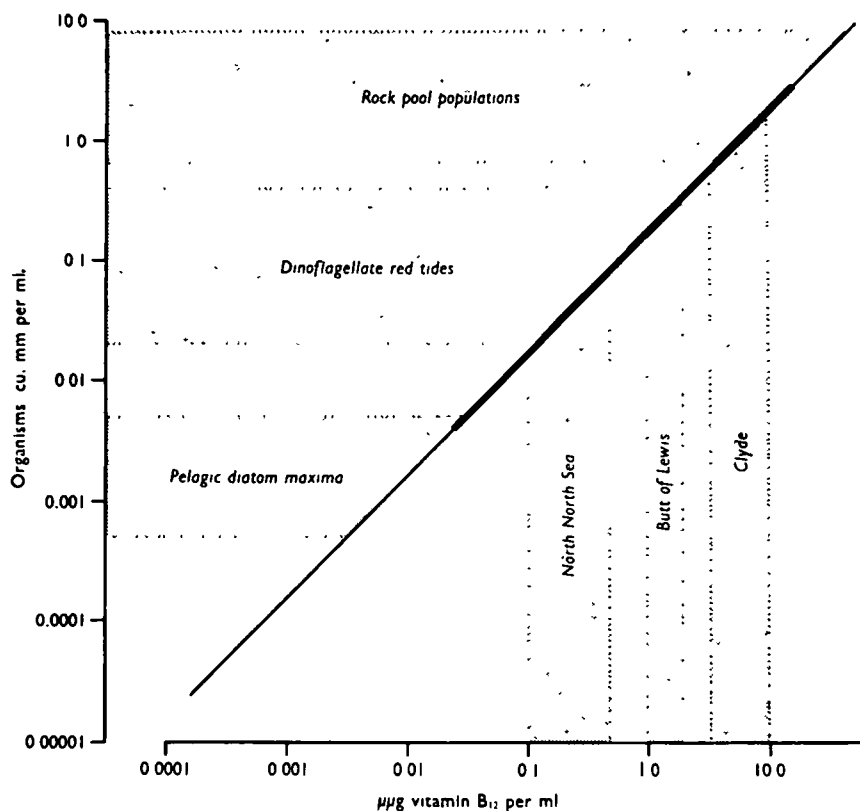


FIG 18. Dosage/yield ratios for *Monochrysis* and vitamin B₁₂ on logarithmic co-ordinates. Heavy line, experimental; thin line, extrapolation. Yield calculated as volume of living cells per ml. On the same scales are indicated for comparison: (1) levels reached by some natural populations and (2) some measurements of vitamin B₁₂ in the sea. (Data and references in Ref. 99. Reprinted with permission from Ref. 100, Fig. 1.)

to P and N and other important nutrient variables. So little is known about the whole problem that the early assertion of Doctor Droop,^{77 79 101} that vitamin B₁₂ in seawater is unlikely to be a limiting factor, is controversial. I would like Dr. Droop to express his views.

DROOP: The heavy part of the line in FIGURE 18 represents a number of experiments on *Monochrysis lutheri* (a chrysophyte flagellate from the supralittoral); the lighter extensions are extrapolation. The line represents the regression of yield on vitamin B₁₂ concentration. The vertical shaded areas are various measurements of vitamin B₁₂ in the sea, as reported in the literature up to 1957. The lowest is 0.1 µg/ml. The horizontal shaded areas represent typical blooms of organisms. The lower one is the *Skeletonema* spring maxima in the

Clyde. The upper margin represents a cell density of about 25 cells/mm³. The next area above represents red tide organisms, and the upper one, again, *Monochrysis* in supralittoral pools.

The scales are both logarithmic. The lowest B₁₂ measurement is some 50 times higher than would be required to produce (on a yield basis) the highest population of *Skeletonema* which we have had in the Clyde or any other area. Working from that, and having regard for the sizes of diatom populations generally, the calculated amount of vitamin B₁₂ required to produce them, and the amounts of vitamin actually being measured, I concluded that it was unlikely that vitamin B₁₂ would be of ecological importance as there would always be enough for those that wanted it. The supposed level of B₁₂ requirement was based on my results^{99,100} with *Monochrysis*, Hutner's⁷⁷ with *Euglena*, and Lewin's¹⁰¹ with *Stichococcus*, and was of the order of 3 molecules/ μ^3 of living alga.

We need to look at other areas of the sea where we may get rather lower figures for vitamin B₁₂. My thesis is that limiting concentrations are likely to be of an order lower than those at present being measured.

HUTCHINSON: What do you mean by "not of ecological importance"? Is it in the same sense that light or oxygen are not of ecological importance?

PROVASOLI: Not limiting, in other words.

HUTCHINSON: But ecologically important to the species that need vitamins?

PROVASOLI: Yes, correct.

DROOP: One serious criticism of my calculations and inference is that they are based entirely on yield measurements, and on very few species. On the other hand, in the face of natural competition, the growth rate of an organism obviously has an overriding influence on its success or failure. In fact, circumstances may be such that the rate measurement would be the more appropriate of the two. It is possible that the B₁₂ concentration needed for maximum growth rate is well above the minimum requirement. This would, or might, put some of the sea measurements into the critical range. J. E. Ford, at Shinfield, demonstrated just this with the fresh water chrysomonad *Ochromonas malhamensis*.¹⁰²

FIGURE 19 shows *Monochrysis* again, and Ford's *Ochromonas* curve. Ford found that the important concentrations, when a rate measurement was made, were considerably higher, probably in the region of 100 $\mu\text{g/ml}$.

The upper curve is Ford's result with *Ochromonas*, and the lower two, mine with *Monochrysis*, though under very different conditions: *Ochromonas*, in the dark on glucose; *Monochrysis*, in the light on a mineral medium. The measurement in each case is divisions per day—

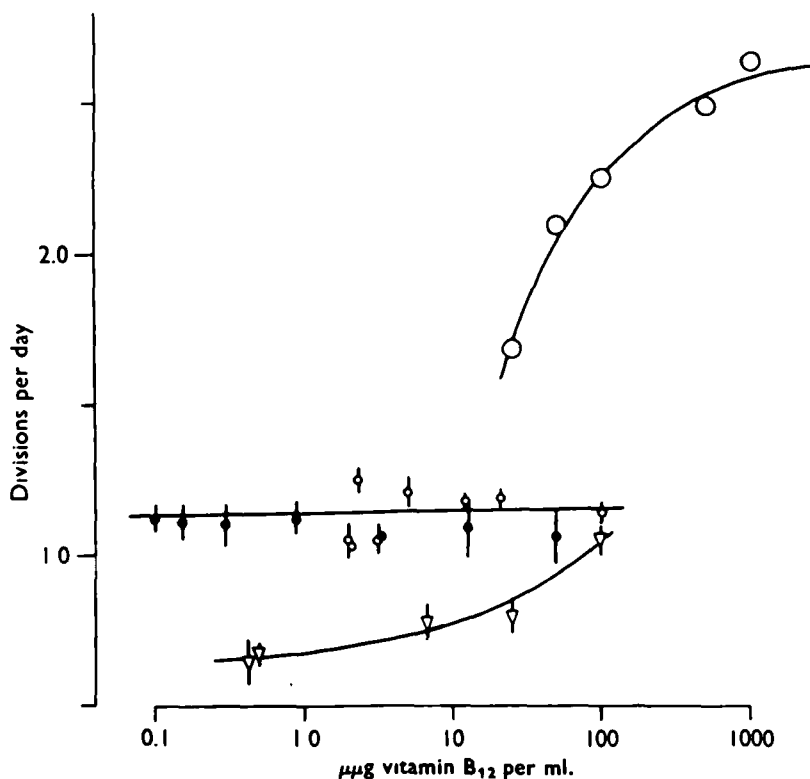


FIG 19. Influence of vitamin B₁₂ concentration (dosage corrected for carry-over) on the initial rate of division in *Monochrysis* (Three experiments: filled circles, 15C, small open circles, 20C; triangles, 25C. 95% fiducial limits indicated). Large open circles, the same, in *Ochromonas*, for comparison (Ref. 102). (Reprinted with permission from Ref. 100, Fig. 4)

that is, a growth rate measurement against vitamin B₁₂ concentration. Growth rate of *Ochromonas*, as I said, proved sensitive to vitamin concentrations up to 100 μg/ml, well within or above the ecological range. But in the experiment with *Monochrysis*, I was unable to demonstrate any effect on initial growth rate by concentrations down to 0.1 μg/ml. The details of these experiments may be important; they have been published.^{100,102} However, on the face of it, it would appear that *Monochrysis* is still insensitive to the range of concentrations measured in the sea, even on a rate basis. Neither *Ochromonas* nor *Monochrysis* is a pelagic organism. There are two temperatures represented here, 15C and 20C. We can ignore the one for 25C.

PROVASOLI: However, it is very interesting that you find a differ-

ence when the temperature is higher. That is the zone where one would expect the effect of increased light to be seen.

DROOP: Doctor Riley, would you care to say something about your results on *Skeletonema*?

RILEY: The work I would like to report on is thesis work by Mrs. Wood of our laboratory, a student who has been examining the growth of *Skeletonema*, both in batch culture and under chemostatic conditions. In the first place, in the ordinary kind of batch culture work, *Skeletonema*, unlike the *Monochrysis* that Doctor Droop has been talking about, is affected in its growth rate by the natural sea water concentration of B_{12} . The growth rate is reduced at a concentration of less than about $8 \mu\text{g/ml}$, which is well within the ecological range, and in most of our coastal waters we could then expect *Skeletonema* growth rate to be somewhat reduced by B_{12} deficiency.

As to the absolute requirements, there are also some differences. If I may summarize some of the work that has been done, it can be said that if we estimate B_{12} requirements in terms of molecules of B_{12} required to produce a cubic micron of cell tissue, some of the results reported in the literature for various organisms range from three to five molecules/ μ^3 . *Monochrysis* is one of the lowest of these, with a requirement of about three molecules/ μ^3 ; *Skeletonema*, determined separately by two different investigators, is of the order of 13 molecules/ μ^3 so that *Skeletonema*, fundamentally, has a higher requirement under these conditions than *Monochrysis*.

The really significant point that I want to make, though, is that there is a vast difference between the results one obtains when trying to grow *Skeletonema* under chemostatic conditions and those one gets in batch culture. Instead of a requirement of about 13 molecules of B_{12} , under chemostatic conditions the requirements are an order of magnitude higher, of the order of 100 to 150 molecules.

I must say that these chemostatic experiments leave a little to be desired. Mrs. Wood experimented with a new technique which caused a lot of difficulty from time to time. I won't go into the technical difficulties, except to say that they did lead to a certain amount of variability in the final results. However, this did not obscure the fact that there is a vast difference involved here, which simply means that in order to maintain an active state of growth in the chemostat, as contrasted with batch culture growth where the cells increase to a maximum possible population at which time the growth rate levels off, the B_{12} -rate requirement under chemostatic conditions is very much greater.

DROOP: My rate measurements were done on both small and large populations. In the early stages of a culture from a small inoculum, the very small number of cells uses up an insignificant amount of

vitamin so that the concentration does not vary throughout the experiment. It was only at a later growth state that one got over to the shoulder of the growth curve. The conditions were not chemostatic, but they were constant as to nutrient concentration during the course of the experiment because of the very small number of cells involved.

When you, Doctor Riley, were comparing rates, you said my experiments on *Monochrysis* were not chemostatic experiments. Yield limitation by virtue of vitamin depletion doesn't occur in my experiments because I was just measuring the rates at the beginning of growth, in very light cultures.

KRAUSS: How do the growth rates in a chemostat compare to those in the test culture? Are they similar or different?

RILEY: They are quite similar. We are talking about an order of one to two generations per day.

STRICKLAND: By how much did the rate slow down when the concentration decreased below $8\text{ }\mu\text{g/ml}$ —really drastically?

RILEY: I cannot answer that specifically because it depends on the state at which rate is measured. At the beginning of an experiment there is a certain initial rate which rapidly begins to level off as the B_{12} concentration decreases and, depending on whether the growth is measured after the first day, first two days, or first three days, the results will be somewhat different. But, calculating growth rates for the first two days, there is a reduction of about 50 per cent when the B_{12} concentration is reduced from 8 to $1\text{ }\mu\text{g/ml}$.

STRICKLAND: And that doubling time at $8\text{ }\mu\text{g/ml}$ would be about 12 hours, would it?

RILEY: Something in that order.

STRICKLAND: Going up to 24 hours?

RILEY: Yes.

HUTCHINSON: This whole figure of three molecules per cubic μ represents the concentration in the maximum population that you get in a batch culture?

DROOP: Yes. Calculated from the average volume of the organism, the yields in cell numbers, and the concentration which produced them—calculated, actually, from the regression of log yield on log vitamin concentration.

HUTCHINSON: Was this also confirmed by Mrs. Wood?

RILEY: She did not confirm this for *Monochrysis*.

HUTCHINSON: But she got the same sort of result for *Skeletonema*?

RILEY: Yes.

PROVASOLI: But with a higher value?

DROOP: About three to four times as high. It may be even higher still, Doctor Riley, because I notice that Mrs. Wood's results, in

which she quotes 13 molecules, were based on a nonlinear regression. She took the top value. If she had taken the lower one, it would be three times as high.

HUTCHINSON: A material occurring in one hundredth of the dilution of B_{12} could not act as a significant biological substance, presumably.

RILEY: One additional point: Many of us feel that diatoms, in order to persist in the sea, must be in a fairly active state of growth approximating, at least in a rough sort of way, chemostatic conditions. If, indeed, the *Skeletonema* requirement is of a magnitude such as Mrs. Wood observed in her chemostatic experiments, the B_{12} could be a significant factor in the sea in determining whether *Skeletonema* can grow in a particular water mass or not.

According to her results, there would be sufficient B_{12} in the rich coastal waters to support a flowering of *Skeletonema*. But in the outer coastal waters where *Skeletonema* does not thrive, and where the concentrations of B_{12} are much lower, it could, indeed, be one of the factors that is involved in limiting the population in these areas.

KRAUSS: With regard to the number of organisms in each of the cultures, Doctor Riley and Doctor Droop, were the same number of organisms dividing? In other words, we may be dealing here with yield versus growth rate, and they are two different situations. How comparable were they?

DROOP: From the measurements that we made on the growth rates, the cultures contained 2^{13} - 2^{16} cells/ml. That is about 8,000 to eight times that much. When there are very low concentrations of B_{12} , the curves begin to flatten off in culture before there has been very much time to get a growth rate measurement, because at least two points are needed. My figure is between 2^{13} and 2^{16} cells/ml.

RILEY: I can't give you those figures immediately. I think they were more or less comparable with what Doctor Droop obtained.

DROOP: They are extremely low figures by culture standards. Normal cultures of *Monochrysis* go up to 2^{20} , 2^{22} cells/ml or more.

FOGG: Do we know yet the function of B_{12} in metabolism?

DROOP: One established function is the resynthesis of labile methyl groups of methionine from "one-carbon" precursors,¹⁰³ but it is certain that it is also involved elsewhere. All one can say is that the vitamin is involved at a very fundamental level.

LUND: Did I understand Doctor Riley to say that *Skeletonema* in nature would not be able to produce a crop of a size comparable to what is seen in nature unless it is growing at the same rate as in the chemostat? If so, what was this rate?

RILEY: I assure you I did not intend to say that. We get flowerings in nature in which the generation time is very much less than it is

under these schematic conditions. What I did mean to say was that the yield of cells from a given amount of B_{12} , under chemostatic conditions, would indicate that a large flowering of *Skeletonema* could not be produced in nature except in the coastal waters where the concentrations are fairly high—say of the order of one or more $\mu\text{g/ml}$.

PROVASOLI: Doctor Riley, in your paper with Mrs. Vishniac⁹⁵ you thought that the termination of the *Skeletonema* bloom was probably due to lack of nitrogen. In the light of the new chemostatic data on the minimal need of B_{12} , do you consider that the low level of B_{12} reached toward the end of the *Skeletonema* bloom was also a limiting factor?

RILEY: The lowest level reached would reduce the growth rate, but probably would not reduce the yield.

PROVASOLI: So that the nitrogen still seems to be the more important factor of the two in Long Island Sound?

RILEY: Yes, at the lowest level something like 3 or 4 $\mu\text{g/ml}$ of B_{12} is present, which is more than the highest level commonly recorded in the outer coastal waters.

PROVASOLI: Yes, but would that be sufficient to maintain continuous growth in the chemostat at the lowest level?

RILEY: Yes, it certainly would. Mrs. Wood had several experiments in which the input was of that order of magnitude, and she was able to establish a steady state.

PROVASOLI: For my own clarification, it is my understanding that the chemostat data were obtained by decreasing the level of B_{12} in the input until the decreased growth rate resulted in the progressive washing out of the cells in the overflow. Is that correct?

DROOP: Your work was on the shoulder of the curve with the flow rate just below the washout rate; isn't that correct?

RILEY: Yes, that is correct.

PROVASOLI: Do you think that quantitative data obtained with the chemostat can be applied with immediate relevance to the natural situation? I ask this because I have no doubt that the *in vitro* data concerning *qualitative* requirements can be confidently used in ecology. I wonder, however, how far we can go in extrapolating laboratory data on concentrations of nutrients or growth rates to the situation in nature. Would we simulate natural conditions better with flowing or stagnant culture?

RILEY: Let me try to answer it this way. A considerable variety of flow rates were used with a range of concentration of B_{12} from one to 10, and, under these circumstances, the requirement varied no more than about 50 per cent. In each case, a steady state, or almost a steady state, was established and the yield varied only within fairly narrow limits.

DROOP: One would think that the conditions of a diatom falling through a body of water is more like the situation in a chemostat than that in a batch culture. I would like to say something about *Skeletonema* in this respect. Mrs. Wood's regressions were nonlinear, and her results were not altogether consistent. There is a good deal of variation in the yield obtained from an identical medium at different times. My own experience in cultivating *Skeletonema*, particularly in the effect of fresh medium and the physical state of the medium, suggests to me that flow rate itself may depress the growth of *Skeletonema*, irrespective of nutrient concentration, and that Mrs. Wood may possibly be measuring the effect of flow rates rather than that of B_{12} concentrations.

MCLAUGHLIN: Doctor Gold and I have been interested in the development of an on-board ship assay for vitamins and other essential micro-organic nutrients. In our first attempt to use C^{14} as a research tool for assay, a dinoflagellate was used which was isolated in 1959 from an endemic population located in an area called Delancy Cove, Larchmont, New York. At the time we isolated it we didn't know its name, but since then it has been speciated.¹⁰⁴

The ecology of the area is such that sewage pipes drain into this cove, and there are four large pipes which drain off rainwater from the land. The bottom of the cove contains a sunken scow and the beach is a sand-pebble type.

We chose this area because of the endemic population of the dinoflagellate and the fact that this population would, under certain conditions, go into a "bloom" condition. The area is also graced by a boat club which maintains a swimming pool and keeps records of the water temperature, etc. for the surrounding area.

We charged Doctor Kenneth Gold with the problem of studying the nutrition and physiology of the organism and trying to use it as a laboratory tool for practical assays of natural waters. Of particular interest here is one result: the effect of B_{12} on the assimilation of $C^{14}O_2$. Results as reported in this thesis,¹⁸ and in a paper submitted for publication,⁸⁸ indicate that B_{12} -depleted cultures take up (assimilate) less $C^{14}O_2$ than those with adequate B_{12} . It appears that when B_{12} is added over a concentration gradient to B_{12} -depleted cells, they assimilate more C^{14} in the presence of increasing B_{12} .

STRICKLAND: Is this incubated 24 hours with C^{14} ?

MCLAUGHLIN: No, with B_{12} . The C^{14} is then added for four hours, then harvested and the assimilated C^{14} measured. We have only started developing this laboratory technique, and so far it seems promising, but I am still groping about with the problems inherent with an *in vitro* technique.

FOGG: Did appreciable cell multiplication take place?

MCLAUGHLIN: No, there is no appreciable multiplication during this short-term run.

STRICKLAND: Did you try to add C^{14} and B_{12} at the same time?

MCLAUGHLIN: Yes, we tried adding both immediately on collection, and assimilation is the same with or without B_{12} . Some waiting period with B_{12} is necessary for differences in assimilation to be recorded. In these experiments, the period was at least 24 hours. We have not determined the shortest period which will give us meaningful differences. As I have indicated, this is a preliminary trial with this particular organism, used because of its euryhalinity and good growth in our particular basis.

STRICKLAND: Could I make a practical observation. In our 1961 bag experiment,¹⁰⁵ I believe we had some $3 \mu\text{g/ml}$ of B_{12} , initially, and it supported a perfectly enormous crop of mixed diatoms, certainly at least three times the crop that would ever be seen in normal coastal water. In so doing, however, the B_{12} concentration decreased markedly but did not disappear.

PROVASOLI: And did you know how much B_{12} was produced in the meantime by the bacteria in the bag.

STRICKLAND: No, but the bloom was very rapid—five days—and one would not suppose bacterial production to be very significant in the few days concerned.

PROVASOLI: I was amazed by the tremendous growth of bacteria observed by Jones *et al.*,¹⁰⁶ in the first 24 hours after seawater is put in the light and dark bottles. It is true, Doctor Strickland, that you are working with a large bag and a better surface/volume ratio, which would tend to minimize surface effects, but I still wonder how far the effects are eliminated.

STRICKLAND: I think the bacteria are in my bag all right, but they are working on the detritus and the plants would have been in the sea anyway. I am just pointing out, given that amount of B_{12} , what size of bloom certainly can be produced.

GOLDMAN: In culturing lake waters with vitamin B_{12} additions alone, I have found a 50 per cent increase over the controls in Alaskan lakes, and about a 10 per cent increase over the controls in two New Zealand lakes.¹⁰⁷ This is obtained by simply measuring the change in rate of carbon uptake after the addition of B_{12} . I have no value for natural B_{12} concentrations in the fresh water, however.

PROVASOLI: Doctor Guillard, would you present Menzel's data on vitamin B_{12} in the waters of the Sargasso Sea?

GUILLARD: I don't have their Bermuda report here with me, but the information has been published by Doctor David Menzel and Miss

Jane Spaeth.⁹⁶ The amount of B₁₂ reported was very small, less than about 0.03 mμg/liter from May to October, up to 0.06 or 0.1 mμg/liter during the winter. What was that figure you gave Doctor Riley, for the minimum B₁₂ concentration that provided for rapid growth of *Skeletonema*?

RILEY: That was 8 μg/ml.

GUILLARD: In short, as far as the Bermuda report is concerned, the B₁₂ concentrations found in the waters off Bermuda are large compared to your value of 8 μg/ml.

I have a few notes here concerning some questions I believe I was asked. When I run a bioassay, I first prepare an inoculum consisting of cells in depleted medium, but not completely resting; that is, I grow cells until they no longer divide because of lack of B₁₂, and use these cells as an inoculum before they begin to die. In our assay experiments, a sample of natural seawater is treated with activated charcoal to remove all the vitamins by adsorption. The charcoal is first washed so that it doesn't poison the seawater.

LUND: What else is removed by the charcoal?

GUILLARD: Oh, all kinds of things, no doubt, but I am not interested in that, for the following reasons: (a) The adsorption removes the vitamin, as can be proved by adding B₁₂ and then showing by bioassay after charcoal treatment that it has gone; (b) if the complete medium (including B₁₂) is prepared with charcoal-treated water, the algae, even pelagic ones, grow pretty much as they do in water not charcoal-treated, showing that the water is not toxic.

To get a dose-response curve one would add to portions of such charcoal-treated water, for example, 0, 0.5, 1.0, 1.5, and 2.0 μg/ml of B₁₂, and inoculate with perhaps 100 cells/ml, taking cells from a depleted culture. If one counts cells at intervals there after, a family of curves is obtained shown schematically in FIGURE 20. If the vitamin value (cell number) of each curve is plotted against vitamin concentration, the dose-response curve, as in FIGURE 21 is obtained.

I am told by Mrs. R. M. Cassie, of the New Zealand Oceanographic Institute, that one can calculate a value for the asymptotes of the curves shown in FIGURE 20 and, thus, get a statistically better regression line than by plotting the maximum values observed, as I have done.

The dose-response curve goes through zero, because in the flask without B₁₂ the cells used as inoculum usually divide once or twice and then die.¹⁰⁸ Unfortunately, as shown in FIGURE 20, cells in the flasks having B₁₂ also die after a time, so that it is necessary to count cells at frequent intervals, instead of just once or twice during the experiment. At least, this is the case with the diatoms I have studied. There is no

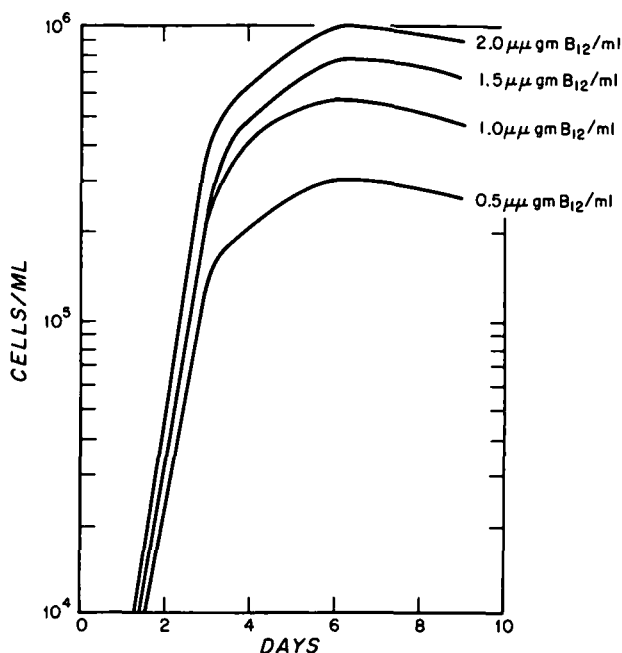


FIG 20. Schematic representation of the growth of populations of diatoms in a bioassay experiment for vitamin B_{12}

Ordinate: population density, in cells/ml. *Abcissa*: days after inoculation of the cultures with 100-1000 cells/ml from a B_{12} -depleted culture. The concentration of B_{12} in the culture vessels is shown to the right of each curve. The curve for the flask without added B_{12} is not shown because the inoculum does not grow.

evidence in FIGURE 20 that indicates the rate of cell division during the first days of growth is different in flasks having different B_{12} concentrations.

KRAUSS: That is just the point I brought up before. The rate is identical, but the yield is something else.

GUILLARD: Right, that is what I find. However, I must agree with Doctors Riley and Droop that the regressions are nonlinear for some organisms. It has happened often enough to convince me that the regression curves are concave upwards sometimes, but, in a paper in preparation,¹⁰⁹ I have drawn straight lines through the points anyway.

STRICKLAND: We get a good yield with *Amphidinium carteri*.

GUILLARD: I have never tried to do a bioassay with *Amphidinium*.

STRICKLAND: We get yields approximately proportional to the initial concentrations of vitamin B_{12} , and, when it is depleted, the cells do not die or seem to lose pigment for several days.

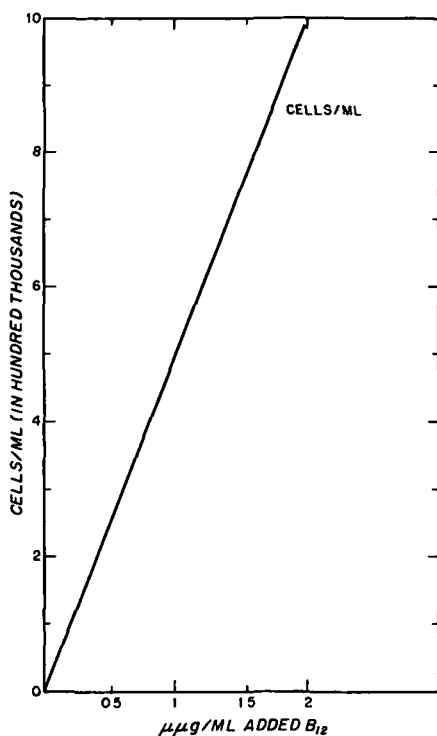


FIG 21. Schematic dose-response curve, obtained from the data shown in Fig. 18. The maximum population (ordinate) found at any time in each B_{12} concentration is plotted against B_{12} concentration (abscissa).

GUILLARD: I have wondered if B_{12} might not be destroyed during some of our experiments. There is a further possibility to explain why the cells die. The number of cells put into a flask at the start of an experiment is at least two or three orders of magnitude smaller than the final yield, so I don't think that the total amount of B_{12} available per cell can change much in the first day or so after inoculation, but perhaps the inoculum absorbs all the B_{12} out of the water in a very short time.

DROOP: There is some evidence of such absorption by *Ochromonas*. Actually, uptake of vitamin B_{12} by *Ochromonas* takes place by a process of sequestration or binding of the free vitamin. I am not quite sure of the figure, but heavy cultures of *Ochromonas* will, I believe, bind something like 90 per cent of added vitamin B_{12} very quickly.¹¹⁰

PROVASOLI: How quickly? A matter of hours or days?

DROOP: Two hours in Ford's experiments.¹¹⁰

GUILLARD: This may explain part of the discrepancy between the results obtained by Mrs. E. A. Wood of Yale University and me (see page 000). Experiments such as I have just described did not show reduced growth rate below $8 \mu\text{g/ml}$, as she found in continuous culture.

PROVASOLI: That perhaps explains why the division rate at the beginning is the same no matter what the concentration of B_{12} . Since the B_{12} is quickly absorbed it cannot become limiting in the first phases of growth.

OPPENHEIMER: Could the initial depletion of the B_{12} affect the sequence of growth? Doctor Riley mentioned that three molecules of B_{12}/μ^3 cell are needed.

GUILLARD: This is clear. The figures we got¹⁰⁹ were from about five to 15 molecules of B_{12}/μ^3 of diatom protoplasm. But, by the time the highest yield is obtained, the diatom cells are in very poor condition. Therefore, I think this maximum yield is not of great ecological significance. I also think it may well be that at crucial stages in the life cycle the diatoms require a higher B_{12} concentration than they do at other times, or are more sensitive to low concentrations. I have no definite evidence for this.

In summary, there are two points that may be interesting. First, no matter how you regard the evidence, there are no differences as large as an order of magnitude in the B_{12} requirements of any species studied so far. There is no evidence that any of the algae discussed here can synthesize part of the B_{12} they need, as some organisms do for thiamine, for example. The more interesting point, though, is: Why don't the species that have no B_{12} requirement dominate the ocean?

MYERS: Don't you think that you are asking very different questions than Doctor Riley? Were the cells at zero time on the curve in FIGURE 20, like the cells on the top of the curve?

GUILLARD: No, they are usually not that bad.

MYERS: But you said the cells at the top of the curve wouldn't grow.

GUILLARD: That is right.

MYERS: Therefore, the initial cells are similar to the cells on the top of the curve. So aren't you studying a transition?

GUILLARD: I wouldn't say that.

MYERS: Aren't you studying the transition between cells that don't have enough B_{12} to grow, and cells which have used up the added B_{12} and, again, cannot grow. It seems to me that you are studying the wave that occurs in a population when a given amount of B_{12} has been added. Isn't that the answer you obtain?

GUILLARD: Yes, except for one thing. If one starts with an inoculum from a culture in which the cells are not yet depleted of B_{12} , a small

enough inoculum so that no appreciable amount of B_{12} is added, the results are about the same.

RILEY: This is one of the very difficult points about this work. If one depletes the cells as thoroughly as one would like, the chances are that one will kill them and get no growth. On the other hand, if one wants to deplete them as much as possible and still get active growth, one tries to use an inoculum that is obtained along toward the end of the log phase, when the cells are just beginning to be depleted. It is possible to establish some arbitrary rules for this that would work out, more or less, but this is one of the difficulties involved in standardizing such experiments.

STRICKLAND: There seems to be something fundamentally different between a diatom and an *Amphidinium*, then, Doctor Guillard, because *Amphidinium* is remarkably stable, if it is provided with excess nitrogen, phosphorus, and so on. When it has used up its B_{12} , it sits in the bottom and swims around and keeps its color. The diatoms definitely do not do that.

PROVASOLI: Perhaps, it is a real difference, and may go beyond the fact that dinoflagellates swim and diatoms don't. For instance representatives of the two organisms in question differ in their ability to survive in stock media. In general, diatom cultures bleach very quickly and have to be transferred at least once a month or earlier. Cultures of many dinoflagellates remain alive for months: While the majority of cells may die and bleach at the bottom, quite a few cells keep on swimming. Bill Wilson at Florida State University told me that a culture of *Gymnodinium breve* remained alive in a closed test tube for a whole year.

DROOP: It all boils down to the point that we can't yet grow diatoms. That is my opinion.

HUTCHINSON: Is it known what the ratio of cobalt in vitamin B_{12} is to the total amount of the element in seawater?

PROVASOLI: Extremely small, and this brings up an interesting point. Vitamin B_{12} can be employed as a source of cobalt for non- B_{12} requiring blue-green algae.¹¹¹ Apparently, vitamin B_{12} is a very efficient source of cobalt because only about one one-hundredth as much cobalt is needed if it is given as B_{12} rather than as a mineral salt. This indicates that B_{12} can serve a dual ecological purpose; as a growth factor or as a source of cobalt.

HUTCHINSON: You don't think that Skoog's work is really a demonstration that starting with B_{12} is the best way for an organism to get a supply of B_{12} ?

PROVASOLI: No, I think that it might be a convenient way of providing soluble cobalt, just as it has been found that hemin and

hemoglobin are very useful sources of iron because of their solubility in the alkaline media for tissue culture.

HUTCHINSON: Isn't this almost the same thing, that taking up porphyrin is the best way for an organism to make iron-bearing porphyrin? Is there any independent evidence of cobalt metabolism not involving B₁₂?

PROVASOLI. Offhand, I don't think there is any evidence. The only reason for thinking that cobalt may have functions other than being incorporated in B₁₂ is the analogy with iron. Iron, besides being needed for porphyrins, in protists at least, is needed for the ferri-chrome growth factors, and ferrous iron is a cofactor for some bacterial aldolases. Perhaps I am obsessed by the problems of preventing precipitation of many metals at the high pH of seawater. Natural organic chelators like the porphyrins are better than most artificial chelators because of their high specificity toward one metal, and unusually low ionization constants. It is even conceivable that vitamin B₁₂, being such an efficient source of cobalt, may become more rapidly poisonous to organisms sensitive to cobalt. We have some results, not fully confirmed, which indicate that.

DROOP: High concentrations of vitamin B₁₂ in the order of thousands, instead of hundreds, do retard growth.

TECHNICAL PROBLEMS IN SAMPLING

Discussion leader:

T. BRAARUD

*Institute for Marine Biology
University of Oslo
Oslo, Norway*

BRAARUD: We shall begin by dealing with a technical problem that is comprised of two parts: How are we to describe populations in the sea and how can we find time-saving and satisfactory means of estimating populations in cultures? I propose that we first discuss work on populations in the sea.

There is no single satisfactory method that gives information on the total phytoplankton population indigenous to seawater. To describe such populations in the water, we have to use several different methods and try to combine the information gathered in order to get at some, not reliable but at least *some*, information on the various living organisms. Quite recently, some of those present have reviewed the methods currently used for quantitative determination of populations in the sea,¹¹²⁻¹¹⁴ so I will not at this time give a complete review of the situation. I suppose several of you will have comments to make on some of the methods which have been improved recently. There is also E. J. Ferguson Wood's method,¹¹⁵ which is going to be used in the Indian Ocean Expedition and which I hope we will hear a good deal about from Doctor Oppenheimer who has used it, not for plankton but for sedimentary algae microorganisms.

Then we have to consider the main obstacle to the study of populations in sea water, the total population, and that is the lack of satisfactory ways to preserve the naked flagellates. We have been facing this problem for a long time and it has caused us considerable concern, because we have never felt quite happy about the validity of our population studies since so little has been known about the possible role of the naked flagellates. Various methods have been used to estimate the naked flagellates, but they have not given a very consistent picture. It is still an open question and that is the main problem we are going to deal with here.

At the last meeting of the Plankton Committee of the ICES International Council for the Exploration of the Sea, it was decided to take the initiative in this matter. It was decided to ask Doctor Mary Parke,

who has done such excellent work on some of these groups, to chair a small committee, which would include an organic chemist who might assist in seeking a compound for preserving the naked flagellates in a satisfactory way. This is a matter which might be discussed here, and any suggestions would be received with gratitude.

All of our present methods are unsatisfactory in one way or another, but they may serve a purpose if one takes their limitations into due account. Still more important, people working on populations in the sea are aware of how inadequate the sampling methods are. Economy decides to a great extent the accuracy of our estimation of plankton populations. Available observations demonstrate patchiness and great variation within small distances, and I think it would be very useful if a few special studies were made that would cover a greater number of stations within a small area, instead of the large grid patterns that we ordinarily use. This should be done both in oceanic and in coastal waters, and the results should be published so they are readily available and act as a warning against pitfalls when observations are made at great intervals in space and time.

One point I think we should discuss here is the publication of observations which are made by any of these methods. In most countries, it is very difficult to have extensive tables of biological observations published. My experience is that when a paper is over 10 years old, the essential part of it is not the text but the tables. When one considers what it has cost to collect planktonic data, the cost of the publication is comparatively small, and one should accept the duty to have these tables published.

As an example, I may mention the international study of the North Sea in 1912.¹¹⁶ When we made a more extensive survey of the same area in 1948,¹¹⁷ we could not use much of the text of the 1912 study which at the time of publication was very stimulating and valuable, but the tables could be used and worked up in the same way as those for the 1948 observations. Again and again one finds that the text soon becomes dated while the tables continue to be useful.

PROVASOLI: The National Science Foundation in a recent ruling has approved the use of grant funds to pay part of the printing cost for non-profit journals. This ruling is helping several learned societies to increase substantially the number of printed pages, thus reducing the lag in publication. The main factor behind the decision is the one you mentioned, Dr. Braarud. So much money and time has already been spent to acquire the data that the cost of publication is comparatively small. Nobody benefits from buried research results.

GOLDBERG: This problem of publication of data is not a monopoly of the biologists. It occurs in chemistry and geology, also. Recently, I published a paper¹¹⁸ on the distribution of feldspars in sediments of the

South Pacific, and the tables, which ran to about five or six pages, were rejected by the journal. However the tables were put on record in the ADI Auxiliary Publications project, Photoduplication Service, Library of Congress, and microfilm or photoprint copies may be obtained by sending \$1.25.

Apparently, this type of data publication is going to become more common, as so much data are coming out. There will be central depositories for data, with copies at a price, available to all who wish them.

PROVASOLI: I would still prefer the tables to be near the text and printed in regular journals. Unfortunately, many editors are not as discriminating as they should be. Much printing lavishness and untidiness could be eliminated and texts tightened, but, above all, why don't we bring back to original size the many yearly progress reports which are blown up into full-sized publications. With the increasing cost of journal subscriptions we should accept only the very best for efficiency.

HUTCHINSON: Isn't the main solution to have all the tables reproduced by photooffset?

PROVASOLI: Yes, this might be one solution.

HUTCHINSON: Actually, although a majority of publishers hate to admit it, it doesn't cost any more to do a photooffset page than it does to do a printed one, even to insert it in a textbook, and there are a number of journals now which insist on having all their tabular matter submitted in the form that can be directly photographed. I just sent off one in the last two weeks that has the tables prepared for offset and they will publish all the tables if they are intelligent journals.

It is largely a question of editorial policy and of admission that it really doesn't cost any more. It is an old tradition that anything done as a figure is twice or three times the cost of the printed page, but that has ceased to be the case.

PROVASOLI: You are so right. Very few journals, unless pushed, will consider adopting new techniques to effect substantial economies in printing. So far as possible, they prefer to ask for higher society dues, contributions to page costs, government subsidy, etc. Much could and would be solved by efficient editorial policies. General economies and astuteness are needed because, in every discipline, there are fields, for example, taxonomy and morphology in biology, for which abundant illustrations or tables are indispensable. The elimination or reduction in size of the publications would be a mistake, and is not warranted even when no grant support can be obtained for this type of work. The progress of science should not be permitted to become consciously lopsided because of transitory fashions.

KOCZY: Doctor Provasoli, I agree with you in many respects, but I would prefer to get tables on IBM cards directly. Otherwise, I have to

transfer the tables onto IBM cards in order to make statistics and make correlations. If printed tables are put on IBM cards, and a copy of the set of cards can be purchased for \$1.25, or even \$5, I would prefer to pay for the cards and get a table in such condition that I do not need to pay a girl to read through the table and make IBM cards at a cost of, say, \$120.

HUTCHINSON: Yes, but if you are trying to write a book, which is an occupational disease that I have been suffering from, the number of \$5 bills which you have to dispense will become perfectly prohibitive on that basis.

PROVASOLI: Tell me, Doctor Koczy, with the IBM cards don't you have the difficulty of programming, in the sense that you may want to program one way and others another way?

KOCZY: No, that is a misconception. The card isn't programmed. The card is only made up on some standard computer form and from that form we can program, as we want to afterwards. So, we have only to standardize on how to make tables or how to make IBM cards. I think one of our duties is to do that soon, before it is too late. The cards can be put in the IBM machine and the printed data sheets can be as long as desired, all nicely typed.

BRAARUD: This question of a data center is being worked on in all fields. In this country there is, for example, the Biological Data Center in Rhode Island. To my mind, it seems more satisfactory to have the data printed, even in the cheapest way, because then the author is really responsible for what is presented in as good and as reliable a way as possible.

FREMONT-SMITH: Doesn't this mean we need both—the tables in the journal or volume, and the cards available, also? It is not an either/or, and neither of these is impossible to handle.

PROVASOLI: And at a reasonable cost, once we set our minds to it and cut out the useless material.

ALLEN: I would like to discuss a couple of items that we have been working on during the past year. In the first place, we are interested in the problem of collection of samples. It is well known that phytoplankton, including the naked flagellates and other representatives of the nanoplankton, exists in the sea in a very patchy distribution. If one puts down several water bottles side by side they may come up containing quite different populations. It seemed that the only way to cope with this was to use some method which would collect a large sample, subdivide it, and deliver into one's hands for further use a representative sample of a very large body of seawater. We ended up with a pumping method, which is not a new idea; many people have devised pumps. However, we set ourselves the engineering problem of

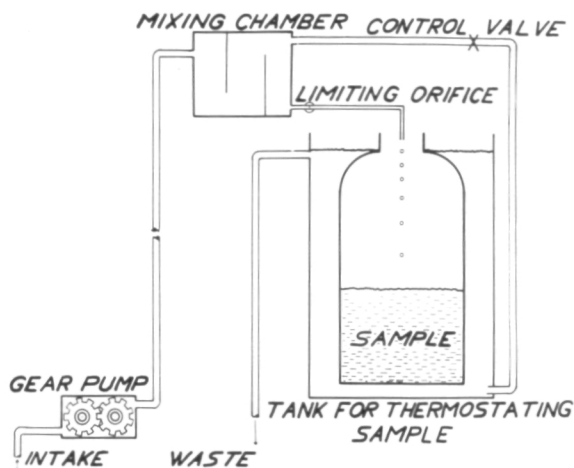


FIG. 22. Plankton pump arrangement.

developing a pumping method that would deliver physiologically-active phytoplankton, and in this I think we have succeeded.

FIGURE 22 shows the schematic plan of the system. We have a gear pump, which is immersed in the water. This sends a sample on deck to a mixing chamber which has two holes in it, a large hole and a small hole, which means we can collect about 1/100 of the sample, use the rest of it to circulate around the sample bottle to keep it at the same temperature as the seawater being collected, then send it out to waste.

The gear pump is made of Hastelloy C and Teflon and operates at a low speed, so that the sample will neither be chemically contaminated nor mechanically damaged. The actual setup, minus housings, is shown in FIGURE 23. The apparatus in the foreground is intended to be submerged in the water. In the background is the mixing chamber and a water meter to show the total quantity of water which has been put through the samples.

To test this apparatus, we found, from tests on cultures, that the most sensitive criterion of damage to cells was their rate of photosynthesis. We had samples which showed 100 per cent viability by cell counts, but if we ran the pump a little too fast we would get at least temporary inhibition of photosynthesis. In testing the apparatus on natural populations from San Francisco Bay, and comparing the rate of photosynthesis as measured by C^{14} uptake before and after going through the pump, we obtained the result in TABLE 4, which shows that passing through the pump has had no bad effect whatever on the population.

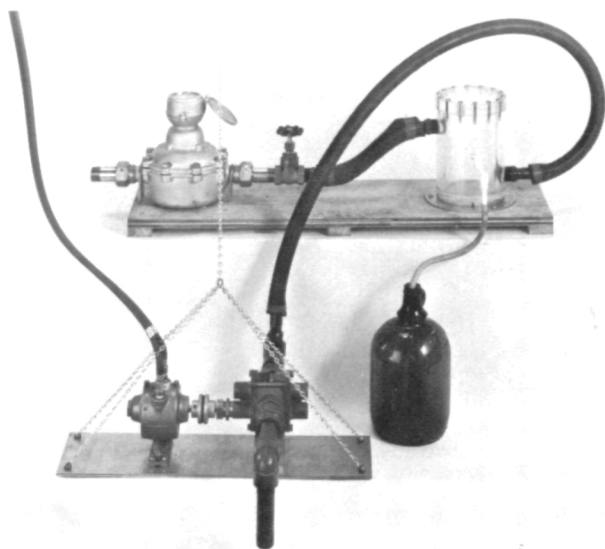


FIG. 23. Plankton pump photograph.

The third figure shown in each experiment gives the result of putting the material through the pump, then filtering through a pilot plant-type Millipore filter, which has a large area and, hence, a small pressure drop across each unit area of the filter. The filter is not allowed to get quite dry, and the sediment is gently resuspended with a

TABLE 4
EFFECT OF COLLECTION PROCEDURES ON
PHOTOSYNTHESIS OF PHYTOPLANKTON

	Treatment	$C^{14}O_2$ fixed, c/m
Expt. 1	original	$186,000 \pm 4,000$
	pumped	$247,200 \pm 14,400$
	pumped, filtered, and resuspended	$161,300 \pm 12,600$
Expt. 2	original	$11,450 \pm 500$
	pumped	$12,000 \pm 1300$
	pumped, filtered, and resuspended	3940 ± 400

vinyl policeman. This latter technique is probably not quite ready for general use, but, under favorable circumstances, a large proportion of the organisms can be recovered in good shape.

KOCZY: Why is the plus or minus variation so large—why is there such a large spread?

ALLEN: Actually, I don't know. There were only four bottles in each test, so the results are not of much statistical significance, anyway. I would like to see the apparatus tested over a wider range of conditions, particularly on tropical phytoplankton, which may be more sensitive.

PROVASOLI: I would like to know how this device compares with Doctor Tonolli's phytoplankton bar.*

VOLLENWEIDER: I don't know. The situation is a great deal different. I should like to emphasize the problems of collection. The most striking fact is that the horizontal and vertical distribution of zooplankton and phytoplankton is very different from place to place. Sometimes in extreme cases it is possible that there can be rapid changes and within a few minutes during net or bottle collection different kinds and density of species will vary greatly.

We are a little bit skeptical at the moment about the values of all plankton sampling, not so much as to the counts we can make using any kind of device, the microscope or electronic counters, but sampling on the spot.

ALLEN: One thing we have thought of doing with our pump apparatus, but haven't yet, is to monitor the flow through the large orifice with a sensitive nephelometer and, thus, obtain a picture of the distribution.

PROVASOLI: Doctor Tonolli had a specific fish-shaped sampler whose fins are governed by a temperature, a pressure, or a salinity recorder. In this way, one could sample in a layer of water constant for one variable.

VOLLENWEIDER: In lakes, if you go out and stay in one place, you will find after one or two hours, very big changes. These changes may be due to the fact that you have internal wave action. That is one possibility. Another possibility is that you have migration of some sort. If you have internal waves, you have the same effect also on phytoplankton because they are so very small.

PROVASOLI: The phytoplankton would be more subject to external forces because they cannot swim as powerfully as the zooplankton.

VOLLENWEIDER: This brings up the same problem that we discussed earlier about the concentrations of the plankton in specific water masses. Sometimes we find lakes which at half a meter are very

* Tonolli, V. Istituto di Idrobiologia, Novara, Italy.

dense in phytoplankton population. If you put a bottle in and draw out a certain population of phytoplankton, you will find that you can repeat the sampling in a short time—I mean after a quarter of an hour—and you will get quite a different sampling.

BRAARUD: Doesn't the scheme of the sampling have to be varied according to the habitat where one is working? In the lake, it may be necessary to have very dense spacing; in the oligotrophic part of the ocean, one may, for practical reasons, be satisfied with greater distances between stations.

VOLLENWEIDER: Truly, one must decide and one must have some experience as to what is the local situation. Surely in the open ocean it will be quite different, but assuming that you have, for instance, local coastal variations of water types, I am sure that the same difficulties arise as we have in a large lake.

BRAARUD: In that case, the thermogram is very helpful and one can adjust the spacing somewhat according to the stratification.

STRICKLAND: It is also advisable, I suggest, to determine a profile of the turbidity of the water in the euphotic zone. This can be done with commercially available turbidity meters which have a horizontal housing with a light source at one end and a photo-cell at the other. One should always take a water sample at any depth which shows an extra high turbidity as this may indicate a dense phytoplankton population. Such a procedure is now used routinely by Russian marine productivity workers in both the Atlantic and Pacific and, I am sure, by other people as well.

PROVASOLI: And it is done with a source of light and an enthalometer, in other words, on the other side.

VOLLENWEIDER: I am looking for information on techniques to determine turbidity rapidly because turbidity may be influenced by minerals.

KOCZY: Have you considered echo sounding?

VOLLENWEIDER: We are using echo sounding for zooplankton.

KOCZY: Echo sounding is worse than a scattering meter.

VOLLENWEIDER: No, no; echo sounding for zooplankton is now an excellent instrument. You have to work with about 60,000 cycles and then you can detect dense zooplankton population.

KOCZY: Yes, and shorter wave lengths, corresponding to 100,000 cycles, produce another type of stratification; with 30,000 cycles, there is still another stratification because of resonance phenomena. I believe the interrelationship between acoustical impedance of plankton suspension and wavelength has had little thorough investigation. For that reason interpretations of echo-sounding records seems to be worse than light scattering.

BRAARUD: Let's not go into echo sounders here because that is not really applicable to phytoplankton; for zooplankton it is excellent.

KOCZY: In some respects.

STEEMANN NIELSEN: In Danish waters where there is strong stratification. Mr. F. Hermann has made a rather nice apparatus we have used during recent years. It is a transparency meter; but, at the same time, it measures temperature and collects a water sample. We get water samples from exactly the same water where we measure the transparency, and this has been of real use for our work. If we first measured the transparency and afterwards collected a water sample by means of an ordinary water bottle, we often didn't get the sample from exactly the same depth.

VOLLENWEIDER: Another possibility which I am studying now will perhaps be useful. It is a horizontal transparency meter which measures specific spectra. So you get constant information whether you have minimal clouds or you have heavy clouds, because it is possible to see, absorption peaks.

PROVASOLI: Wonderful. This is real progress.

BRAARUD: Doctor Lund, do you have some remarks to make on methods?

LUND: No, I don't think so, but I do think there is another quite different problem from the one we have been discussing. It is the problem of preserving samples and recognizing objects. It is quite clear that, at the present time, we cannot identify small flagellates, particularly, and some other algae, without the use of an electron microscope. The work done at Plymouth is a very good example of this.

When it comes to ecology, this is not very practical, but here we are concerned perhaps with a combination of two things: a desire for correct taxonomy, and a desire merely to identify. These can be quite different aims, and I would like to suggest to the committee you mentioned, Doctor Braarud, that they should look into finding out methods for identifying these very small objects, using ordinary light microscopes. It doesn't matter how the identification is made, what method is used, what bit of material is left, as long as one can distinguish between these bits of matter.

I don't think this is at all impossible. One of the troubles at the moment, as I see it, is that once one has an electron microscope there is such enjoyment to be had out of this instrument, such marvelous things to be seen, that one forgets all about one's poorer brothers who do not have electron microscopes, or who are trying to do ecology and cannot take every little specimen, transfer it to an instrument, and look at it before going any further. This probably has arisen in other aspects of biology, and it is a remarkable fact that one can learn to

identify objects by characteristics which would turn the taxonomists hair gray or make it fall out altogether.

BRAARUD: This is a very important problem, I think, and in our laboratory, we have been facing it for a long time. Every new student who starts studying populations faces the same problem: First, he must distinguish between forms and, later, he must try to identify them.

Doctor Halldal¹¹⁹ has developed a very small micropipette by which one can pick up one or more of such small objects and transfer first, to a research microscope and, if necessary, later, to the electron microscope.

In the working tables, the organisms may first be given preliminary characteristics and later be identified. Having access to an electron microscope is a great advantage, as it may then be possible to describe or identify forms which one otherwise might distinguish but not be able to describe satisfactorily.

ALLEN: This comes close to a problem that I have talked about to various people during the past year or so, and I would very much like to have the opinion of the taxonomic experts here as to whether this is a sensible thing to do. More and more people are going out and looking at more and more parts of the sea, and I am thinking particularly of the international effort in the Indian Ocean. If people who are not experienced in the taxonomy of these groups are going to collect and look at the nannoplankton forms, particularly those that do not preserve well, (and the literature for their identification is really rather scattered and a little hard to come by), would it be at all feasible to prepare something like a field guide for the identification of phytoplankton, at least at the generic level?

BRAARUD: I am very skeptical of guides for phytoplankton, because even when one has worked on phytoplankton for many years, one finds variations within the species which were assumed to be very stable. We have a very sad situation with *Thalassiosira gravida*, which Doctor Steemann Nielsen and many others here know to be a very good species. It now turns out that this species includes many different forms which look much the same, and certainly have a different ecological response also.

Taxonomic knowledge has to be based on a good botanical background and specialization, and I consider it preferable that people who lack the right background refrain from taxonomic work. One should, in any case, warn against publication of observations based upon inadequate identifications.

PROVASOLI: What is the alternative, if the material is not preservable and cannot be sent to a specialist?

BRAARUD: This is a matter which should be taken seriously; how we shall provide students of phytoplankton with an adequate background in general taxonomy and in the elements of physiology, since I think that is a necessary prerequisite for a broader advance in the field.

PROVASOLI: You mean interdisciplinary teaching?

BRAARUD: Yes.

GOLDBERG: I would suggest that much of this identification work can be done by a machine. With the developments of recognition machines today (for example, the Russian translating machines, or the devices used in the identification of high energy nuclear events at the Radiation Laboratories at Berkeley, California), the possibility that such techniques may be applied to the study of plankton populations is appealing and worthy of serious investigation. One could utilize the tremendous knowledge of taxonomists, such as Doctor Braarud, in the memory program of such recognition devices, to compare with photographs of organisms sent in by workers from laboratories all over the world.

PROVASOLI: I think it has great potential, especially for species that can be differentiated by external shape. But if we have to resort to internal details, like chloroplasts, or number and location of other organelles lying in different planes, then we would need three-dimensional scanning.

ALLEN: And I don't see how a machine could match the detail the microscopist observes in internal structure.

KOCZY: In micropaleontology, where they deal with skeletons, it can be done.

GOLDBERG: If it can be done in micropaleontology, then it would appear promising that at least a semi-quantitative description of living plankton populations could be obtained.

BRAARUD: Good pictures can be obtained in micropaleontology, but there one deals with comparatively large forms, or such solid fractions as coccoliths which may be studied in the electron microscope. In the case of recent phytoplankton, the situation is quite different.

GOLDBERG: It couldn't be done solely on external shape.

BRAARUD: No.

HUTCHINSON: Surely the good taxonomer always knows that the thing is different before he knows what the characters are.

MCLAUGHLIN: One of the problems I've recently encountered may help clarify the taxonomic classification. At the time I isolated a dino-flagellate, *Glenodinium halli*, I sent it to a taxonomist, Doctor John Lee at the American Museum of Natural History. He called and asked; "Why don't you send me a single-cell clone isolate?" "I thought I had," I replied. He said: "There are two different trophic forms in this culture; you must've made a mistake."

I reisolated it and, again, came the same answer, "Don't waste my time. Send me a clone." The organism had two trophic forms: It varied from a linear dimension of $6\ \mu$ up to $24\ \mu$, and it had a life cycle—including a cyst form.

After being convinced that I could pick up one cell with a micro-pipette and establish a culture, Doctor Lee thought that he would probably find one of these forms in the literature with a name attached. The other two shapes he saw didn't appear in the literature, so he assumed I had given him mixed cultures. He was perturbed—and I had spent hours making sure that in isolation I moved only one cell from well to well to well.¹⁰⁴ These variations in size of cells is something we had never dreamed of—at least I hadn't—and when one puts them through an electronic counting device, such as the Coulter Counter, one gets objective proof of volume variations representative of size variation, sometimes four-fold (FIGURES 24,25,26,27,28). I have a

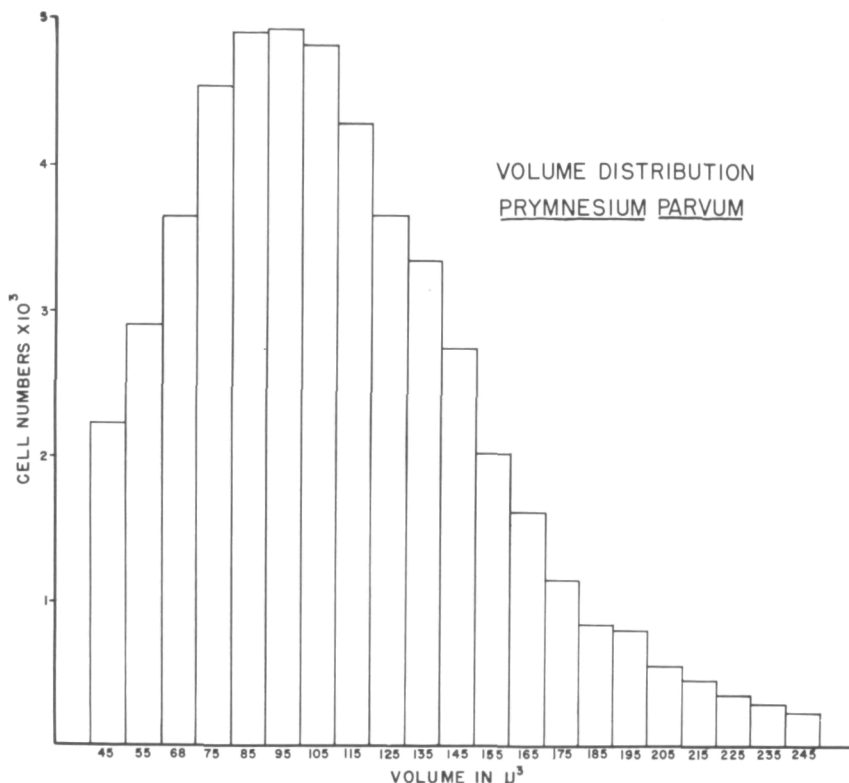


FIG. 24. Volume distribution of *P. parvum* grown in ASPs for 10 days. Volume and cell count recorded with Coulter Counter Model B.

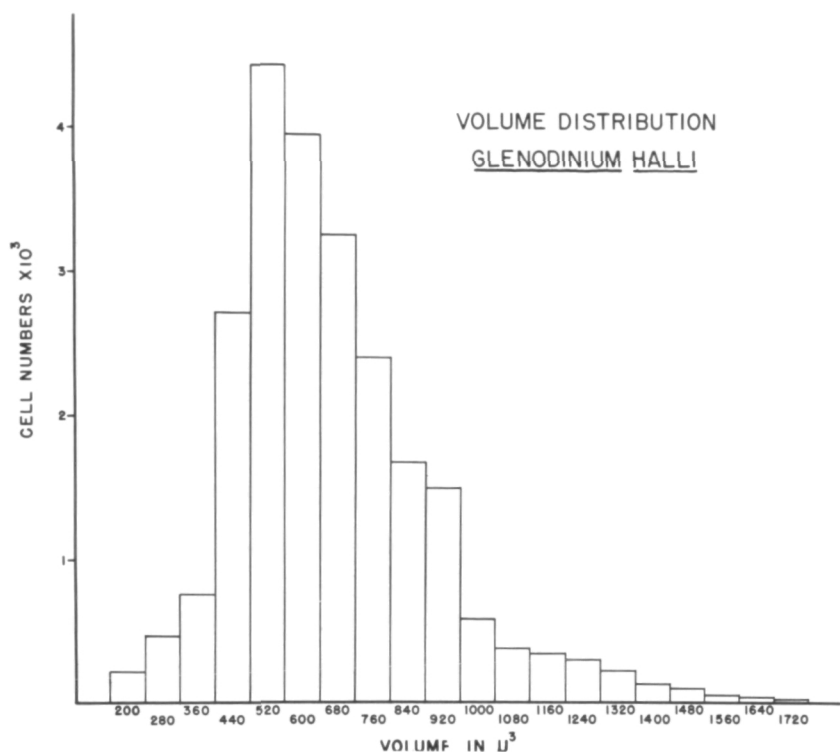


FIG. 25. Volume distribution of *Glénodinium halli* grown in ASPs for 16 days. Volume and cell count recorded with Coulter Counter Model B.

Pyrophacus in culture; Doctor Braarud, are you familiar with the genus?

BRAARUD: Yes.

MCLAUGHLIN: It looks like a Chinese hat, or a space craft—it has a flat bottom, the apical end coming to a point—yet, in its life cycle it has a Gymnodinioid form. Again, when I looked at the cultures I thought I must have picked up two in the same pipette; I re-isolated it and, sure enough after its division stage it grew into a Chinese high hat, to the confusion of old-line taxonomy. The moral is that every culture for taxonomy should be derived from a single-cell isolate. Then, let the taxonomists map these clonal variations, and they will give us some foundation for identifying natural populations.

BRAARUD: You make a very good point in stressing how crude the taxonomy is in these fields, because so many species have been described on the basis of one specimen.

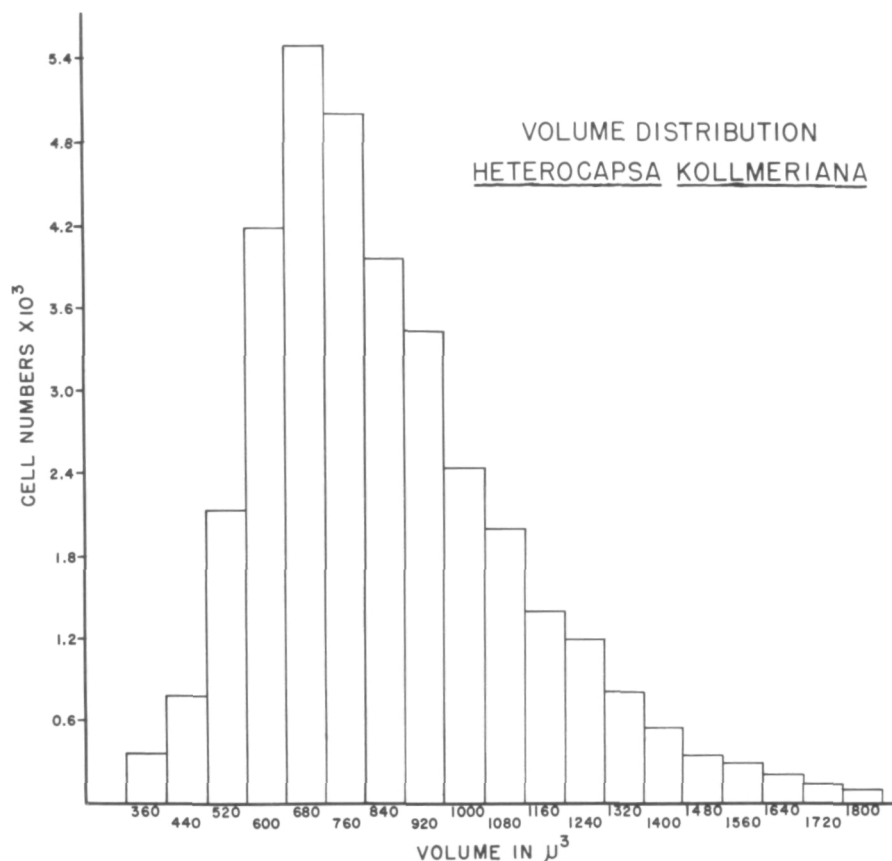


FIG. 26. Volume distribution of *Heterocapsa kollmeriana* grown in ASP₈ for 12 days. Volume and cell count recorded with Coulter Counter Model B.

MCLAUGHLIN: We have our hands full with only one of these crazy cultures.

BRAARUD: But that is the first attempt.

MCLAUGHLIN: Oh, yes.

STEEMANN NIELSEN: To drive a car, one has to have a driver's license; perhaps we should have a license to give names to specimens. (Laughter) Perhaps it would be wise if not everyone were allowed to put names to specimens.

STRICKLAND: Can the rest of us use "sp." when in doubt?

STEEMANN NIELSEN: Yes, that would be much better. I remember that when I worked with the genus *Ceratium* I couldn't use most of the

published works. The work of only a few other specialists who really had been studying *Ceratium* could be used; they had the license.

STRICKLAND: I don't know if this is particularly relevant. We had a problem this year, in a kinetic experiment, of trying to count 80 samples a day of very small flagellates, and we found it extremely difficult. We solved the problem by a method which may not be particularly new but perhaps it might be of some interest. We photographed the flagellates alive in a hemocytometer and, by "freezing" them in this way, we could come back and count at leisure. The photography part, at least, kept pace with our experiment. FIGURE 29 shows *Monochrysis lutheri* photographed on the hemocytometer. Generally six of the nine large squares can be included.

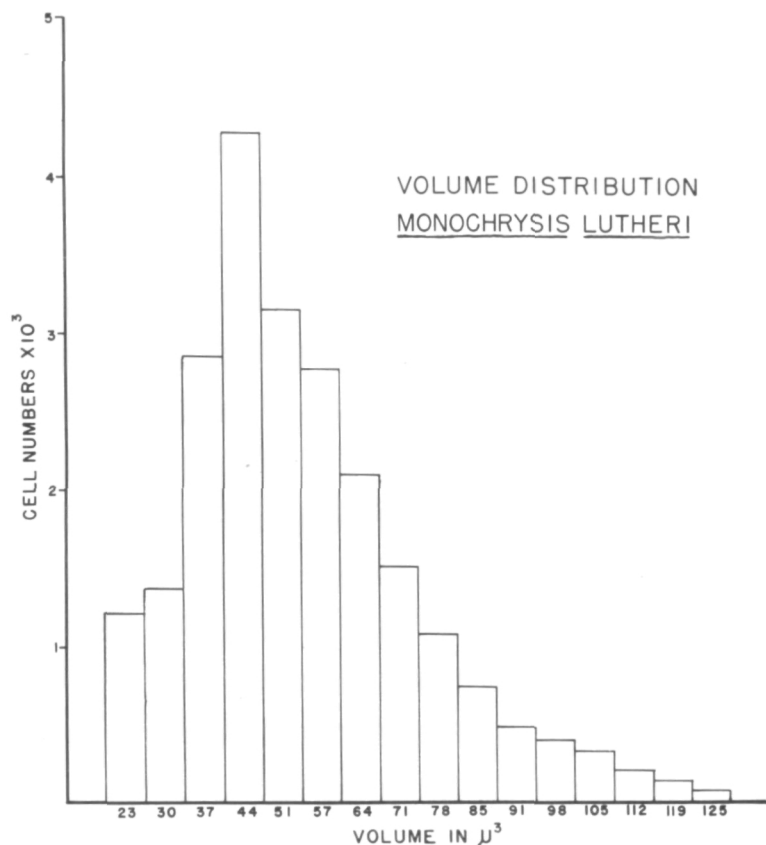


FIG. 27. Volume distribution of *Monochrysis lutheri* grown in ASP₈ for 12 days. Volume and cell count recorded with Coulter Counter Model B.

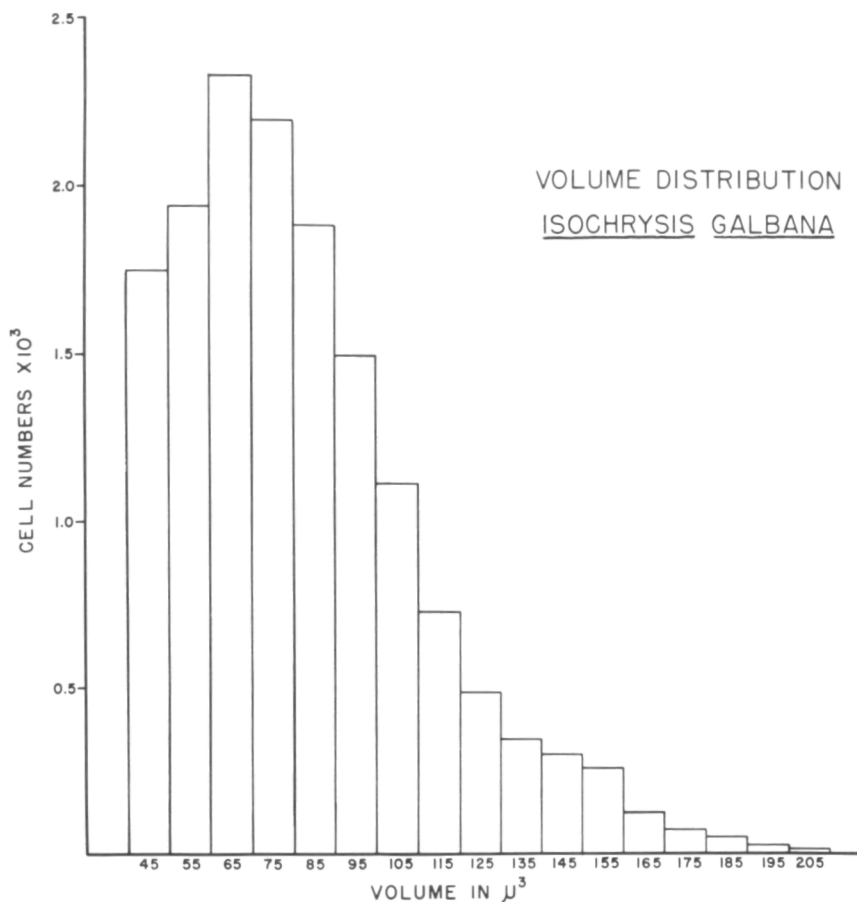


FIG. 28. Volume distribution of *Isochrysis galbana* grown in ASP₈ for 8 days. Volume and cell count recorded with Coulter Counter Model B.

With cells too large for a hemocytometer, we have used a sheet of plastic drilled with a number of small holes which are filled and covered with a cover slip. The cells are killed and allowed to settle.

GUILLARD: What type of film did you use?

STRICKLAND: A fairly fast panchromatic 35 mm film. Six out of the nine squares can be seen on the hemocytometer.

PROVASOLI: Don't you think it is an understatement to say you could count the animals at your leisure with that kind of photograph?

MCLAUGHLIN: How accurate or reproducible are the counts under the hemocytometer?

STRICKLAND: Oh, quite statistical. Considering the cells are only about $3 \times 7 \mu$, I don't think the resolution is too bad. *Skeletonema* is

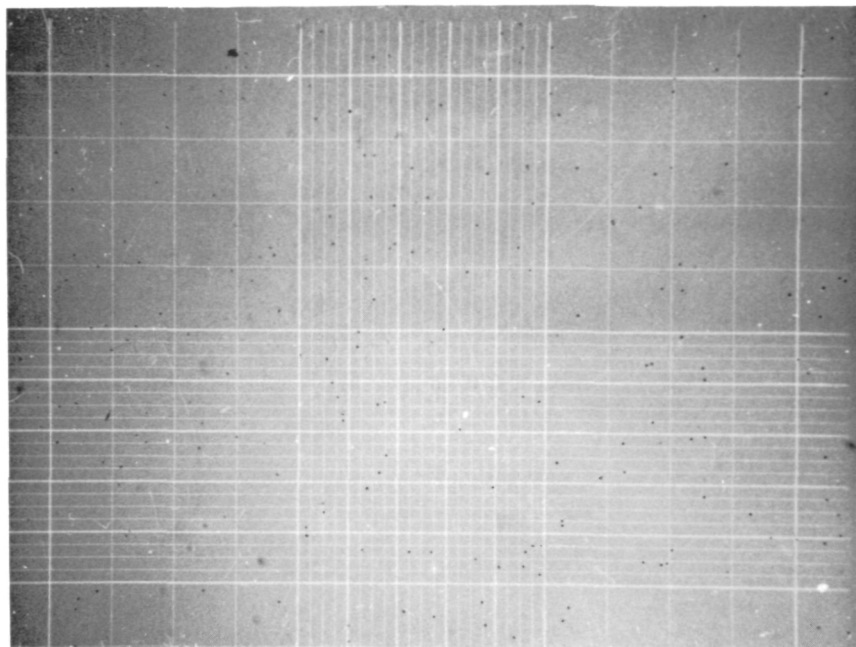


FIG. 29. *Monochrysis lutheri* cells in hemacytometer.

shown in FIGURE 30. The cells are settled at the bottom of a small hole in a sheet of plastic. One projects this negative onto a calibrated screen and, with a ruled grid, can count fairly accurately. With larger cells it is much simpler.

MCLAUGHLIN: I have been using the Model B Coulter Counter for *Monochrysis lutheri*. We find that in the 15 second counts, our replication for counting, and the Coulter Counter is still not very satisfactory hemocytometer.* This type of accuracy has been demonstrated by Mattern *et al.*¹²⁰

STRICKLAND: Yes, but we could only afford to take one drop of solution for counting, and the Coulter counter is still not very satisfactory for a chain-former such as *Skeletonema*.

MCLAUGHLIN: Do you kill your cells before adding them to the hemocytometer?

STRICKLAND: No, the cells in FIGURES 29 and 30 are alive.

PROVASOLI: Did you employ a stroboscope flashlight?

*Some aspects of this work are supported by the U.S. Public Health Service under grants B-1198 and R. G. 7022; National Science Foundation Grant No. 9562.

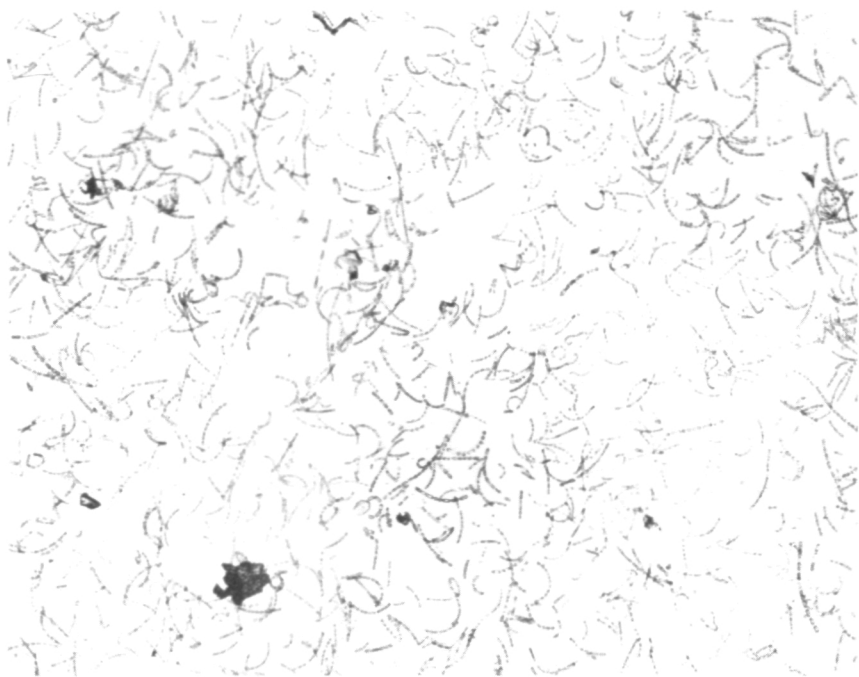


FIG. 30. *Skeletonema costatum* cells in counting cell.

STRICKLAND: No, not really; an exposure of 1/125 second "freezes" the cells in motion.

MCLAUGHLIN: On replicate counts, what do you get—10 per cent or 20 per cent?

STRICKLAND: A good poisson distribution.

MCLAUGHLIN: What are the total cell counts per milliliter?

STRICKLAND: Fairly large. Figure 30 showed about 20 cells/hemocytometer square, or 20×10^4 cells/ml.

MCLAUGHLIN: Couldn't this one drop be diluted into the medium and put through the Coulter Counter?

STRICKLAND: I tried it, but when one starts doing that one gets too low a count for the Coulter.

MCLAUGHLIN: There is at least a 10 per cent error in our hemocytometer counts. When the Coulter Counter is checked against the red blood-cell hemocytometer count, in the Coulter count there is about 90 per cent more accuracy than the hemocytometer count.¹²¹⁻¹²⁴ The Coulter machine cannot be used for *Skeletonema*, but is good for *Monochrysis*, which is analogous to red or white blood cells.

STRICKLAND: I can assure you that our counts are quite easily re-

produced. We are looking for comparative changes of number, so even if they are all 90 per cent of what they should be it is sufficient. The Coulter Counter is not adaptable to a very small sample, Doctor McLaughlin, but can be used with adequate samples of a unicellular organism. But this is not a very useful technique when there is a very small amount of sample or when one is working with some chain forms.

DROOP: Isn't the critical word here, "poisson"; that the more one counts the more nearly a normal distribution is obtained? In the Coulter Counter, very many more cells can be counted. That may be the only difference.

BRAARUD: I think we should return to our original subject. I have Doctor Wood's paper¹¹⁵ on a fluorescence method he proposes to use on the Indian Ocean expedition to count phytoplankton, and I was a little concerned about two details.

The first is that it may be tiresome to count with fluorescent light, and the other is the taxonomic aspect. Doctor Oppenheimer has used this method for studying populations in sediments, and I think it will help if he describes his experience and explains the method for those who don't know it.

OPPENHEIMER: The method is certainly not new. Strugger¹²⁵ described the fluorescent technique and the autofluorescence of chlorophyll quite well. Doctor Wood, who has used the technique for some years, and I were interested in determining the abundance of unicellular phytoplankton in a sedimentary environment containing sand grains and clay.^{126,127} Under ordinary microscopy it is difficult to make such counts. But with the fluorescent technique,³⁷ one can see through sand grains and clay particles. The apparatus consisted of a high-intensity mercury arc lamp filtered for 4000 Å, a quartz condenser, and standard microscope optics. A counting chamber was made with a quartz slide with two # 1 cover glasses cemented on top to form an opening of about 0.5 cm wide and the depth calibrated. A cover glass was placed on the chamber and the living sample introduced at the side. At 4000 Å, chloroplasts in the living cells autofluoresce a bright red.

PROVASOLI: Doctor Wood uses dyes as well, doesn't he?

OPPENHEIMER: In another technique¹²⁵ acridine orange may be used as a dye to distinguish living and dead organisms.

ALLEN: We looked at this a little and found that the autofluorescence of a lot of living organisms is rather low. As you all know, the chlorophyll fluorescence increases about 10,000 times when the organisms are killed.

OPPENHEIMER: That depends on the strength of the light used. In our system, the fluorescence was quite apparent. We made excellent counts of particles to 2μ.

PROVASOLI: Some time ago we used UV fluorescence to follow disappearance of chloroplasts in streptomycin-treated *Euglena*. Our UV source was weak and at first we could barely see any fluorescence, until we thought that the glass prisms of the binocular head diluted the red light to a invisible state. Apparently it did so, because when we replaced it with a monocular tube which decreased the glass path we could see clearly the shape of the chloroplasts which were fluorescing brightly.

ALLEN: The fluorescence of chlorophyll in living cells is rather low. This is not a function of the size of the cell, or of the U.V. supplied by the lamp, since the fluorescence of chlorophyll can be excited by any light absorbed by this molecule or by the other photosynthetic pigments (hence, both blue and red visible light are effective). In living cells, the energy available for chlorophyll fluorescence is only that which is not being used for the normal processes of photosynthesis. When the cells are dead, or photosynthesis is inhibited, these processes do not occur, and fluorescence of chlorophyll increases as much as 2,000-fold. (High intensity UV, or acridine dyes with blue, or UV light, can result in photosensitized killing and, hence, increase fluorescence.)

However, fluorescence of chloroplasts in living cells can be readily observed (as has been reported here) and, by vitally staining with very dilute acridine orange, some very striking effects can be obtained. However, since the acridine dyes are photosensitizers, observations must be made quickly. The change in color of the acridine orange fluorescence corresponding to the death of the cells is noticed on longer observation.

OPPENHEIMER: As mentioned previously the autofluorescence of the living chloroplasts was quite evident. For example, I am circulating a color plate that has been published³⁷ that illustrates the fluorescence of various samples we examined. The rather interesting thing here is to see the fluorescing organisms on both sides of quartz sand grains and accurate counts of attached organisms can be made.

In one plate you can also see fecal pellets containing chlorophyll passing through a Copepod. Aragonite Foraminifera, shown in one picture, fluoresce a light green. The upper right-hand corner of the plate illustrates the reaction mentioned this morning, the degradation of chlorophyll in the blue-green algae, which changes from a bright red fluorescence to a very bright yellow fluorescence after about five minutes of exposure. The other organisms with chloroplasts do not show this same effect. Many objects can be detected with the autofluorescent technique that would normally be completely missed with regular microscopy.

STRICKLAND: Can you tell us where you get the UV lamp?

OPPENHEIMER: We were using the Reichert UV microscope lamp with an Osram HB-200 bulb.

ALLEN: What kind of filter did you use in making the photographs?

OPPENHEIMER: We used Kodachrome II daylight film with 5 min exposure. The UV was filtered with a "Wild" Fluorescence Filter set B.

BRAARUD: I can't quite understand how it is possible to count flagellates that are moving.

OPPENHEIMER: Our work was conducted with a sedimentary environ where movement of the organisms is somewhat retarded.

BRAARUD: But it is supposed to be used for plankton, also.

OPPENHEIMER: The phytoplankton in water must first be concentrated by centrifugation and then counted in a chamber. With such treatment the organisms are not very motile. The centrifugation is done immediately after the samples are collected.

PROVASOLI: In flagellates, I saw the bright fluorescing chloroplasts apparently swimming alone, and I could not see the body. It was really strange and wonderful at the same time.

ALLEN: If an organism is combined with very dilute acridine orange, it looks simply gorgeous.

OPPENHEIMER: It is very difficult to stain inshore material or sediments for fluorescence studies because of the presence of detritus. I have tried several different fluorescent dyes. They all react the same; they will stain carbonate and many other particles which look very similar to microorganisms.

STRICKLAND: I thought the dyes only stained living material.

OPPENHEIMER: That is what the literature would lead one to believe.

ALLEN: The living are stained one color and the dead another color, according to the literature.

OPPENHEIMER: The dyes are efficient only in pure cultures or in clean water, because they also stain carbonates, organic detritus, bits of grasses, and so on.

DROOP: There is no specific test for this?

OPPENHEIMER: No. The only thing we have found to be specific is autofluorescence. We find that chlorophyll does not fluoresce in cells dead for more than a few hours.

ALLEN: May I mention an observation made not on a microscopic one-cell organism, but by people who have measured fluorescence on a larger scale than essentially the photosynthetic organism. They find that more significant than the size, is the fact that often the normal pathway of utilization of the light is blocked; all it can do is fluoresce, and 2,000 times the enhancement of fluorescence has been reported.

MCLAUGHLIN: In Doctor Wood's paper,³⁷ he said that recently dead organisms will give the chlorophyll fluorescence, and that the distinction between living and dead organisms is not always clear when acridine orange is used.

BRAARUD: I am concerned about the taxonomic part of it also. Doctor Wood¹¹⁵ writes that if one has a table of the commonest forms, it is easy to identify them and supplement them with new forms one finds. According to my interpretation of his paper, the plan is for each ship to have equipment and a person who works up material in between stations. According to my experience with Norwegians, it takes quite a long time to teach people taxonomy, but he may have had a different experience. I doubt if the identification will be very valuable if it is going to mean extra work in connection with the counting of different particles. It will be interesting to see the first tables, but I understand that the observations he presented to you at the First Conference¹²⁸ did not include tables.

OPPENHEIMER: The work of Wood and me was conducted immediately after collection at low magnification, but with adequate light all objectives can be used to identify the diatoms by their shape and chloroplast structure. It is often difficult to determine the species, but easy to determine genus. For routine work the 16X objective was used which would allow us to see about a 2μ fluorescing particle. The technique produces a magnification effect, as fluorescence acts somewhat like a photomultiplier. For species or genus identification a 43 or 97X objective must be used. With the high-intensity lamp it was quite easy to use the high-power objectives, and we routinely used Zeiss NA 1.0 40X oil immersion phase contrast, which is an excellent objective that can give a clear 1000X image.

DROOP: Is it possible to switch over to the bright field?

OPPENHEIMER: Yes. Our microscope lamp also had a prism which would allow one to shift from regular light to ultraviolet. Thus, by shifting to bright light, phase microscopy can be used.

ALLEN: I would like to mention something else. One method that is rather widely used for estimating standing crops of phytoplankton, and by which some people try to find out something about the composition of populations, is to estimate the pigments. At the First Conference, I mentioned some doubts I had about existing methods for these determinations, doubts which, I should say, are shared by a number of others. There is currently a good deal of work being done on the improvement of the present standard method, based on the extraction of filtered material with 90 per cent acetone and measurement of the absorption of this material at several wavelengths, then solving simultaneous equations for the amounts of the various pigments present.

However, no matter how much this method may be refined, it is still sensitive to the presence of decomposition products of the pigments found in dead cells and detritus. It would be preferable if one could separate the different chlorophylls and determine each one separately, and a number of chromatographic methods have been developed to do this. However, these methods, like any other methods for the determination of unstable molecules such as chlorophyll, should be applied to fresh material. So, when Doctor Shirley Jeffrey, who has developed one of the chromatographic methods,¹²⁹ came to my laboratory a few months ago we began to see if we could develop a chromatographic method that could be used on a ship. Having liquid sloshing in chromatographic jars at sea may at first sound a little crazy. However, we came across a little device called a Chromatobox, manufactured by Research Specialities Company in Richmond, California. It is made of polypropylene and costs in the neighborhood of \$10. Inside the box is a trough to hold solvent, and devices for holding the paper in place. The sample is spotted on a paper strip—these can be bought already cut—and the strip is rolled up, together with a dimpled Teflon strip, and held in place with a sort of napkin ring. Solvent is added, the lid put on and, with the solvents Doctor Jeffrey uses—in this case 4 per cent n-propanol in light petroleum—in 10 minutes one has a well-developed chromatogram. The device seems to be insensitive to motion.

FIGURE 31 shows two chromatograms developed after shaking 80 times a minute on a shaker. On the right is a synthetic mixture of chlorophylls *a*, *b*, and *c*; on the left, an extract of the brown alga *Egria*. Moreover, the chlorophylls and carotenoids can be eluted from the paper with 80 to 90 per cent efficiency; all that is then needed for their determination is a colorimeter. It would be perfectly feasible to keep up with the sample collection at sea. Determination can be done with a rugged colorimeter that won't go out of order on a ship. Some standards would be required for work with such an instrument; someone should get busy on the preparation of chlorophylls for standards.

KRAUSS: Are you assuming complete extraction?

ALLEN: That is a problem. However, it might be mentioned that extraction can be greatly improved by freezing and thawing the sample before it is extracted.

Another useful point is that samples are usually collected with Millipore filters. Then the standard procedure calls for dissolving the whole filter in 90 per cent acetone. The chromatographic method does not work well in the presence of dissolved Millipore filter. However, a filter of similar characteristics and pore size that does not dissolve in organic solvents has recently been developed by one of the

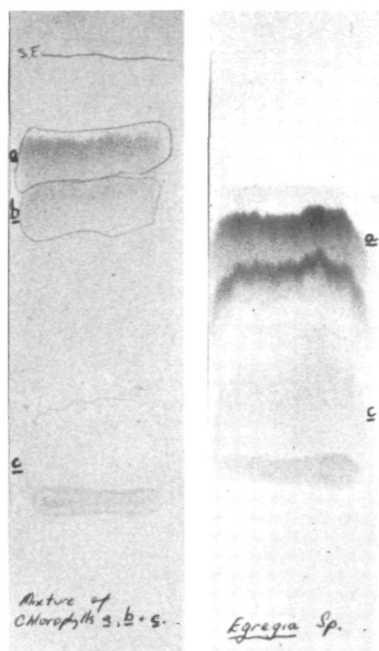


FIG. 31. Algal pigment chromatograms. a. (right) and b. (left).

competitors of Millipore. It is called Polypore. It can be obtained on special order in the same size as the Millipore filter holder, and it works very nicely. It is supplied by the Gelman Corporation, 106 North Main Street, Chelsea, Michigan.

SPENCER: I have a little information which is perhaps relevant here.¹³⁰ It concerns the adverse effects of dispersed Millipore material on the efficiency of 90 per cent aqueous acetone as an extractant for the chlorophylls of the phytoplankton. The data have been collected by comparing the performance of the Creitz and Richards' method¹³¹ with an exactly comparable technique using glass-fiber filter paper. (Whatman GF/C). Regular replicate bi-weekly samples taken from the Menai Straits have been analysed by both methods for about 18 months.

At times, when the phytoplankton population was low, there was usually no significant difference in the results returned by either method except that on a few occasions there was some suggestion of a retention failure by the glass-fiber filters. This occurred particularly when *Nitzschia delicatissima* or *Asterionella japonica* formed a significant proportion by volume of the standing crop. On the other

hand, at one period, when 5 to 10 μ flagellates made up some 90 per cent by volume of the standing crop, the glass-fiber filters showed no evidence of a significant retention failure. During the whole of the spring diatom flowering in the Menai Straits in 1962, however, the Millipore method gave results that were consistently lower than the results returned by the glass-fiber paper.

The variation of retention effect is difficult to imitate with cells grown in culture because it only occurs to any great extent when the cells are in a particular physiological state. It seems to be most marked when a conventional batch culture is within some portion of the phase of decreasing growth rate, and not to occur throughout the rest of the growth cycle. This intrigues me because of its possible implication with respect to the physiological state of the cells in the natural blooms.

GOLDMAN: Was this an AA or HA Millipore filter?

SPENCER: The AA type filter, as recommended by Creitz and Richards.

STRICKLAND: What causes the difference in values.

SPENCER: I don't know. The only suggestion I can make is that the dispersed Millipore material is highly hydrated and is, thus, dehydrating the acetone and so decreasing its efficiency as an extractant.

STRICKLAND: Is pigment being lost through the membrane, do you think?

SPENCER: Oh, no! The effect can be shown on aliquot portions of a culture, the cells being collected by centrifuging and comparing with either 90 per cent acetone alone, or the same extractant which has had a Millipore filter dissolved in it. It is, I believe, generally recognized that a certain amount of water in the acetone increases its effectiveness as an extractant.

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SUMMATION

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FOGG: We started off with Doctor Steemann Nielsen who reminded us that the organic production, which it is fashionable to concentrate on these days, occurs as different species, and these vary both with space and time. He illustrated the main points about the distribution of marine phytoplankton by taking species of the genus *Ceratium* as examples. Then Doctor Lund told us about the distribution of freshwater forms, using *Asterionella* as his chief example.

There seemed to be the paradox that, in the continuous medium of the oceans, species are often localized in particular places, whereas in the discontinuous medium of lakes, the flora seems to be rather cosmopolitan. This arose, I think, from differences of emphasis by the two speakers, for the main features of distribution in marine and freshwater environments, for zooplankton perhaps, as well as phytoplankton, seem to be the same, with temperature standing out as the apparent principal controlling factor, although salinity can sometimes be of over-riding importance. Some of the most widely distributed freshwater forms seem unable to survive desiccation and we know nothing about how they are dispersed: the time-honoured legend, involving their transport on the feet of water-fowl, evidently has no evidence to support it. Perhaps dispersal is so slow as to be perceptible only over geological periods of time. If so, micropalaeontologists are the best people to investigate this.

Although temperature received considerable emphasis as a factor leading to particular species occurring in particular places, it was clear that we still have much to learn about its cause and effect. The temperature requirements of a particular species in nature are not necessarily the same as those which have been found by culture techniques and even, as with *Skeletonema*, different optimum temperatures in culture are found by different workers.

A first possibility here is that different strains are being studied or that differences in media may cause changes of optima. A great deal remains to be done with taxonomy, and even when organisms appear to be morphologically identical it is possible that they are different

physiological strains. The temperature factor in the sea is undoubtedly complex and bound up with hydrographic and nutrient conditions. In addition, temperature effects on the organisms themselves are complicated and dependent on other factors. The temperature coefficients of different metabolic processes must vary considerably, so that, if the temperature is altered the balance of metabolic processes is changed. For example, if the temperature coefficient for nitrogen assimilation is higher than that for carbon assimilation, as we have actually found to be the case with *Anabaena cylindrica*,¹³² then, an increase in temperature, will cause the nitrogen content of the organism to go up, given an ample supply of nitrogen. On the other hand, if nitrogen is in short supply, nitrogen deficiency will be apparent more rapidly at the higher temperature and growth will be retarded.

This seems to be what Doctor Jones observed with *Carteria*. Thus, it doesn't seem to me that the optimum temperature for cell division is necessarily that which gives the balance of metabolic processes which will lead to the organism's successful growth in competition with other species in nature. Altered metabolic balance as a result of temperature changes could lead to more dense cells, for example, which, as Doctor Oppenheimer pointed out, might effectively eliminate a species even if its cell division rate were at the same time enhanced. It is possible that a change in temperature, altering the properties of the cell in such a way, might be compensated by variation of other factors such as light intensity and nutrient conditions. Looking at it in another way, effects of temperature, as Doctor Guillard pointed out, may also depend on the previous history and physiological condition of the cells. Clearly, one is dealing with a complex of factors and can't consider the effect of temperature per se.

Doctor Braarud returned to the question of the distribution of species by asking what the essential difference was between coastal and oceanic plankton forms. The discussion of this led to consideration of flotation mechanisms, since it seems that a successful oceanic species must of necessity possess an efficient means of keeping afloat. Several different methods of doing this are available to plankton, and the particular mechanism varies according to species. Some diatoms evidently regulate density selectively accumulating the lighter ions in their vacuoles, but it appears that this mechanism doesn't operate in all diatoms and it seems to be altogether out of the question for diatoms growing in oligotrophic freshwaters.

Production of fat was mentioned as a means of flotation but from my own experiments¹³³ I am inclined to think that the appearance of large quantities of fat in diatoms is an indication of breakdown in cell organization. I am not sure, even, that it has ever been shown that a diatom which has accumulated large quantities of fat is viable. Fur-

thermore, the view was expressed that, because of other concomitant changes, diatoms which have accumulated fats actually have a higher specific gravity than normal cells. It is indisputable that the freshwater plankton form, *Botryococcus*, floats by virtue of the large quantity of lipid material which it contains. But this genus is odd in other ways, and there otherwise seems to be precious little evidence for fat production being a generally effective means of flotation.

Another mechanism of flotation, the production of gas bubbles within the protoplasm, seems limited to a very small number of plankton forms. It undoubtedly occurs in many of the blue-green algae¹³⁴ but otherwise seems to occur only in a few protozoa and bacteria.

Mucilage production is another possible means of lowering mean specific gravity but has the disadvantage of restricting turbulence in the vicinity of the cells. Thus, flotation is bound up with nutrient supply. Discussion of this led to the subject of turbulence patterns in general, and here it became clear that we know very little about small-scale water movements around cells and this obviously may have considerable effects on the supply of nutrients.

Doctor Hutchinson pointed out that we know nothing about the thermal expansion of protoplasm and that it is conceivable that abrupt changes in specific gravity may occur with changes in temperature. This is something which might be of great ecological importance.

The question of the energy relations involved in alterations in specific gravity was raised. As very little information is available on the subject, we got rather at cross-purposes in the discussion and I think that perhaps it is not a very timely question to pursue; lowering of specific gravity by fat accumulation would involve energy consumption, but if it were achieved by formation of gas bubbles, these might easily be produced by exergonic reactions.

Before leaving the subject of the buoyancy of plankton and its ecological significance I should remind you of the excellent discussion of this by Doctor Lund.¹³⁵

A short consideration of the relative abundance of size groups, taxonomic groups and ecological groups followed. Doctor Steemann Nielsen made the generalization that in the sea one finds that smaller species predominate under oligotrophic conditions, which is what one would expect, having regard to the fact that nutrient uptake efficiency is dependent on surface/volume ratio. In freshwater one finds the same tendency, but desmids provide a notable exception and bring us back to the realization that we know all too little about movements of materials in the immediate vicinity of cell surfaces and how elaboration of form may be significant. On the question of whether major groups of phytoplankton algae have predilections of particular conditions, Doctor Braarud emphasized that the evidence which we have at

present may not be very good. Nets are inadequate sampling devices. Many forms of phytoplankton are not preservable and some only become apparent in enrichment cultures.

Then, in a discussion of the effects of light, we started off with Doctor Halldal summarizing recent work on phototactic responses. He pointed out that there are probably three mechanisms involved, with different action spectra; the one for orientation or direction having its maximum in the blue; the one which determines the optimal light intensity in which organisms congregate, having an action spectrum resembling that of photosynthesis; and the third system, which is yellow sensitive, antagonizing the effect of the photosynthetic one.

We passed on to consideration of the effects of ultraviolet light. There are many apparently conflicting results but it soon became evident that different people were talking about different things. Different experimental organisms were being used, as well as different wavelengths of ultraviolet and different experimental conditions, including not very obvious things such as inorganic and organic constituents of the medium which may selectively absorb ultraviolet and undergo photochemical reactions.

Turning to the effects of visible light, Doctor Krauss reminded us, drawing his examples from among the species of *Chlorella*, how various the reactions of an alga to light and darkness may be. Doctor Jones showed how, to complicate matters still further, the effect of light intensity on a single species is highly dependent on nutrient conditions. Again, there was emphasis that it is not sufficient just to study the effect of varying one factor, that others must always be taken into account. Doctor Myers then gave us a much-needed homily on the subject of growth and photosynthesis in relation to light intensity. He stressed the importance of the physiological history of cells and showed us how curves for growth and photosynthesis, although superficially alike, may represent quite different things, because the former is determined with algal material adapted to the various intensities used, whereas the latter is determined with samples from a single population of cells adapted to the one particular intensity under which it was grown. This explains the apparent anomaly that a cell can photosynthesize for a short period at a rate much greater than that at which it can grow.

There was some discussion of chromatic adaptation, which, on the phenotypic level, seems to be confined to the blue-green and red algae and not of great importance in planktonic forms. On the genotypic level, of course, the pigmentation of all the major groups of marine phytoplankton is adapted to the wavelengths which predominate in the surface waters of the oceans. The question was asked, whether in those algae which do show phenotypic chromatic adaptation, a pre-

cursor can be switched to chlorophyll or phycobilin synthesis by illumination with light of an appropriate wavelength. While this particular thing has not, as far as I know, been demonstrated, Fujita and Hattori¹³⁶ have shown that *Tolypothrix tenuis* forms phycocyanin or phycoerythrin from a common precursor, according to whether it is illuminated with red or green light. Doctor Allen described the recent finding that, for effective utilization of light in photosynthesis, not one but two, or possibly three, light reactions having different wavelength requirements are necessary. She made the comment that this doesn't have much to do with ecology, but the physiologists have still a lot to learn about these wavelength effects.

Doctor Strickland emphasized once again the need for standardization in measurement of light intensity and wavelength distribution in studies of the effects of light on plankton and described his attempts to produce an artificial source simulating natural light in the sea. It is certainly most desirable that there should be some degree of standardization, but I fear he has chosen a hard row to hoe. There is need for standardization in the handling of plankton for productivity determinations, too. Doctor Goldman drew our attention to the drastic effects which even brief exposure to bright surface light may have on the rate of photosynthesis of algae which have been growing in subdued light. This possibility, which follows on from what Doctor Myers was saying, is often overlooked in field work. Determinations of productivity of the radiocarbon method should be started at dawn or dusk unless the samples can be taken and the tracer added automatically *in situ* at the depths at which measurements are to be made.

Doctor Braarud reminded us that, besides laboratory experiments, we need considerably more studies on the light factor in field ecology. This brought us back to phototaxis again. It was pointed out how this can lead to collection of dinoflagellates, in particular, at a well-defined depth in the water, and from this we went on to a general discussion of tactic phenomena and moved rather away from the effects of light. Our ignorance of the effects of factors such as pressure on the activities of phytoplankton is very apparent.

In all this discussion of the effects of light, I missed any mention of the possible effects of alternation of light and dark periods. There is some indication that photoperiodic effects do occur in algae, Pirson,¹³⁷ for example, has demonstrated something of this sort in *Hydrodictyon*. I think this possibility is something we ought to bear in mind far more than we do; there are certainly photoperiodic effects on metabolism and day-length may be important in determining distribution, as it is for flowering plants.

Finally, we came to a consideration of the nutrition of phytoplankton, concentrating almost exclusively on the role of vitamins, particu-

larly B₁₂. I am sure that this was not because no problems relating to mineral nutrition remain, rather it reflects a relative paucity of inspiration in this field at present. Doctor Provasoli put before us a valuable table showing the distribution of vitamin requirements among the algae. The question came up of how representative such tables are and Doctor Droop confessed to selecting vitamin-requiring species for his studies. Many people, such as myself, on the other hand, avoid anything that seems to be exacting, so perhaps on balance we have a reasonably accurate idea of the proportions of vitamin-requiring species in the various groups. Determination of vitamin requirements is very much dependent on experiments with pure cultures, and workers in this field are particularly troubled by the impossibility of proving a negative, and the thought that their so-called pure cultures may have bacteria in them which do not respond to the media used for testing. We need to know much more about the relations between algae and bacteria. It may be that, in nature, phytoplankton takes its vitamin B₁₂ directly from epiphytic bacteria, so that discussion of the relation between productivity and the concentration of the vitamin in the bulk of the water is irrelevant. The great differences observed by Doctor Riley between the concentrations of vitamin B₁₂ necessary for the growth of *Skeletonema* in batch and continuous cultures, point to the need for caution in extrapolating from laboratory cultures to the natural environment and underline, once again, our ignorance of the effects of the microenvironment of the cells in determining the supply of nutrients. The effect of additions of vitamin B₁₂, reported both by Doctor McLaughlin and by Doctor Goldman, in immediately enhancing uptake of C¹⁴ from bicarbonate, is most interesting for little is known of the metabolic role of this vitamin, and I am not aware that there has ever been any suggestion that it is implicated in photosynthesis. Doctor Guillard seemed to me to sum up our uncertainty regarding the ecological importance of vitamin B₁₂ in the question of why algae which do not require an exogenous supply of this factor do not dominate the oceans.

II. SYNECOLOGICAL PROBLEMS

SEASONAL CYCLES AND SUCCESSION OF SOCIETIES

Discussion leader:

G. A. RILEY

*Bingham Oceanographic Laboratory
Yale University
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RILEY: In most oceanic waters, from the subtropics to the polar regions, we observe a phenomenon known as the seasonal succession of phytoplankton. As the season progresses, one or a few species of phytoplankton will be dominant at a particular time and then will be replaced by other species. This goes on throughout the year, and the cycles repeat more or less from one year to the next.

There are a number of questions we can ask about seasonal succession, and to some of these questions, hopefully, we will find answers today.

One question that is commonly asked is: How is it possible for a particular species to appear seemingly out of nowhere, and how does it survive during the rest of the year when seemingly it does not exist? We have already discussed this question and it is apparent that in some coastal waters there are resting spores that permit survival. We also know that species often do exist in the water in an unrecognizable but viable state and, further, that often our apparent failure to find a species is simply that we do not look for it quite as thoroughly as we might. It has been my experience, working on Long Island Sound, that of over half the species which are really important dominants can be found throughout the year in some sample or other if one searches for them thoroughly enough. Most of these species have rather broad tolerances and can survive through most of the season, although there may be one or two times during the year when they are really dominant.

Often, too, species that are present on a less continuous basis are likely to be subarctic or subtropical forms which are more continuously present elsewhere and are occasionally brought into an area and

achieve limited dominance during a shorter season when conditions happen to be favorable.

Then there are questions of how the growth rates of individual species vary with environmental conditions, and how these variations bear upon the problem of seasonal succession. We have to consider a large array of environmental conditions, of course. Some conditions, presumably, are relatively simple, involving only the physiological responses of the cells to changing conditions of light, temperature, salinity, or inorganic nutrients. But a full discussion of seasonal succession undoubtedly involves more complicated problems of auxotrophy and the stimulation or inhibition of one species by another through the production of external metabolites.

I hope we may discuss seasonal succession from several different viewpoints: Perhaps as descriptive aspects of seasonal succession in the sea; also, by analysis of our observations to determine what bearing they may have upon the question of how environmental factors affect seasonal succession; and, finally, as to the importance of data from our laboratory experiments with pure cultures upon the problem?

I know that there are a number of people here who have worked and thought about these problems, and I would like to hear from some of them. Doctor Braarud, would you like to comment?

BRAARUD: Yes, I would like to supplement what Doctor Riley said by pointing out the very interesting, and certainly very important, approach to the study of changes in populations which he has made through what we would call the dynamic ecological approach, whereby he has also considered the effect of zooplankton grazing, etc. This is, doubtless, a very important feature in the annual cycle of phytoplankton in any water mass. This approach also demonstrates that there are really two features of importance. One is the need for collaboration between the various fields of oceanography in order to understand the details of the whole picture; the other is that in order to really exploit such a method as Doctor Riley has used, it is necessary to obtain much more basic information on productive rates, grazing, selective grazing, and so on. So, again, we come back to a need for a combination of laboratory work and field work.

In Norway we have been unable to start such an ambitious project as Doctor Riley's, because we have only studied populations of phytoplankton and haven't had such good collaboration as to the zooplankton. Thus, the conclusions that can be drawn are much more limited. But, even so, the problems related to the autecology of each species can clearly be seen. One comes back to the question of the reaction of each species to the environment, whether it is related to physical changes of the environment or to changes produced by the organisms, whether phytoplankton, bacteria or zooplankton.

If we are to obtain an insight into succession, we must gather more experimental data; the discussions of these past few days have demonstrated clearly that one single factor is not decisive, but, rather, that there has to be a correlation between the effects of various factors.

I don't know of any good example of succession that can be traced back to a single factor. In some cases it seems to be temperature, but we have too little information to really draw any conclusion. So I feel that the problem of succession represents a synthesis of practically all the other problems one might raise about phytoplankton culture.

I would also like to point to the extreme cases of succession where one gets a predominance of one species. Such is the case of blooms of diatoms, coccolithophorids, or red-tide organisms among the dinoflagellates. The latter are being studied closely and may reveal some of the mechanisms in the competition between the species. It may not be at all as simple as we have thought. The red-tide problem, which occurs so regularly, may be the best example, and Margalef's studies,¹³⁸ where he has applied information theory on the description of succession in Vigo bay, is a very interesting approach. It may bring out the problems better than other approaches, and it would be interesting to apply it to oceanic conditions.

There is one more point I would like to make. In order to make further advances in the field, it seems desirable to obtain many more observations from offshore waters, from weather ships, for instance, where studies are made throughout the year. They should include zooplankton observations.

JOHNSTON: I think Doctor Braarud has described the difficulty of coping with two situations that change simultaneously. Seawater is changing throughout the year, and so is the plankton composition. It is possible to try to look at these separately. One organism grown in seawater samples taken at different times in the year, may give a measure of the variability of the seawater. Different types of phytoplankton grown in seawater samples will give a measure of the suitability for the different types.

I have attempted this kind of experiment, but it is very difficult because it is hard to set up adequate controls. I have tried to grow *Skeletonema* all the year round in different seawaters and, in a very broad way, I think *Skeletonema* betrays the usual pattern of succession in the water. Growth is poor in wintertime, good during the spring, falls off greatly in the summer, and returns to fairly good in the autumn.

STRICKLAND: Was this done at normal sea temperature or at one temperature?

JOHNSTON: It was all done at one temperature, and with enriched seawater. There is no possibility of phosphate, nitrate, silicate, trace metals or chelation affecting the experiments. The variable is something other than these.

STEEMANN NIELSEN: Did you use the same light intensity?

JOHNSTON: Yes. It was done in the shore laboratory.

BRAARUD: If we who study natural phytoplankton had one species only, this method would be excellent. But, since there is such a varied spectrum of ecological types in the sea, expressed by the succession of species, I think that while your approach, Doctor Johnston, may give some very interesting information it cannot tell the whole story.

JOHNSTON: No, but the two variables—the seawater variable and the changes in phytoplankton population—are being separated.

FREMONT-SMITH: Is it possible that there is some kind of biological clock that regulates a kind of seasonal change?

JOHNSTON: I don't think it is a clock mechanism, because we have eliminated variables such as light. I give the organisms 12 hours of light and 12 hours of darkness; always the same light.

FREMONT-SMITH: But the clock doesn't have to be light-dependent; there are a good many other clocks than light.

JOHNSTON: A dinoflagellate such as *Peridinium trochoideum* does not show very much variation throughout the year. It seems to grow much the same way regardless of the kind of water. But if a diatom such as *Skeletonema* is used, it shows differences in each season.

PROVASOLI: I think Doctor Johnston's approach is very valuable despite its limitations. Its merit is that it determines the biological value of waters for single species of algae, and, therefore, establishes for which organisms the waters are bad or good. These two terms often have been employed indiscriminately and without qualification. This is unwarranted because, as we discussed earlier, no environment is so completely inimical as to not include life of some sort. We cannot call water bad or good without further qualification merely because it is less productive for the species we seek.

True, as Doctor Braarud said, the method determines the biological potential of waters by the growth response of a single species, while succession is a phenomenon involving many interacting species. Because of this complexity, we need to dissect out as many variables as possible before attempting more complex experiments.

Since water quality changes continuously during the growth of the various species involved in a succession, we need to test, seasonally and spatially, the biological quality and potential of the water masses to determine these changes. This is what Doctor Johnston tried. Essentially, each sample of water was enriched with all the nutrients so far known to be essential for phytoplankton growth; that is, vitamins,

trace metals, silica, phosphorus, and nitrates. Since the quality of the water is tested in the presence of abundant nutrients, any depression or stimulation of growth cannot be attributed to deficiencies in nutrients; it should be caused by inhibitory or growth-promoting substances, probably produced by the previous population.

JOHNSTON: That is so, and the seasonal effect can be demonstrated in another way. We know that open sea water has its spring diatom peak several weeks or even one or two months later than inshore waters. By drawing waters from different areas at one time it will be found that the growth of *Skeletonema* depends also on where the water comes from, and that growth is related to the previous history of the water.

STRICKLAND: Can *Skeletonema* be grown in open water?

JOHNSTON: *Skeletonema* grows excellently in open water.

STRICKLAND: Is it ever found in open ocean water?

JOHNSTON: Yes, but it has never been dominant among the phytoplankton. It comes up if enrichment cultures of open ocean water are made, but it does not grow as a dominant organism in the phytoplankton.

STRICKLAND: Have you any comment on that?

JOHNSTON: The general comment is that, so far, all my enrichments bring the water to equal a state of early spring. If a great many nutrients are added to water that is loaded with *Ceratia* and *Rhizosolenia*, the spring forms, which haven't been seen at all, come up and grow in abundance.

It is a very complex situation to interpret. Other variables such as cell size, rate of multiplication, and so on, are also involved. I don't think *Ceratia* and *Rhizosolenia* are killed off. I think the faster-growing forms like *Skeletonema* are encouraged and, in the relatively short duration of the cultures, they completely swamp the larger, slow-growing *Ceratia* and *Rhizosolenia*.

PROVASOLI: In this type of experiment, seawater containing its living population of phytoplankton was enriched. I have conducted experiments for the bioassay of filtered samples of seawater with bacteria-free cultures of *Skeletonema*. In my experiments, some samples of seawater were found to repress and others to enhance the growth of *Skeletonema*, even though all the samples were equally enriched, showing a wide variability in biological potential of the waters.

JOHNSTON: And a special one, as well, linked with the seasonal change.

GUILLARD: Doctor Johnston, do you use the growth rate or the crop of *Skeletonema*, as the measure of the suitability of the particular seawater?

JOHNSTON: I measure the final crop afterwards. With bacteria-free *Skeletonema* I think it takes about five or six days; with unialgal *Skeletonema* or unialgal *Peridinium*, about eight to 10 days; so it is a combination of both.

FOGG: Is the inoculum heavy or light? The amount of inoculum may make a considerable difference.

JOHNSTON: The inoculum is somewhere around 5,000 to 20,000 cells/10 ml of seawater.

BRAARUD: When you say off-shore water is used, what does that mean?

JOHNSTON: Off-shore means anything from two hours' to two days' steaming from Aberdeen in many directions—as far away as the Faeroes Islands, or even Iceland.

MCLAUGHLIN: How is the water prepared?

JOHNSTON: It is filtered through ordinary filter paper to take off the phytoplankton and any zooplankton that may have been caught, because one zooplankton organism in a 10 ml sample wreaks havoc. Any possible bacterial supplement to the culture as might result from the death of the plankton must be removed.

PROVASOLI: Don't you filter with Millipore?

JOHNSTON: No. I am only concerned with removing any possible gross organic supplements.

PROVASOLI: Leaving all the very small flagellates, if they are present?

JOHNSTON: Their mass will be very small.

STRICKLAND: But when they are autoclaved a growth factor might result.

JOHNSTON: They are autoclaved so they don't grow.

PROVASOLI: Yes, the quality of the seawater is being altered because it is being enriched with living organisms that have been cooked.

JOHNSTON: I imagine the mass of a few flagellates is very small, is it not?

PROVASOLI: I don't know, but it is a variable that has to be taken into consideration.

DROOP: Natural biologically-active substances are usually of unknown potency, so I think everything possible needs to be removed.

OPPENHEIMER: I would like to bring up a problem in marine microbiology that might be of interest. The adverse effect of raw seawater on the growth of some terrestrial organisms, especially *Escherichia coli*, is probably well known. Unaltered fresh seawater will kill *E. coli*. If the water is filtered by bacteriological filters or steam-sterilized, something unknown is removed which, apparently, is the lethal material. The *E. coli* will grow quite well in either filtered or heat-sterilized sea-

water. It is quite possible that filtration or heat sterilization may also affect growth-promoting substances. As Doctor Droop says, traces of biologically-active compounds may be most important to growth studies and, therefore, all precautions should be taken to remove them or, if natural sea water is required, to preserve them as much as possible. Saz¹³⁹ recently reported the presence of trace organic materials from the sea which are antibacterial, and Jones¹⁴⁰ has found that part of the inhibitory effects of *E. coli* are caused by heavy metals.

JOHNSTON: I think I partially misled you. Doctor McLaughlin asked me what kind of filter paper I used. It was 542 filter paper, which is a very, very fine grade. It won't take out all the small organisms, but it goes down to a mean pore size of 0.4 to 1.1 μ . It will remove barium sulphate.

STRICKLAND: What happens to vitamins in seawater when it is sterilized. The loss of vitamin B₁₂ in ordinary water is appreciable with each sterilization. Has anybody actually measured the decrease of B₁₂ every time a sample of seawater is cooked for 15 minutes?

DROOP: I believe they have done so, at Shinfield (National Institute for Research in Dairying, Shinfield, Reading, England), but I don't know what the results are. I think there are far more important things about sterilization in relation to *Skeletonema* than the mere loss of certain percentages of vitamins.

STRICKLAND: The loss might be quite appreciable, might it not?

DROOP: It may be.

GUILLARD: I think Doctor Elizabeth Wood at the Bingham Oceanographic Laboratory, Yale University, may have experimented with B₁₂. The amount lost is probably measurable, but not large, probably not 50 per cent.

STRICKLAND: That is quite a lot when one is dealing with a threshold amount of B₁₂.

DROOP: But B₁₂ is added after autoclaving.

JOHNSTON: FIGURE 32 shows the sort of pattern obtained in my first field survey in July and August, 1956. It can be seen that, by and large, as one goes into oceanic waters better growth is obtained.

FIGURE 33 gives rather the same sort of picture, taken one month later. Growth increases from less than 40 cells/mm³ coastal water off the Scottish coast and the Faroe plateau to good growth in the oceanic waters. The complicated situation around Iceland relates quite well to the thrust of oceanic water around both the southeast and the southwest of Iceland. Both FIGURES 32 and 33 show considerable detailed relationships with indicator zooplankton, salinity, and, incidentally, with the natural phosphate and oxygen contents to some extent.

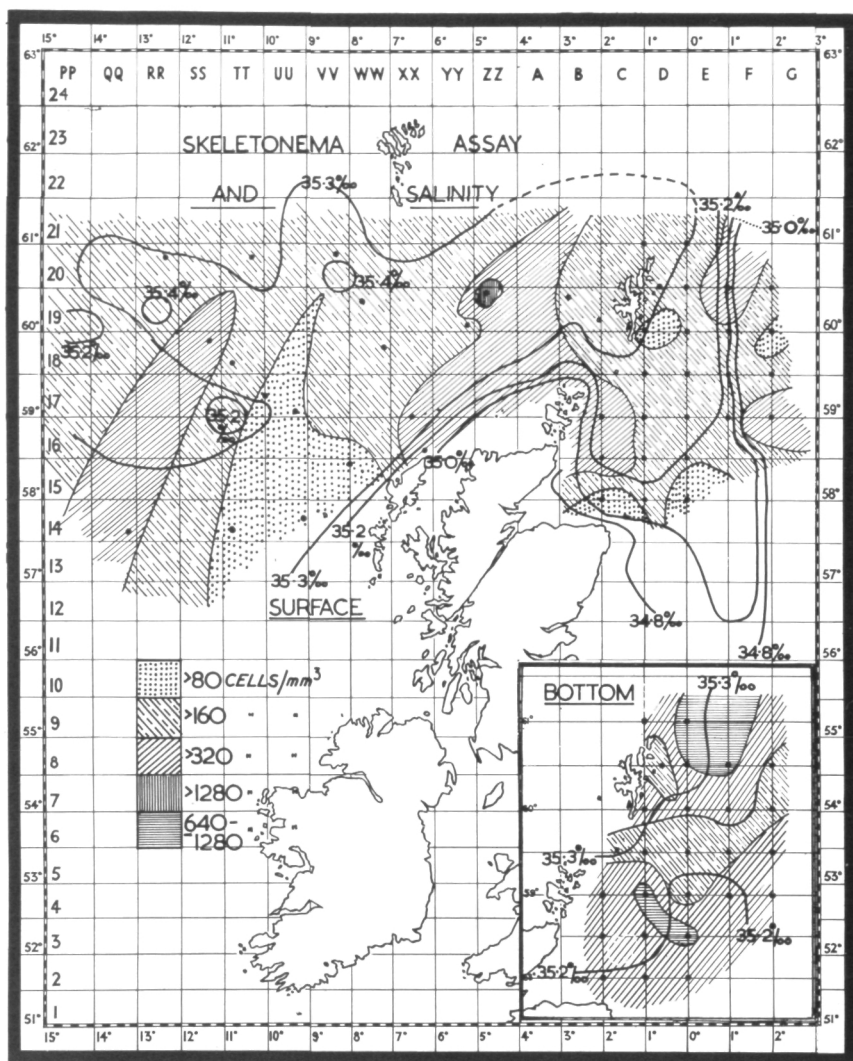


FIG 32. Unialgal *Skeletonema* assay. N. North Sea—Faroe Is. July-August 1956. Surface samples. (Fig. 2. Johnston, 1963a.¹⁴¹ Permission, Journal Mar. Biol. Assoc, United Kingdom).

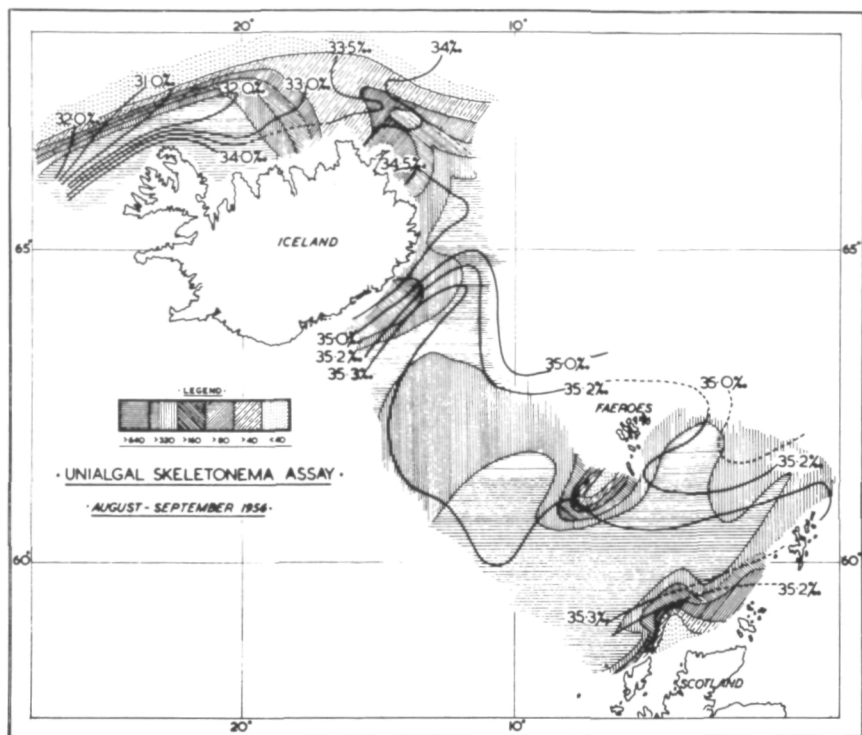


FIG. 33. Unialgal *Skeletonema* assay. N. North Sea—Iceland. August-September 1956. 10m samples. (Fig. 3 Johnston, 1963a.¹⁴¹ Permission, Journal Mar. Biol. Assoc., United Kingdom).

Natural nutrient contents, however, play no part in the assay because the amounts of added nutrients would swamp those originally present.

BRAARUD: What was the total population in these waters?

JOHNSTON: There is no apparent correlation between my assay result and dominant phytoplankton species. I have, however, built up what I believe is a convincing correspondence of the pattern of phytoplankton abundance with that of *Skeletonema* growth. FIGURE 34, showing phytoplankton abundance in the same area, is based on Standard net hauls by the "Explorer." The data is supplied by the Fisheries Laboratory at Iceland, and from Hardy Plankton Recorder data supplied by the Scottish Marine Biology Association Oceanographic Laboratory in Edinburgh.

STRICKLAND: Does FIGURE 33 indicate that in winter, the open ocean water sustains *Skeletonema*?

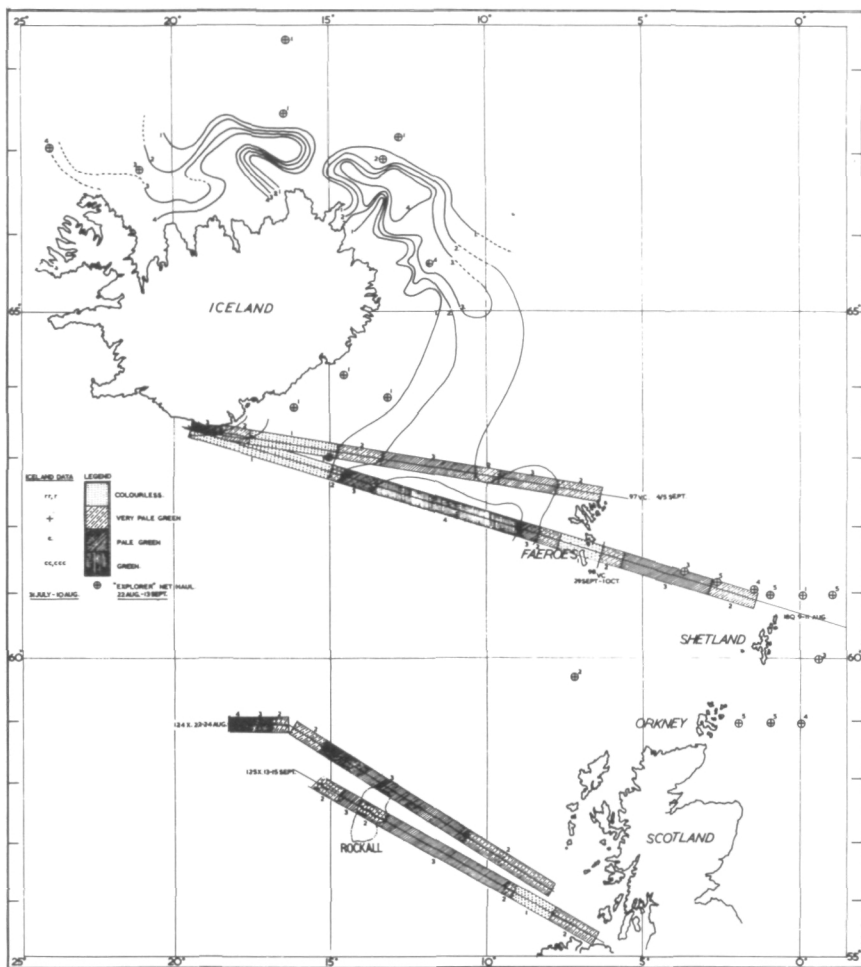


FIG. 34. Composite chart of phytoplankton abundance based on "Explorer" Standard net hauls, data from the Iceland Fisheries Laboratory (31/7/56 to 10/8/56) and Plankton Recorder data from the Oceanographic Laboratory, Edinburgh. (Fig. 5. Johnston, 1963a) (Ref. 141. Permission, Journal Mar. Biol. Assoc. United Kingdom).

JOHNSTON: No, it is not as simple as that.

STRICKLAND: Does it mean that in summer the inshore water contains something which appears to antagonize the growth of *Skeletonema*?

JOHNSTON: That is roughly right, yes. If the work had been done in spring it would have been a different story. Inshore water would be found to give much better growth than offshore water.

MCLAUGHLIN: Am I right in saying that in the spring you took water in which *Skeletonema* will grow, and followed this water mass out so that, later on in the year, it can be picked up out in the open ocean and still identified?

JOHNSTON: No, that cannot be done. I have grown *Skeletonema* in inshore and offshore waters before the spring bloom, and have found the water to be poor. It won't grow *Skeletonema* very well. Once the spring bloom starts, sea water becomes very patchy with very intense peaking in the assays. Enormous growth of *Skeletonema* is found in favorable patches, reaching three or four times higher culture densities than the best in these summer pictures, and one mile from that spot in the sea there may be very little growth. I have some phytoplankton data which shows that in spring the biggest amounts of phytoplankton are obtained in Standard net hauls, as well as the biggest variety of species between stations, and the biggest proportion of "negligible" for net hauls. There are blank stations and rich stations to a greater extent in spring than at any other time of the year.

The occurrence of phytoplankton shown in TABLE 5 pretty well explains itself. The peak occurrences of "negligible" phytoplankton, that is, net hauls yielding less than one recognizable full cell per routine subsample, are found in the spring and in the autumn. Seemingly, while some areas are in full growth, others nearby are quite barren.

GUILLARD: The evidence seems consistent with the hypothesis that there is a different amount of inhibition at different times or places, rather than any enhancement of growth. From studies of cultures with artificial seawater and with "good" natural seawater, we have an idea of the final crop that should be attained with the usual nutrient additions. I noticed that the maximum crops you observed were of this magnitude, the others were lower.

DROOP: What order was that?

GUILLARD: About 1 million cells/ml.

JOHNSTON: That is right, 1,000 cells/mm³.

DROOP: Many times that amount can be obtained in a good culture.

JOHNSTON: In the assay, I do not aim for the ultimate in growth enhancement by adding a very elaborate nutrient mixture. One might go too far and find no differences. I add the usual simple nutrients and harvest all at the same time, often before optimum growth. The suggestion that I am measuring inhibition isn't true because if seawater is added to a good artificial medium it won't invariably produce inhibition. In fact, if different kinds of seawaters are added, most of them will produce an improvement in growth.

GUILLARD: In effect, then, growth rate is being measured.

DROOP: A bit of lag, a bit of growth rate, a bit of yield.

TABLE 5
OCCURRENCE OF PHYTOPLANKTON
(Percentage of hauls in which the group is dominant)

A. Scottish coastal belt (10 to 60 miles offshore).
B. Fladen ground.
C. Fair Isle.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
<i>A.</i>												
No. of samples	1	10	27	14	28	17	10	41	15	17	19	7
Negligible	✓	0	0	28	4	0	0	0	12.5	0	16.7	0
Diatoms	(100)	100	100	92.7	58.0	24.5	13.3	0	33.4	18.7	49.0	21.7
Rhizosolenia	✓	0	0	4.2	10.2	7.8	19.0	62.4	12.6	45.8	27.7	0
Dinoflagellates	✓	0	0	3.1	27.8	67.7	67.7	37.6	41.5	35.5	6.6	58.3
<i>B.</i>												
No. of samples	1	4	15	17	8	9	13	13	7	3	2	2
Negligible	✓	0	11.7	14.1	16.7	16.7	0	0	0	0	0	0
Diatoms	(100)	75.0	84.4	51.4	55.4	38.3	0	0	0	33.3	41.7	50
Rhizosolenia	✓	0	0	10.7	22.2	28.3	24.8	75.0	33.3	33.3	0	0
Dinoflagellates	✓	25	3.9	23.8	5.7	16.7	75.2	25.0	66.7	33.4	58.3	50
<i>C.</i>												
No. of samples	0	1	7	8	10	11	12	9	10	3	5	0
Negligible	-	✓	0	16.7	0	8.3	0	0	25.4	0	33.3	-
Diatoms	-	(100)	66.7	75.0	66.6	30.5	6.7	0	6.3	33.3	27.7	-
Rhizosolenia	-	✓	33.3	8.3	16.7	18.0	53.2	62.5	18.5	0	39.0	-
Dinoflagellates	-	✓	0	0	16.7	43.2	40.1	37.5	49.8	66.7	0	-

Diatoms excluding *Rhizosolenia* sp. (mainly *alata* and *styliformis*).

Values are the 5-year means (1954-1958).

"Negligible" means less than one cell per subsample examined.

(Based on Scottish phytoplankton records compiled by T. E. Lovegrove, Marine Laboratory, Aberdeen.)

JOHNSTON: Quite.

DROOP: There has to be a compromise.

STRICKLAND: They all contained B₁₂, didn't they?

JOHNSTON: My experiments were with unialgal *Skeletonema* which contains some bacteria. The assay is not perfect, but the picture for bacteria-free *Skeletonema* still shows much the same sort of story. For the bacteria-free assay, all the components of the usual artificial seawater used for maintaining *Skeletonema* must be added; I have used about one-tenth concentration.

GUILLARD: I think, probably, we all have observed this phenomenon. Even in Woods Hole, when we make medium with dock water throughout the year sometimes the algae in the culture collection behave badly for a while.

ALLEN: We have frequently found artificial seawater better for growth than our local inshore water.

JOHNSTON: FIGURE 35 shows growth of bacteria-free *Skeletonema* in the month of June. The poorest water in June is not closest to the shore. There is quite rich growth, not necessarily the top growth, to-

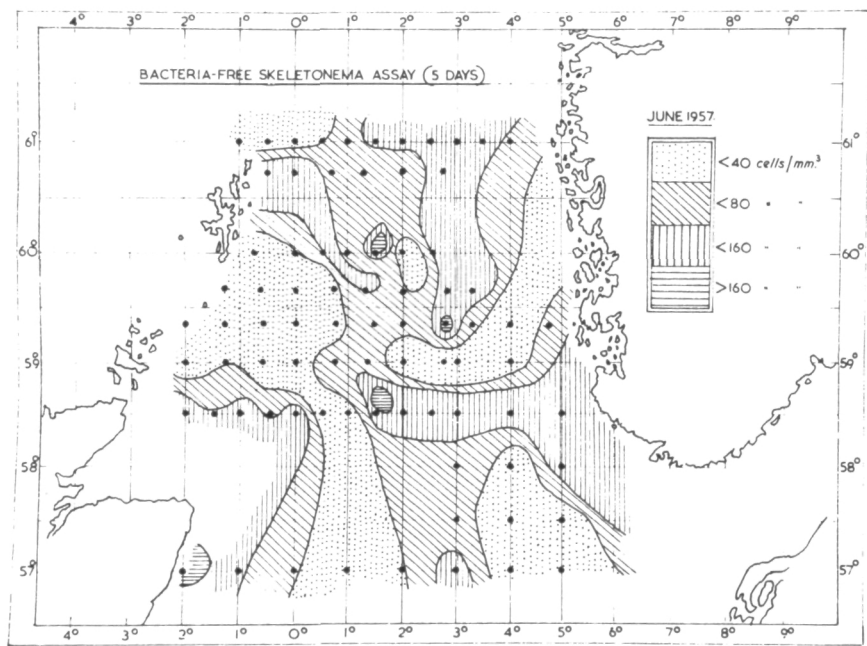


FIG. 35. Bacteria-free *Skeletonema* assay. North Sea. June 1957. Surface samples. (Fig. 1. Johnston, 1963a) (Ref. 141. Permission, Journal Mar. Biol. Assoc., United Kingdom).

ward the shore where there is a lot of turbulence and, even in June, the diatoms are just starting to bloom. In the center of FIGURE 35 there are patches that are supporting better growth. Perhaps over to the left the growth has already gone past several months and the seawater is poor.

BRAARUD: You have not used *Coccolithus* for a similar type of experiment?

JOHNSTON: No, I thought I was doing plenty when I did it for *Skeletonema*. It takes considerable time and effort. There are 132 assays, in FIGURE 35.

BRAARUD: In view of Kramer's and Ryther's studies¹⁸⁷ of the nutritional demands of *Skeletonema* and *Coccolithus*, it would be very interesting to run the two simultaneously.

JOHNSTON: I have only compared *Skeletonema* and *Peridinium trochoideum*. Both were bacterized. There is a sort of reciprocal relationship between the growths of the two organisms, but it is not easy to be dogmatic. *Peridinium* grows at a slower rate than *Skeletonema* and, if the cultures are bacterized, the seawater and the bacteria both change with time. So, if *Skeletonema* is allowed to grow for eight days, and *Peridinium* for 16, one can't be sure the water has not changed by the time *Peridinium* is ready to be harvested. Ideally, both have to be done at the same time.

JONES: Have you tried growing *Skeletonema* in intertidal waters where one would expect to find a lot of organic decomposition products?

JOHNSTON: No. My work is supposed to be related to fisheries and we keep away from such environments. (Laughter).

DROOP: Have you considered humus as one of the possible entities in what you are measuring? I find that *Skeletonema* in so-called synthetic media very frequently responds to humus. In FIGURE 36 growth curves show a two-fold enhancement with humus.

In the assays my colleague, J. Doyle, and I did, it was convenient to standardize the inoculum for a two-fold effect. FIGURE 37 shows fractions from a Sephadex column, the original material being the acid-precipitable, or "humic," part of soil extract. On the right, is relative cell yield—relative to that of the control with no addition. By "yield," I mean the maximum the growth curve attained. All the fractions, more or less, doubled the yield in that particular experiment. On the other hand, all the fractions were more potent than the parent material—the histograms on the left represent the reciprocal of the relative potency of these same fractions: the smaller the histogram, the more potent the fraction. All were more potent, but that is neither here nor there; I just wanted to mention that one does get an effect with humus.

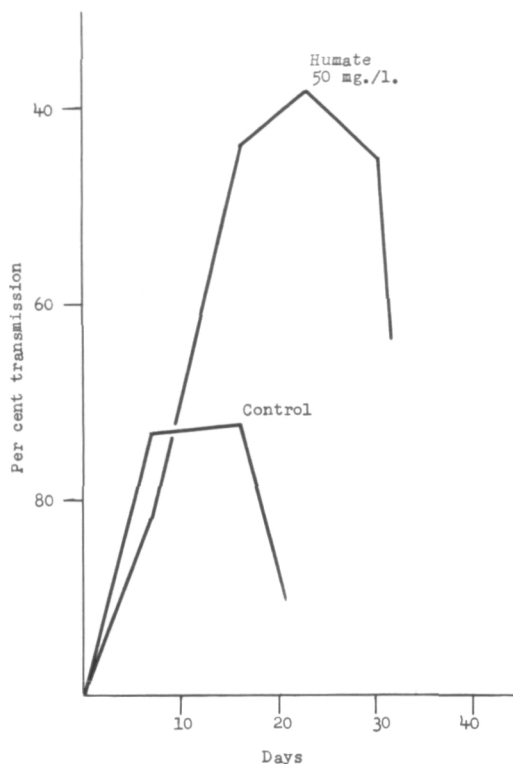


FIG. 36. Growth of *Skeletonema* in a defined medium (p. 76, Ref. 186) with and without the 'humic' fraction of soil humus. Growth as per cent transmission.

JOHNSTON: I have made some deductions based on Duursma's¹⁴² analyses of dissolved organic matter in seawater. He gives figures for the carbon and the nitrogen content of the dissolved organic matter from which quite a big change can be shown in the ratio of carbon:nitrogen between deep oceanic water and coastal water. In coastal waters the ratio of carbon to nitrogen is large; in deep oceanic waters it is much less. This is because inshore waters are plant-dominated, whereas deep waters are animal-dominated, relatively speaking. There could be an important difference between the "animal manure" in deep oceanic water and the "vegetable compost" in shallow coastal waters.

STRICKLAND: This Sephadex yield contains excess vitamin B? Growth stops for some reason—or is it cut off at a given time?

DROOP: It stops for some reason.

STRICKLAND: But not after running out of nutrients.

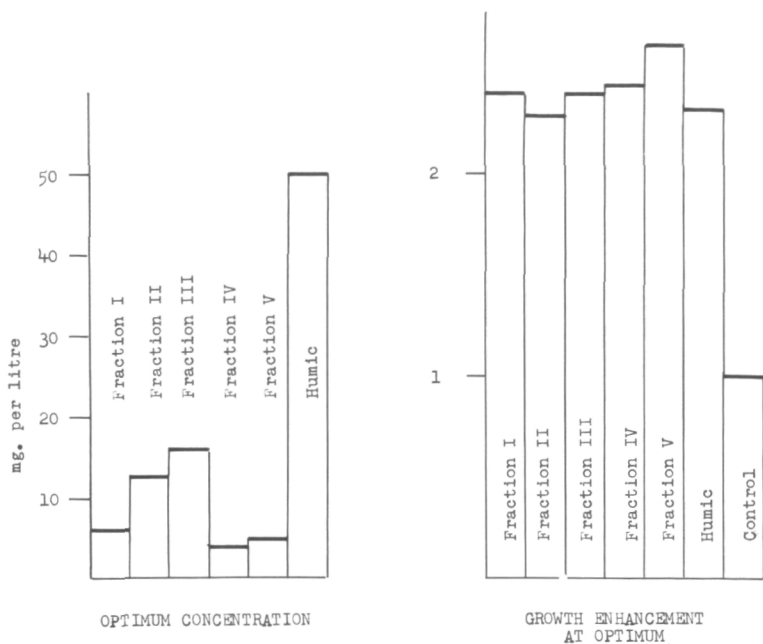


FIG. 37. *Skeletonema* assay of fractions of 'humic acid' off 'Sephadex 75.' Fractions numbered in order of decreasing molecular size. A: optimum concentration, i.e. concentration of fraction giving highest yield. B: growth enhancement at optimum concentration of fraction relative to the control having no additions (measurement: per cent transmission).

DROOP: Oh, no. I am convinced that the type of medium I used enriched with seawater, doesn't always give the best effect with *Skeletonema*. There are redox effects that we haven't discussed yet (and I am not sure this is the place to do so), which determine the yield obtained.

FOGG: Since Doctor Droop has mentioned humic acid, may I mention glycolic acid? As we see it at the moment, glycolic acid comes out of algal cells during photosynthesis and, until a particular concentration has been built up in the medium, it is the main product of photosynthesis. After this concentration has been achieved, it may be reabsorbed, producing an effect on the lag phase. We have done experiments with a planktonic *Chlorella*. If a heavy inoculum is used, growth will start without a lag phase but, with a very dilute inoculum about 1 cell/mm³, there is a lag phase of one or two days. If 1 mg/liter of glycolic acid is added to the culture medium, the lag phase with the dilute inoculum is reduced or abolished, and growth in cell numbers

begins straight away.¹⁴³ This does seem to be a specific effect. We have tried various substances, such as glucose, Krebs cycle acids, and other organic acids which may increase growth, but they do so by increasing the relative growth rate, not by shortening the lag phase.

I should say that our experiments are carried out under light-limited conditions. So it seems that if a cell is put in a large volume of medium, it will first of all devote its photosynthetic capacity to building up the glycolic acid concentration in the water. Then it can start growth immediately, using all its photosynthetic capacity for producing cell material. I think this sort of effect could be important under natural conditions. We have practically no data on glycolic acid concentration in the sea and the way it may vary with season. But, if a particular water body were poor in glycolic acid and the inoculum were small, then I think growth might never take place because the cell wouldn't be able to build up the necessary concentrations.

STRICKLAND: You have not tried this experiment with anything except *Chlorella*?

FOGG: *Chlorella pyrenoidosa* is all we have tried so far, but it is a genuine planktonic strain.

KRAUSS: What is the minimum level of glycolic acid required to initiate growth?

FOGG: We haven't yet tested a great range of concentrations. 1 mg/liter does nicely; 20 mg/liter seems to be slightly toxic and doesn't produce the effect.

STRICKLAND: I have determined glycolic acid in Departure Bay on several occasions, and it apparently does not get above 0.2 mg/liter, which is the limit of detection in seawater by the direct routine I used.

FOGG: That is about what we find in lake waters, so we really ought to try slightly lower experimental concentrations.

STEEMANN NIELSEN: I would like to go back to what Doctor Johnston was saying about the North Atlantic. Have you also made experiments with water from lower depths where no production at all has been going on? There may very easily be two effects. In such new water where plankton has never grown, something may be lacking or something in the water may inhibit the plankton. Therefore, I would very much like to know if you have done experiments with water where we know that there hasn't been any plankton production for years.

JOHNSTON: Yes, I have made *Skeletonema* assays at all standard hydrographic depths at typical positions in the North Atlantic, Arctic, and Norwegian Sea waters²¹⁸ (TABLE 6). Deep water is quite good for *Skeletonema* growth, better than at some lesser depth.

STEEMANN NIELSEN: Better than the surface water in the spring?

TABLE 6
UNIALGAL SKELETONEMA ASSAY

Station No.	1	2	3	4	5
Position	60°35'N 8°00'W	62°25'N 14°12'W	68°00'N 16°27'W	61°21'N 3°10'W	61°08'N 2°10'W
Date	24/8/56	30/8/56	3/9/56	12/9/56	13/9/56
Depth (m)	920	1475	1300	1300	515
	a b	a b	a b	a b	a b
0	980 19	400 22	—	—	—
10	1480 21	1280 19	25 19	520 21	755 21
20	535 22	620 22	—	—	—
30	515 23	20 22	730 16	1300 21	535 24
50	1030 18	1000 18	830 15	610 23	925 20
75	535 22	660 21	—	—	—
100	220 22	15 22	400 16	1300 26	960 21
150	1170 23	30 29	—	—	—
200	40 25	860 24	10 16	1350 24	160 19
300	1060 10	535 14	—	510 25	10 17
400	535 22	540 14	285 19	460 17	160 17
500	620 15	600 13	—	180 18	80 17
600	710 13	285 20	495 19	70 17	
800	710 14	345 14	20 19	315 18	
1000	25 12	345 13	710 15	300 19	
1200		420 13	330 19	140 17	
1500		285 13			

a = culture response, cells per mm³; b = fluorescence, 1 mg quinine bisulphate per liter = 50 units (in distilled water). From Johnston (1963).¹⁴¹

JOHNSTON: In early spring, shallow waters such as those of the North Sea show no difference between surface and bottom waters. That is to be expected. In midsummer, bottom water grows *Skeletonema* better than surface water by a considerable amount, nearly 75 per cent better; that is almost twice as good as shown in FIGURE 32.

STEEMANN NIELSEN: There seems to be an inhibiting factor, and not a factor like glycolic acid.

DROOP: I think I had better show my data on redox effects because it is so relevant here. In FIGURE 38, the horizontal scale is time interval in days between autoclaving and making the inoculation; the vertical scale is yield. The medium is a defined one, the organism, *Skeletonema*. When the medium has no additions, after one or two days a good culture is obtained. After four days, it will not be so good; and after eight days or more the chances are it will be pretty poor. Now, deterioration will not occur if there is soil extract in the medium. The

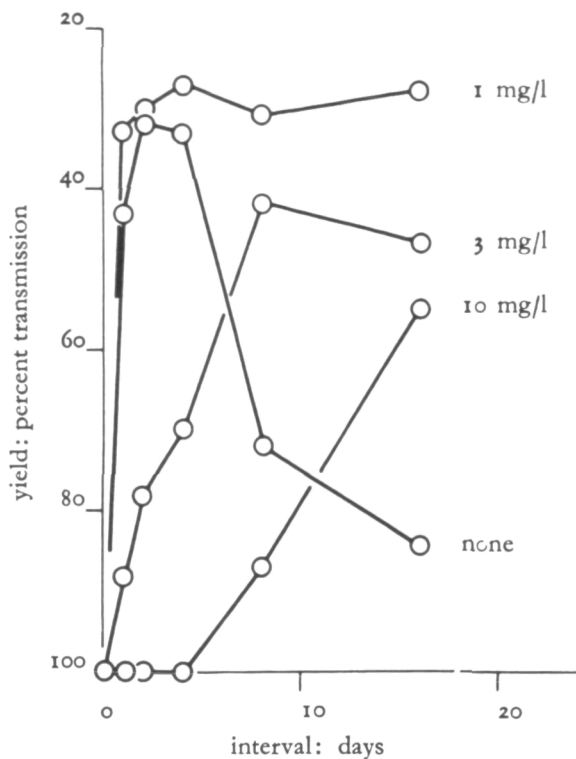


FIG. 38. Final yield in cultures of *Skeletonema* as a function of time interval between autoclaving the medium and inoculating, with indicated amounts of buffered $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ added aseptically at the time of inoculating. (Reprinted with permission from Ref. 264).

effects are also reversed by adding small amounts of sulfide. One curve is 1 mg/liter of hydrated sodium sulfide, one 3 mg/liter, and the third is 10.²⁶⁴

PROVASOLI: Do you use cotton-plug tubes?

DROOP: Yes. Oxygen and carbon dioxide are driven completely out of the medium during autoclaving, and then re-enter exponentially during the following two to four days. Redox potential also drops in the autoclave—farther than the pH change requires. Oxygen, as I measured it in the test tubes, arose from about 60 or 70 per cent to saturation, and pH dropped from 8.5 to 7.4 during the interval in question, that is, between the second and ninth day after autoclaving. It is inconceivable that oxygen tensions of this order could affect the situation; also, the pH range is well within the limits tolerated by

Skeletonema in the medium. But redox is another matter. Now, when sodium sulfide is put into the medium just before making the inoculation, as I was doing, the redox potential drops by about 300 mv, but it rises again within 15 minutes. Its effect is to render a medium that has become stale by standing, as good as new. Something has been done to the medium; some system has been shocked and reduced momentarily and, presumably, it can only be slowly reoxidized. I agree with Doctor Oppenheimer that it may be the ferrous/ferric system that is affected.

PROVASOLI: I can confirm Doctor Droop's observations. We tried to get better yields by aerating freshwater diatoms. If we aerated the medium a few hours after inoculation, *Asterionella* and *Fragilaria* did not grow. We obtained, on the contrary, excellent growth if we let the organism grow for a few days after inoculation in stagnant conditions and then started aeration. We also found that the initial phase of growth is helped by addition of reducing agents.

DROOP: I should say, that mine were unaerated unagitated cultures.

PROVASOLI: Yes, at the beginning. With some diatoms one should take certain precautions.

JOHNSTON: I think I observed the necessary precautions, because, after autoclaving, all my samples stood with the cotton plugs overnight before they were inoculated.

DROOP: That wasn't meant as criticism.

JOHNSTON: No, no, I am quite happy that my conditions were reasonable.

DROOP: Certain deductions can be made as to what is needed in this medium, that is, some system has to be shocked, has to be reduced; one doesn't get these effects nearly to such an extent if humus is present.

MCLAUGHLIN: I think Doctor Fogg gave a clue when he asked the size of the inoculum. I imagine he has had some experience with the fact that if the medium is not optimum for the growth of the organism a bigger inoculum is required. As the medium gets better there is less need for total cells in the inoculum.

As for autoclaving and shelf time, we have found the same thing. When we get better medium by redefining for the particular organism, we can leave it on the shelf for as long as two weeks and still use small inoculum.

PROVASOLI: However, you should mention the fact that we employ screw caps at the Haskins Laboratories.

MCLAUGHLIN: We use screw caps on our test tubes instead of cotton plugs, which, when tightened after autoclaving, does not allow for rapid oxidation. Does this still hold with a higher inoculum,

Doctor Droop? Was yours a minimal inoculum or did you have to go to higher inoculas to get growth?

DROOP: With *Skeletonema*, very often one won't get a good culture if the inoculum is made too large.

MCLAUGHLIN: Would that be due to cells lysing in the inoculum?

BRAARUD: For nearly 30 years we have accepted vertical transport and turbulence as an important factor in delaying phytoplankton development out in the open sea as compared with coastal waters. Ibsen said that truth does not hold more than 20 years, and certainly it is time that this concept was tested, because we know so little about transport. I am very pleased to hear that a new method is being tried. Even if I am not content with *Skeletonema* as an assay organism for oceanic waters, I think the whole approach is very valuable. If one can find that there is an improvement in water as a medium for plant production as the season goes from mid-winter toward spring, then we might drop the whole vertical transport theory.

JOHNSTON: There are two phenomena involved. Upwelling and deep mixing in the oceans bring "old" water to the surface, but the cycle of *Skeletonema* growth in the North Sea refers to relatively shallow water, and that is a different story.

BRAARUD: I am not as interested in the North Sea as I am in the assay of offshore water, which, I think is very important.

RILEY: It is now abundantly clear that there are some difficult and subtle chemical aspects involved in seasonal succession which we shall have to understand more clearly before we can explain everything that is involved. I think it would be fair to say, though, that some aspects of seasonal succession are much simpler. I can cite a particularly simple case. In Atlantic coastal waters we have a major diatom flowering in late winter, of which the two most important species are *Skeletonema costatum* and *Thalassiosira nordenskioldii*. If the temperature is below 2C *Thalassiosira* will be the dominant species; if the temperature is a little above 2C, *Skeletonema* will be dominant. I have seen this pattern of dominance shift several times in different ways during the course of a bloom with one species tending to replace the other.

Along these lines, if one analyzes observations from a number of years simply observing the pattern of succession in different years in relation to the temperature and light and other easily measurable environmental factors, one sees many indications of some degree of control of seasonal succession, by simple physical factors. The total picture would include light and vertical turbulence and various other things as variables. We do get some indication from our observations that may help us to select the problems that might be studied by more

critical methods. The question, then, is whether one can devise experimental work to test these problems a little more carefully.

I am not sure whether this is possible or not. We are dealing with rather subtle differences in growth rates, for one thing. For another thing, it has been made quite clear to us that it is somewhat difficult under laboratory conditions, to duplicate natural conditions in the sea precisely enough to give us the answers we need. *Skeletonema*, for example, grows rather slowly in the laboratory at low temperatures, even though we know that it tends to be a winter dominant in the sea. Doctor Guillard has told us that he is able, by special conditioning, to get *Skeletonema* to grow rapidly at low temperatures. Isn't that correct?

GUILLARD: On a few occasions, yes.

RILEY: Perhaps before we can do very much with experimental work, we have to know enough about growing these forms so that we can condition them in the laboratory in the same way they are conditioned in the sea, in order to give us critical results.

I think Doctor Guillard has had about as much experience as anyone else with growing a variety of these organisms. Could you help us with this discussion?

GUILLARD: There are a number of instances in which the physiological experiments correlate very neatly with what one would expect from observations in nature. For example, *Detonula confervacea* won't live in warm water in the laboratory, and it doesn't live in warm water in nature. If the water is warmed, it makes resting spores. The resting spores are found in nature when the water gets warm. There are other small diatoms that also show some reasonable correlation. It seems to me that when we are interested in the ecology of some region we should look first for these simple explanations. When we find an organism such as *Skeletonema* that has curiosities of behavior, we should study it for its own sake, because it is giving us answers to physiological questions; but we should not let this confuse the ecological situation unnecessarily.

PROVASOLI: Very good! Preconditioning of the inoculum, especially to temperature and light, may be extremely important if we want to approach natural conditions experimentally.

STRICKLAND: *Skeletonema*, conditioned for several months at 19 or 20C, and then put in a incubator at 6C for a long time, just doesn't seem to want to grow readily. So, the conditioning has obviously got to be done in degrees. One has to take an organism that is just growing at 10C and keep it for months, and then turn the temperature down another few degrees, etc. This is a very interesting business. We could find out what the absolute range might be.

PROVASOLI: How is your conditioning done, Doctor Guillard?

GUILLARD: Perhaps I can't help, in your situation. When John Ryther did the first study on acclimatization that he reported⁸—it was on respiration—he transferred a culture from standard conditions at about 20C to 4C and left it, and behold, after a matter of a few weeks, suddenly a beautiful culture appeared. I had done this before for another reason, but subsequently it has not worked even when I have tried to reduce the temperature more slowly. In fact, I have continuously cultured two *Skeletonema* species, my new species (from the Sargasso Sea) and *S. costatum*, in a temperature-controlled bath and taken the temperature down a few degrees at a time, subculturing the same cultures. When the temperature became low, *S. costatum* did not grow very well. It did not go through auxospore production. It may be that the culture has to go through a sexual cycle every now and then as the conditions are being changed, but I have no evidence for that.

DROOP: I think Doctor H. A. von Stosch knows something of that. He has suggested to me that this variation in behavior in *Skeletonema* and other diatoms may be due to genetic recombinations altering one's material between one sexual cycle and the next, so that one is never using precisely the same material in successive experiments.

PROVASOLI: Should we then use only strains derived from one-cell isolations to avoid recombinations.

DROOP: That is no good.

PROVASOLI: Why? *Skeletonema* does not have male and female strains.

DROOP: No, both sexes are produced by each clone, the female gametes, first, and then the male.

PROVASOLI: I see, so we need genetically homogeneous inbred strains.

DROOP: Or a diatom which forms auxospores at infrequent intervals.

MCLAUGHLIN: Were any nutritional studies done at low temperatures? We have discussed the physiology of the organism at 10C, on the phosphate/nitrate ratios, the amount of chelation, and how many atoms of iron are in what condition. But have any real nutritional studies been done in correlation with reducing the temperature? From my own experience, if an organism that grows at 18 or 22C, is adapted to grow appreciably at 12C, it usually requires nutritional experiments—with control of physical conditions and various nutrients.

Doctor Hutner⁶ has raised the temperature of cultures from the normal and found that other requirements vary greatly as the temperature rises; likewise in unpublished work, with a variety of organisms ordinarily growing at relatively high temperatures, he

finds that the organisms may become very fussy indeed at their "lower" limits.

STRICKLAND: What do you find? Does it require more or less nutrients when the temperature is lowered?

DROOP: More. I can say something about vitamin B₁₂ and *Monochrysis*. Very shortly, as one approaches within a degree of the apparent minimum temperature, which is 10C for *Monochrysis*, the B₁₂ requirement goes up fourfold. On the other hand, if it is possible to lower the temperature a bit farther—in experiments I did last year, I found that the requirement is still only four times as high as at normal temperatures.

PROVASOLI: And there are so many nutrients that even going down a few degrees there is no need to increase them.

MCLAUGHLIN: We have taken some cultures in minimum medium and frozen them solid for 48 hours; when removed, they are viable. But if they are in a heavy organic medium, in which they would normally grow at room temperature and freeze them, we do not get viable cultures.

RILEY: One of the problems in succession in the ocean, of course, is the fact that every water mass that we observe is subject to invasion by other species. We do not at any time know whether there is what might be called an internal seasonal succession, or simply the results of random interchange of water. From this standpoint, of course, the fresh water succession in lakes is a simpler and often more profitable subject to study.

Doctor Lund, would you say, for example, that there is a more repeatable seasonal succession in lakes than in the open ocean? Can you pretty well predict what is going to happen from year to year, or are there pronounced variations, too?

LUND: It is difficult to answer that question, and I can only do so on the basis of one very small experience. The lake I know best is Windermere, so I am not talking about lakes in general. In Windermere, the answer is: yes, a lot can be predicted, not perhaps so much in quantity, although some quantitative predicting can be done, but much more so in quality. Although there are differences from year to year in Windermere, there are also astonishing similarities which occur from year to year.

As I listen to this discussion, everything that is said brings up the same sort of problems that we have in fresh water. It is astonishing to me how fresh water people and marine people can, when discussing plankton, talk the same language. The fact that the oceans are so big and the fact that they are so salty gives rise to these awful problems of distribution—these seem, nevertheless, rather minor matters. The overall problems are the same, both generally and in detail. The

continuous line in FIGURE 39 is the diatom *Asterionella* at Windermere, with cell numbers plotted on a log basis, over a period of 16 consecutive years. We have a number of such pictures from other places as well, and it can be seen that *Asterionella* has a pattern which is astonishing for a natural situation. It occurs over and over again. The maxima obtained in the spring, for example, are quite similar, really, so the system is, indeed, remarkably repeatable. Even the other two diatoms *Fragilaria* (I won't specify species) and *Tabellaria*, also have cycles which, though quantitatively much more irregular than that of *Asterionella*, qualitatively show rather remarkable similarities from year to year.

The really striking thing is that whereas this burst of *Asterionella* occurs every spring, it has been going on ever since I have been at Windermere Laboratory, and there is good evidence that it was going on for long periods before that, *Fragilaria* and *Tabellaria*, appear strikingly at times when *Asterionella* is usually rather rare; they have a different cycle. They occur in the spring too but, again, it can be predicted with fair confidence that next spring there will be a big crop of *Asterionella* which will reach at least one million cells/liter, and one can also predict approximately when it will ebb. While this doesn't always take place it is the usual cycle, and it can then be predicted that sometime in the summer, probably rather late, there will be smaller crops of these other two diatoms.

Does that answer your question?

RILEY: Yes.

LUND: The situation in a lake is rather more stable. This is also true for other kinds of algae. I can predict, for example, that next spring during the period when the *Asterionella* burst is occurring, certain small green algae will appear, notably, a small plankton; i.e. *Chlorella*—only Doctor Krauss can possibly tell us what it should be called—which can't be distinguished from Doctor Fogg's *Chlorella* which, I think, came from Torne Träsk, a lake in northern Sweden. Whereas this and other small green algae are seen in the spring, later on, in the summer, *Fragilaria* and *Tabellaria* may also be common, or perhaps the main crops will be the larger green algae, such as the coccoid green algae, colonial forms and desmids. It can also be predicted that in spring the blue-green algae will be very uncommon. In summer and autumn the blue-green will certainly become noticeable and, perhaps, common. One can predict that after the *Asterionella* maximum, a species of *Dinobryon* will appear (Doctor Hutchinson mentioned this earlier), and also *Uroglena*. I am sure I could think of other predictions that could be made, and I would be quite prepared to take bets on the subject.

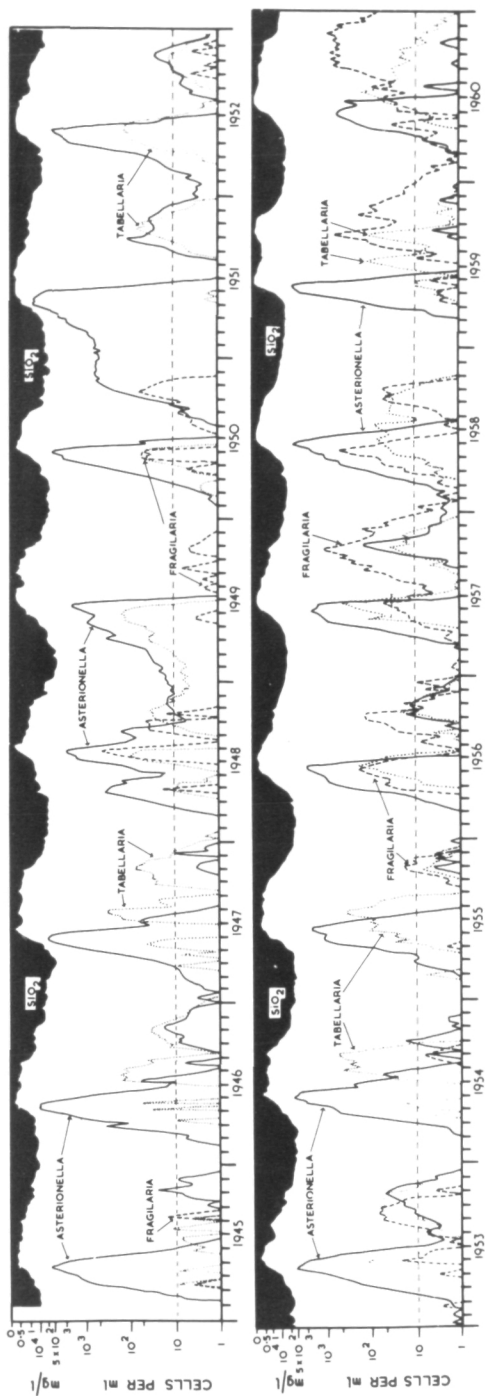


FIG. 39. The periodicity of *Asterionella formosa* Hass (continuous line), *Tabellaria flocculosa* var. *asterionelloides* (Grun.) Knuds (dotted line), and *Fragilaria crotonensis* Kitton (broken line), in the northern basin of Windermere (see Fig. 56) from 1945-1960 inclusive, and the fluctuations in the amount of SiO_2 dissolved in the water. Population density, No. cells/ml plotted on a logarithmic scale. Solid black, SiO_2 /mg/liter. The continuous horizontal line separates populations of over 10 cells per ml from those of less than 10 cells per ml. (Reprinted with permission from Ref. 144).

FIGURE 40 shows the seasonal cycle of another diatom, a *Melosira*. The black "mountains" show the numbers of *Melosira* plotted on a logarithmic scale. Its periodicity in three different lakes occurs year after year with an astonishingly regular cycle. It can be predicted with confidence that *Melosira* will appear in significant quantities when the water mass is mixing in the autumn. The details of the picture are not shown, but one can predict that the organism will disappear when thermal stratification begins to rise in the spring, and it won't be seen again in any quantity until the next autumn. One can be pretty sure of the sort of thing it is going to do in different lakes. In relation to what Doctor Riley said, I had a feeling that perhaps some people didn't wholly agree with his views. If I understood him correctly, he said that if organisms are observed over long periods, certain regularities or characteristics tend to repeat themselves, even in the sea—certainly in many of the coastal waters—and that these characteristics are correlated with certain environmental factors. This, perhaps, supplies a clue as to the causes, or, even if it doesn't, gives us some idea of the sort of experiments which might be carried out. I think this is a very important viewpoint.

If we are going to make progress, I think we must first try to do simple experiments, if possible. I don't want to suggest that someone else should stop doing something complicated, because the vital thing, of course, is that everyone should do what he is motivated to do, and should not be stopped from doing so.

DROOP: What is a simple experiment?

LUND: One example is to show what starts *Asterionella* growth in the spring where there is a clear correlation with a light curve. As the light curve sweeps up in February and March, the population of *Asterionella* also sweeps up. Isn't it possible that this correlation has some meaning? It is very simple to do some experiments to test this that certainly agree with what one expected to find.

This brings me back to another point, which is that we need more and more basic data. For example, the paper of Parsons, Stephens and Strickland¹⁴⁶ on the composition of certain marine organisms is basic data, and it is rather astonishing that such basic data have not been abundant. Once such data starts to accumulate, information such as what is the dry weight of the organisms will be forthcoming. It is astonishing that there isn't much information about dry weights, and that what there is, is so scattered that it is very difficult to know where to find it. So, I would say I have two prejudices. One is that I try to keep my experiments relatively simple, although I may be utterly wrong; second, I am extremely keen on trying to obtain basic data.

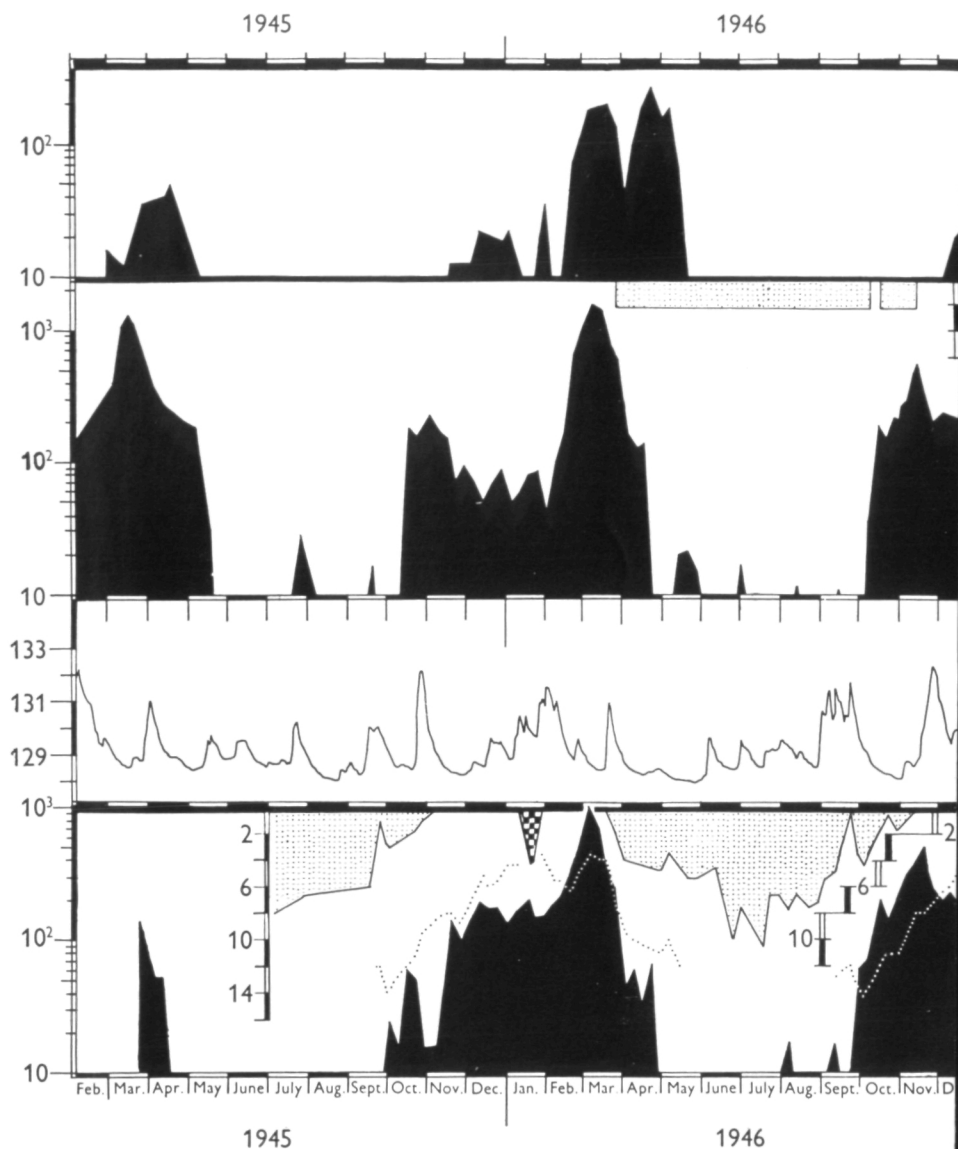
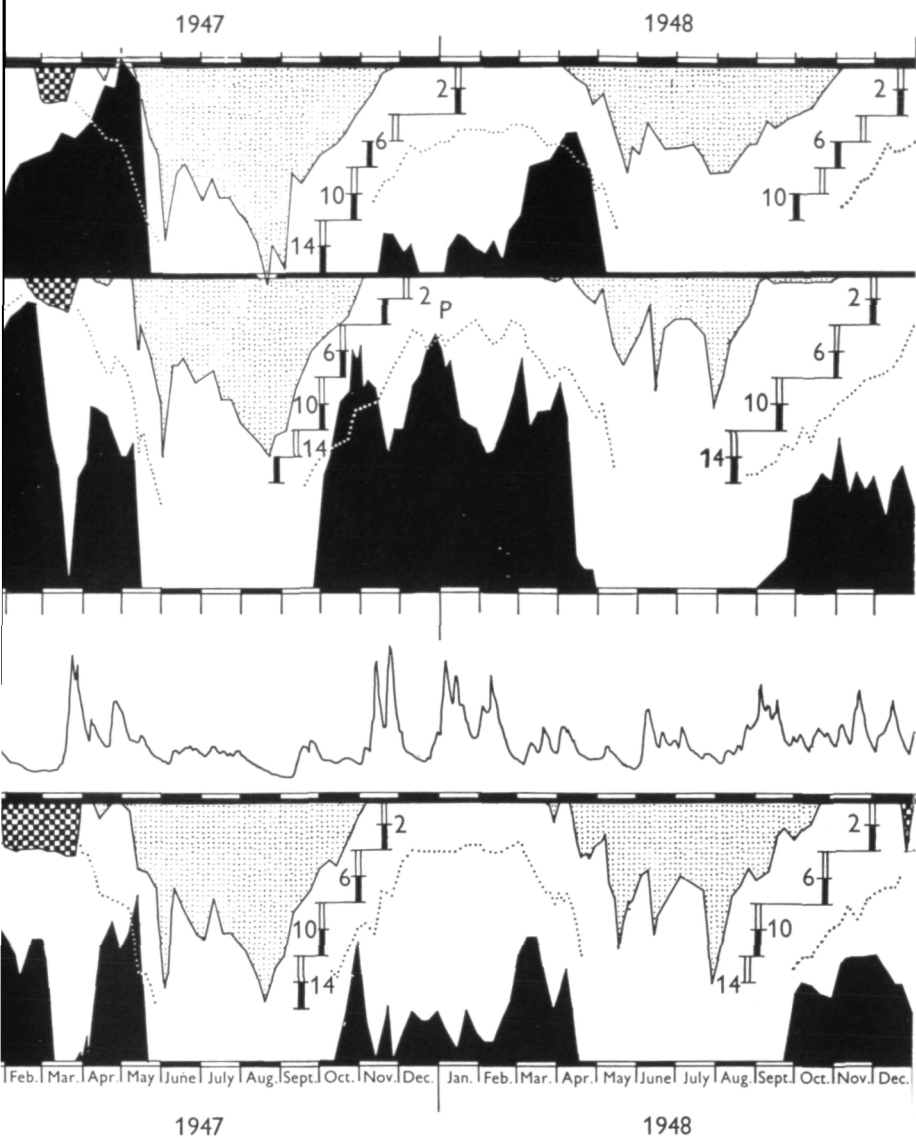
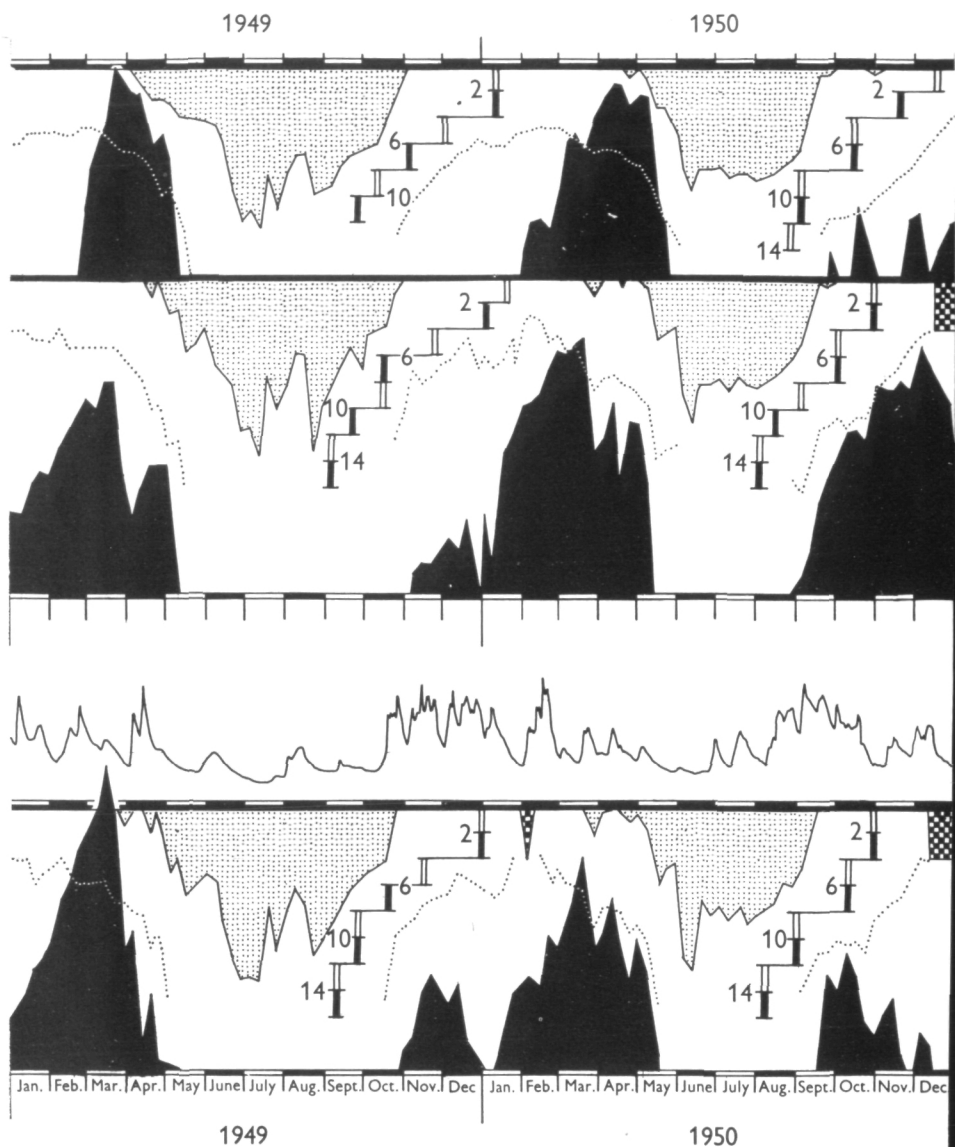


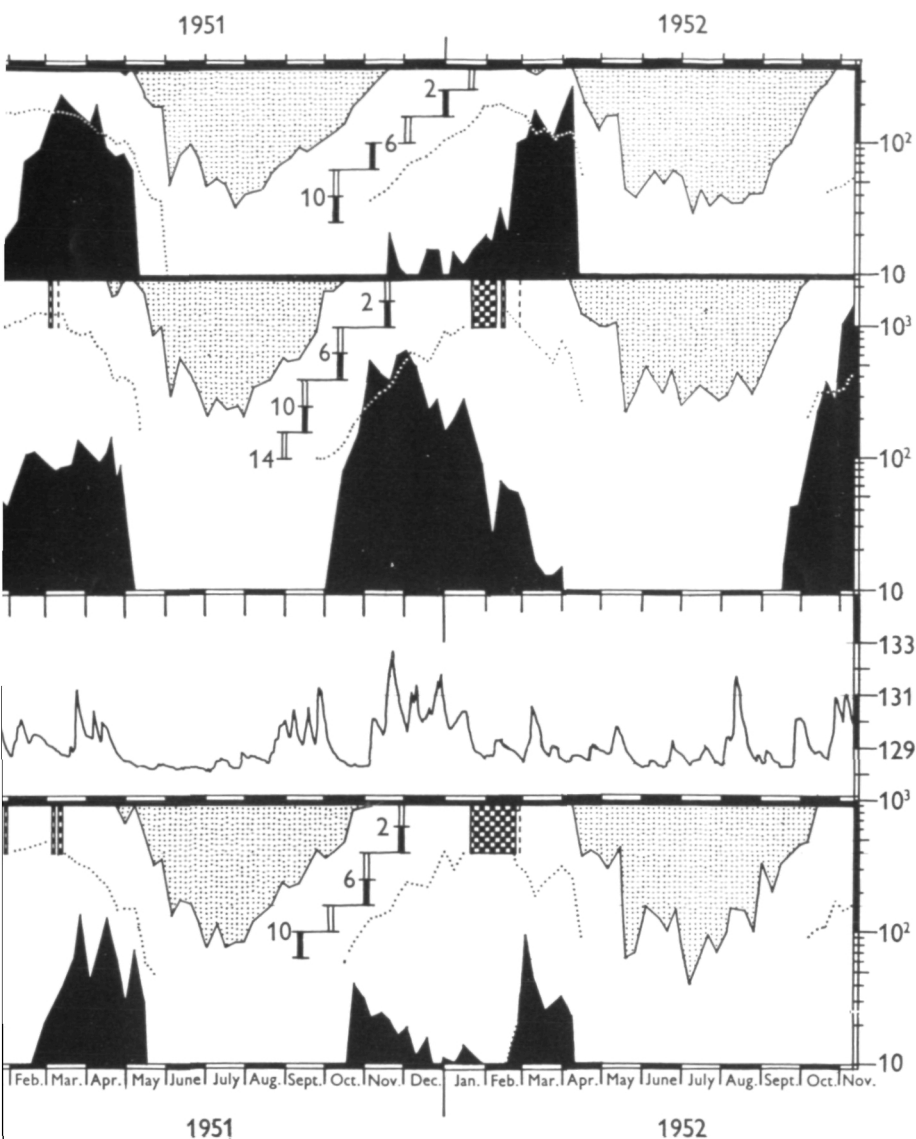
FIG. 40. The seasonal cycle of *Melosira italica* (Ehr.) Kütz. subsp. *subarctica* O. Müll in three lakes in the English Lake District from February 1945 to May 1952. Top graph, the southern basin of Windermere; middle graph, Esthwaite Water, and bottom graph,



Blelham Tarn (see map, Fig. 56). *Solid black*, cells per ml plotted on a log. scale; *dotted areas*, direct thermal stratification expressed as the difference in °C between the surface and bottom temperatures of the water column at the sampling stations; *chequered areas*,



inverse stratification, expressed likewise, in both cases differences below 0.4C not shown and in Esthwaite Water in 1946 the period of direct stratification indicated by horizontal dotted rectangle; dotted line, surface temperature; plain enclosed line, Windermere lake level whose fluctuations are similar to those in the other two lakes.



P, epidemic of parasitism by the fungus *Rhizophydium melosirae* Canter. Maximum depths at sampling stations; Windermere, 60 m, Blelham Tarn and Esthwaite Water, 15 m (Reprinted with permission from Ref. 145).

MCLAUGHLIN: Doctor Lund, could you tell us what other categories you consider basic data, besides dry weight?

LUND: Yes, potential rates of growth.

MCLAUGHLIN: In terms of cell volume or in terms of division?

LUND: It depends on the algae. Some algae can vary greatly in cell volume, some vary very little.

Another is rates of sinking, which we have discussed; this is terribly important. FIGURE 40, which concerns *Melosira* is, in fact, an example of the importance of sinking. One of the absolutely overriding factors controlling the seasonal cycle of *Melosira* is that it sinks relatively fast.

JOHNSTON: Doctor Lund, when you were discussing *Asterionella* you said growth was related to light, and when you talk about *Melosira* you relate growth to sinking. But, at the time of year *Asterionella* correlates with light, wouldn't stability be increasing all the time in the water mass?

LUND: No, when *Asterionella* starts its spring period of increase the water is turbulently mixed at all depths.

JOHNSTON: In the autumn water stability breaks down and light is also decreasing.

KOCZY: What you are saying is: If the correlation between the two factors is measured, the one factor which determines the change of the other may determine something else, which, in its turn, may then determine the change. All the variables have to be considered.

RILEY: I think there are several simple things that can be done. I feel, for example, that the kind of work that Doctor Braarud has done on temperature and salinity optima, which he reported in his paper at the International Congress, can help us a great deal in this problem of seasonal succession. It is also possible that we can get some results of a rougher but useful sort simply by putting natural populations in a bottle and slightly altering the conditions of temperature, or what have you.

This kind of experiment is less satisfactory in one respect, because we do not by any means control all of the conditions we would like to control. On the other hand, it gets around the problem of whether or not our pure cultures are in an adequate physiological state to give us answers to the questions we are asking.

I think the problem of seasonal succession is not insoluble, at least some of the more dramatic aspects of it, but I fear we haven't got very far with it to date.

INITIAL STOCK VERSUS INVASION, GRAZING, AND SINKING

Discussion leader:

T. BRAARUD

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BRAARUD: When we saw Doctor Strickland's very interesting models of curves (FIGURE 57), it was obvious that in order to tell us a still more interesting story he should experiment on members of an ordinary succession in the water where he is working; then, maybe, we will know the whole story of the succession from this area. I think the approach is extremely interesting.

I would like to start by referring to Doctor Lund's Figure (FIGURE 39), showing the regular occurrence of *Asterionella* maximum year after year at the same time, with associated species also occurring very regularly. Doctor Lund said his impression is that limnologists and marine planktonologists speak the same language, but we must admit that in the sea we have other complications, especially with regard to succession and the changes from one year to another. The reason for these irregularities is quite obvious: We have large scale current systems which carry water from one area into the other. The hydrographic conditions in the sea also differ in other ways from those in lakes. We might discuss briefly the importance of the non-nutritional factors which are really determined by the hydrographic situation.

In the agenda, the word "invasion" appears, and it is a very good word for the introduction of populations from other areas. I think we might start with considering this factor for the changes which take place in a special area. My experience in the Norwegian Sea, between Norway, Greenland and Iceland, and in Norwegian coastal waters, is that invasion plays an essential role in determining the specific composition in all of these waters, and I shall give a couple of examples based on recent observations by my collaborators in this area.¹⁴⁷ *Coccolithus huxleyi* is a regular member of the phytoplankton in the Norwegian Sea. Whether it is able to survive the winter is an open question but, according to the data from the Oslofjord, it does not seem to survive winter in that area, and I think

it is doubtful that it survives within the Norwegian Sea, at least the northern part of it. Nevertheless, it appears every year in the Norwegian Sea and presumably is introduced by Atlantic water masses from the North Atlantic south of the Faeroes Iceland ridge. *Thalassiothrix longissima* and *Rhizosolenia styliformis* are two very large diatoms which occur during the period of high production in about June. These species have quite a strange distribution. In some years, they occur together and are directly associated within different parts of the Norwegian Sea; in other years, the *Thalassiothrix longissima* may be predominant in one area, the *Rhizosolenia* in another, in still other years we may find a more varied pattern. Paasche¹⁴⁸ has studied material from different parts of the Norwegian Sea over a two-year period, and has concluded that the relative distribution of these species depends on the initial populations which, during the spring, are being introduced from areas in the North Atlantic where reproduction starts earlier.

The coastal *Chaetoceros debilis*, which is an important component of the coastal plankton at the Faeroes and the Shetland area, and so on, in some years occurs in very great quantities in the central part of the Norwegian Sea in true oceanic conditions. Presumably, this is due to a seeding from the Faeroes-Iceland area and the occurrence is rather irregular. Sometimes it occurs in abundance; at others it may not be present at all, or in very small amounts. This may suffice to indicate how irregular the composition of the phytoplankton is during the spring development in this oceanic area.

In Norwegian coastal waters, we have still more striking examples of how the introduction of populations from outside of the area may influence the composition of the society. In the Oslofjord, with intervals of several years, there are "*Coccolithus huxleyi* summers", when tremendous quantities of this species appear.¹⁴⁹ It occurs in other years also, but not in such numbers. It appears as if the time of the introduction of an initial population from the outside area is one of the main factors that determine whether it will occur in such tremendous quantities as 30-40 million/liter.

On the west coast of Norway, where there is no pollution such as in the Oslofjord, mass occurrences of *Coccolithus huxleyi* may also be seen, and they are conditioned by two things. One is the introduction of initial populations from the Atlantic or the water masses outside the coastal waters; the other is a hydrographic situation which provides a good supply of nutrients at just that time.

Dinoflagellates and diatoms may be taken as examples of such an invasion. Gran¹⁵⁰ described a very interesting situation in the Romsdalsfjord, where one autumn a society of southern species was introduced and survived during the winter; in the following spring, the

diatom society was quite different from what one would ordinarily find.

So much for invasion. One may not think it matters much which species are present when conditions for growth become favorable, but I think the question of the composition of the initial society for development, following winter minimum, may be quite important for the speed of the development, because the different species may have different reproductive rates.

Another important factor for distribution and population dynamics is the vertical mixing which takes place in northern waters. In the Norwegian sea it reaches down to several hundred meters, and along the coast to much less. For a long time we have considered this hydrographical feature as being of very great importance for the start of the development in spring, because certain variations control the time when the waters are stabilized and thus may explain the time difference in the spring development.

A different effect of vertical mixing is the transport of populations from deep layers up to euphotic layers. Most of us who have studied populations have been of the opinion that when conditions for growth are good there will always be a sufficient initial population to take advantage of this situation. During recent studies in the Hardangerfjord,* this factor has been brought to my attention, and I found two effects especially quite interesting. One is that as we compare the composition of the spring society in the innermost part of this fjord and the area just outside the island—it is a very deep fjord, 900 meters in depth—we find that in the inner part of the fjord only four diatom species are present, of which *Skeletonema* is absolutely the most predominant. In the outer part of the fjord at least 17 diatom species occur simultaneously in very great numbers. There may be several reasons for the large number of species in the outer part, but what intrigued me was why there were so few in the inner part.

My reflections are the following: In the inner part, the winter mixing reaches down to only 10 meters so the homogenous layer where vertical churning may take place is very thin. In this area, if populations of meroplankton, which may have occurred earlier in the year, have sunk so that they occur mainly below 10 meters they should have little chance of getting up into the photic layer. Farther out in the fjord, the vertical mixing goes down to 70 meters, and there would be greater opportunity for such vertical transport. *Skeletonema* maintains a population within the area all through autumn and winter and, thus, has the great advantage of having a large initial population for a spring development. As a consequence, it predominates both in the

*Unpublished observations.

inner and outer part, but it is very strange that in the inner part only three associated species were observed. My suggestion is that this poverty in diatom species in the inner part is due to the shallowness of the thoroughly mixed layer there in winter.

If we turn to the oceanic parts of the Norwegian Sea, the winter mixing goes down to several hundred meters. The dilution of the phytoplankton population in the euphotic layer during this period must be very extensive, and it is surprising that initial populations may be maintained for the spring development. Halldal's¹⁵¹ records from station M show that the forms which occurred more regularly during winter were *Coscinodiscus* species. We may suspect them of being able to control their specific gravity, and so may provide a means of counteracting the transport downwards. Smaller species which were not recorded during the winter period presumably may have to be brought up from deep layers to which they have sunk during the unfavorable season for their growth. For such species the vertical mixing must be regarded as having a favorable effect.

These remarks may be regarded as tentative. We have so little information on the composition of the societies in oceanic habitats during the dark part of the year, and likewise about the vertical distribution of the various species, that it would be worth while to provide additional information in order to obtain a better understanding of how the holoplanktonic populations are maintained in waters where vertical circulation takes place.

PROVASOLI: The term holoplanktonic exclusively refers to planktonic?

BRAARUD: Yes, those which don't have any bottom or resting stages.

PROVASOLI: Is that a fact, or is it that we have never found resting stages?

BRAARUD: I mean morphologically discernible resting stages. Of course, that doesn't mean that cells may not be physiologically in a resting stage. In the planktonic diatoms, where resting stages are found, we also see that the distribution is not restricted to coastal areas. *Chaetoceros debilis* is an example of how a coastal species may be carried out and be able to stay and reproduce under oceanic conditions, while *Skeletonema*, which also occurs in coastal waters, has never been found to our knowledge in the open Norwegian Sea.

JOHNSTON: We don't find *Skeletonema costatum* in net hauls from the open ocean, but it has occurred infrequently in enrichment cultures.

BRAARUD: Yes, but the water may not be very far from the coast.

JOHNSTON: The greatest distance from land I have found *Skeletonema*

ma is 28 miles northwest of the Butt of Lewis, Outer Hebrides, and also over Faeroe Bank (which is 60 miles from the Faeroe Islands).

BRAARUD: I have one other small point to make. The question of the importance of initial population for development is well demonstrated, I think, in the areas of the Greenland polar current. There, we find polar water poor in nutrients but with quite large populations of meroplanktonic forms, and this water meets Atlantic deep water which is rich in nutrients but very poor in plankton.¹⁵² In this area, we get a tremendous vegetation which consists mainly of various species of meroplankton, as well as some oceanic plankton species.

PROVASOLI: What are meroplankton?

BRAARUD: Those with resting spores, the coastal species. The polar water may have a beneficial nutritional factor, but even if that is the case, the initial population comes from the polar water and the production is very large. Later, the oceanic waters may develop a large population, even if they are not mixed with coastal waters or polar waters, but it may take a much longer time after stabilization, and the society would be quite different.

PROVASOLI: The hypothesis that high productivity results from mixing waters rich in nutrients and scarce in individuals with waters bringing in the inoculum can be tested experimentally. One could filter off the two populations and inoculate them in each water and then in a mixture of the two.

BRAARUD: The areas are rather inaccessible but the inorganic nutrients have been determined.

OPPENHEIMER: In 1960, Reed Stevens, of the University of Texas Institute of Marine Science at Port Aransas, conducted research in the Antarctic to measure the organic carbon distribution in the Bellingshausen Sea and the Ross Sea.* Since these were two productive areas, we thought we would learn something about rates of mineralization.

We found that the water contained less than 3 mg/liter total organic material, including soluble, particulate, and living. The sediments had less than 0.5 per cent organic carbon, which means that they were extremely poor with respect to other areas for which we have data. The Ross Sea area had a water depth of only a few hundred meters, whereas the Bellingshausen Sea was about 1,500 meters deep.

We found that the ice acted somewhat as a sediment in its ability to collect organic material, and had organic contents up to 18 parts per million. The data suggests that organic matter was mineralized *in situ*,

*This project of Doctor Oppenheimer and Stevens was supported by a grant from the National Science Foundation. The final report to the NSF Antarctic Program was entitled "Distribution of bacteria and organic matter in water sediment and ice of the Ross and Bellingshausen Sea, Antarctica, 1961."

and that the organic carbon does not reach the bottom. Thus in connection with Doctor Braarud's discussion on invasion, it is possible that ice could distribute organisms.

JOHNSTON: Doctor Oppenheimer, what you have just said has a very profound bearing on the technique for determining the age of seawater, using C^{14} .¹⁵³⁻¹⁵⁵ If the C^{14} has not been uniformly distributed, but concentrated in the ice or in the upper water, it will knock the age determination off, and be quite wrong.

OPPENHEIMER: These two antarctic areas, unfortunately, were not the best to study. The experiment should be repeated in a more productive area, such as the other side of the Palmer Peninsula. According to Sieburth,* this is far more productive than the spot we selected.

GOLDMAN: Did you observe the tremendous diatom populations growing under the ice in the Ross Sea?

OPPENHEIMER: Phytoplankton were present in the water when the ice separated. Apparently, the organisms were in the ice. As the ice melted, large plankton blooms occurred both on the surface of the ice and in the cracks.

GOLDMAN: One can literally collect the diatoms in buckets when the icebreakers go through. I wonder if they are not actually maintaining themselves in a better light condition in addition to obtaining some nutrient directly from the ice. The ionic gradient at the ice-water interface would be of interest in this respect, also.

OPPENHEIMER: There is quite a bit of organic material in the ice and, thus, presumably, inorganic nutrients. This brings up a rather interesting bit of philosophy on a method for the capture of energy, especially the energy for enzymatic reaction, in water close to the freezing point where the enzymatic processes within the cell were being conducted at near zero temperatures. If one could speculate on infrared heating, it is possible that the energy from the infrared may be used for enzymatic processes at zero temperature.

GOLDMAN: One of the real problems in dealing with organisms *in situ* is the fact that we don't know the internal temperature of the cells, particularly in the Antarctic where the environments may be zero degrees, and are under terrifically high inhibiting light intensity. The internal structure of the cell may actually be at a slightly elevated temperature.

OPPENHEIMER: I doubt that, because the heat transport must be rapid in cold water. Perhaps Doctor Allen could give us some information. Is there any possibility of infrared activating an enzymatic process without increasing the apparent temperature?

ALLEN: It would seem to me that if this were so, it would be fairly

*Sieburth, J. M., Narrangansett Marine Laboratory: Personal communication.

similar to what Doctor Goldman is saying: that the organism is not in equilibrium with its surroundings.

OPPENHEIMER: It would be almost an instantaneous reaction, and the usual Q_{10} relationship would not be significant.

BRAARUD: Since we are talking about initial populations, cells which are embedded in ice and resting spores in ice have been considered to be essential in giving initial populations in polar waters or in mixed water with a polar component. This idea dates from Nansen's time, and it is obvious that all these coastal forms are found in the outskirts of ice-covered polar currents.

KRAUSS: Isn't there some data with regard to the amount of fixed nitrogen found in snowfall? Aren't the ice packs that ultimately originate from glaciers and, again, ultimately come from snowfall, bound to contain a significant amount of nitrogen?

BRAARUD: The content of nitrates and phosphates in polar water is very low. The original theory was that these waters were especially rich in nutrients because of the addition of fresh water from the Siberian coast, and subsequent transport across the polar basin.

KRAUSS: Generally, snowfall is supposed to bring down with it a certain amount of fixed nitrogen.

ALLEN: More than rainfall?

KRAUSS: Yes, I think it is somewhat higher.

DROOP: It is a matter of ice or snow age whether the nutrients have been leached out or removed as brine.

GOLDMAN: This varies greatly from region to region. For instance, the nitrogen content of rainfall on the Alaska Peninsula is only one-fortieth of the rainfall at similar latitudes in Scotland.

DROOP: How old is the ice of the glaciers? Snow and rain today may have a different composition than when the glaciers were formed.

PROVASOLI: Is there any definite proof that the organisms embedded in ice are viable?

BRAARUD: Yes, according to those who have studied the subject. I have not done so, personally.

PROVASOLI: I heard that some ice is colored by red, brown, and golden streaks and I was wondering if the algae are alive. In what stage are they?

BRAARUD: Resting spores.

GOLDMAN: We tried to culture any organisms contained in ice from the small Antarctic fresh-water lakes. This ice showed strong coloration near the bottom. During freezing, there seems to be a salting out and concentration of the organisms in a brine slick on the bottom of the lakes. However, we found no carbon uptake from a number of pieces of ice we melted down and incubated at 0C in the laboratory.

JOHNSTON: Those organisms may all be in the resting stage, anyway.

GOLDMAN: We kept them for several weeks and they showed no development.

STRICKLAND: Looking at the small amount of work that has been done on division times in the ocean (much of it by Doctor Braarud) I have noticed the remarkable similarity of division times of diatoms, provided they are growing enough to be noticeable. I have not, as perhaps I should have done, studied all the diatoms in Departure Bay over a 5-year period, but we did, as you know, inoculate two "bags" full of filtered sea water, one in 1960 and one in 1961. In both cases, from the initial inoculum of surface water, populations of diatoms grew up and, almost always, the doubling time of the species involved was between 30 to 40 hours.

Thalassiosira rotula occurred both years and this was exceptional as it had the shortest division time, quite noticeably shorter, about 19 hours. *Skeletonema* occurred both years, and both times had a doubling time of 30 to 40 hours. I think the distribution of the species contributing the bulk of the biomass is explicable almost entirely on the dimensions and the initial "seeding" numbers of the cells concerned.

In terms of biomass the spring bloom is characterized in our waters by the fact that plants grow rapidly until the nitrogen disappears.

The big diatoms are bound to win in terms of biomass. If one starts off with six or seven large species that grow at a comparable and reasonable rate, obviously one will never get a large biomass of a small species unless it doubles at a quite impossibly rapid rate. Insofar as the composition of the coastal diatom bloom is of importance in the food chain, and inasmuch as the condition seems to be right for diatoms each year to grow with about the same doubling time, the composition of this inoculum in any given area is probably the determining factor, and it is quite important.

JONES: With regard to the algae in the polar ice, I have a student who is conducting some experiments with various freezing regimes down to subzero temperatures of -179°C . We would be very interested in obtaining some algae from polar ice environments. Are there any that are either in culture or in stock culture?

DROOP: Yes, I think there is a *Chlamydomonas*. I don't know where it came from; it might have been only glacier. R. A. Lewin of Scripps Institution of Oceanography, isolated one. He might know.

PROVASOLI: Doctor Braarud, you mentioned one point which puzzled me very much, and that is the effect of selective grazing in changing the composition of the species. Could we hear more about selective grazing?

BRAARUD: Doctor Guillard knows more about this than I do, but there is some information in Curl and McLeod's paper.³ They observed cases of selective feeding. In the literature on grazing, one finds remarks about species that may be too large and others too spiny for the feeding organisms, but there isn't much information on the question.

I have observed that fecal pellets of copepods may be crammed with the conspicuous corrugated spines of *Chaetoceros compressus*, a demonstration of how even such thick spines do not protect a species from being grazed.

FOGG: Grazing may sometimes be highly selective. Doctor W. T. Edmondson, Department of Zoology, University of Washington, recently showed me the results of a statistical study he has made of the reproductive rate of rotifers in Windermere; he has carried out a regression analysis of the number of eggs per female rotifer per day in relation to numbers of various phytoplankton algae, and he finds a high correlation for the rotifers *Keratella cochlearis* and *Kellicottia congispina* with one particular species, *Chrysochromulina parva*. There is relatively little correlation with other organisms, so that it would appear that these rotifers are eating one kind of alga and ignoring the rest, although the results could include effects of differential digestion and differences in chemical composition of the food organism.*

PROVASOLI: I wonder if selective grazing depends more on size discrimination than on a true selection of a species. Do the rotifers eat the favored sized species?

FOGG: Not in this case.

VOLLENWEIDER: It seems that rotifers prefer a certain kind of food according to Doctor Novick at the Institute of Rome.*

DROOP: Can the rotifer make a mechanical selection as to size? I have one in culture that I have been watching a lot, and I would say that it misses 99.9 per cent of the material that comes in the vortex. Only upon occasion will an alga thrown to the mouth of the gullet actually go down. I can't see what it is, but it might be something to do with compactness.

OPPENHEIMER: Any consideration of the feeding mechanism of such small organisms must involve colloidal aspects and surface charge—Zeta Potential. I have been quite interested in the relationship of bacteria to algae and have published a paper describing how living diatoms do not have attached bacteria.¹⁵⁶ Dead diatoms have a large attached population of bacteria, which suggests that perhaps a difference in surface characteristics between the living and the dead algae can be detected by the bacteria. It is well known that when

*Personal communication.

protoplasm is denatured the surface must change due to the change in molecular structure. Resulting changes in surface potential must, therefore, be considered as one possibility for the attraction or repulsion between unicellular organisms.

I have also watched rotifers feeding, and have observed the same phenomenon that Dr. Droop mentioned. A steady stream of very small particles, including bacteria, passed into the vortex and, apparently, the rotifer was capable of selecting out discrete particles which may be the same size as other particles in the vortex. There is a very good possibility that in any feeding mechanism involving particles of 1 to 5μ , the organism may be able to detect the difference in surface charge between one particle and another. Inorganic particles negatively charged may have quite different effect than, say, a bacterium with a positive charge or a piece of detritus. The rotifer may use such a mechanism during feeding.

The significance of colloidal aspects becomes extremely interesting. In the size range of 2μ and less, the mass effect decreases as the surface effect increases, and sometimes colloidal aspects will overcome physiological activity. This is a wide-open field in which very few people are working.

GOLDMAN: Hasn't it been postulated that diatoms produce a metabolite which keeps bacteria-free?

OPPENHEIMER: Yes, this may be true of certain organisms. The sedimentary environment may contain literally thousands of organisms per square centimeter, where sand grains may take up about 60 per cent of the total volume. In the interstitial space are a large number of diatoms, flagellates, nematodes, bacteria, blue-greens—all living in a composite mixture, brushing against one another, all moving and active. One cannot imagine that extracellular antimetabolites would be active in such an environment. Of course, one cannot exclude the fact that organisms that are being inhibited will not be present. Sediments are extremely fascinating environments to observe. I made a comparison between the different types of sedimentary environments—sands, clays, shells, and so on—to determine the distribution of types of organisms. It was really amazing to see the diversity and the effect of pore space on the type of populations. Clays, for example, apparently exclude the large diatoms and the blue-greens and contain nothing but very small bacteria.¹⁵⁷

I don't know whether this answers your question, but I don't see how an antimetabolite phenomenon could explain this apparent attraction and repulsion of microorganisms. Also, almost all or a good share of the bacteria that were living on the dead or dying diatoms were polarly-oriented, sticking out like a pin cushion, again indicating that there was some sort of an attraction. If nature has endowed the

microorganism with the ability to see the death of an organism by a surface variation, then this is essentially a conservative fundamental property of nature, and enhances the role of bacteria in degradation and mineralization. How do bacteria know when something is living or dead? Nature demands that dead organisms be demineralized. Surface charge differences are a logical explanation.

BRAARUD: Shall we return to the question of initial populations?

JOHNSTON: One might add that the initial phytoplankton population has important consequences on zooplankton and fish stocks. I don't want to go into this in detail, but they are very closely related.

BRAARUD: Yes, because the whole society may change.

JOHNSTON: The whole future of what happens in the sea is related to this.

BRAARUD: In the Norwegian Sea there is a mosaic of water masses, with slightly different character, where the biological season proceeds at a different rate.^{148,158} We have observations for the month of June, when various societies are present in a great number of areas, partly because they have reached a different stage in the development of the summer populations, but also because the contribution of initial populations from coastal areas or polar waters and from the North Atlantic has been different.

In addition there is the difference in latitude, so that light conditions vary. This has been also studied by the Russians,¹⁵⁹⁻¹⁶¹ who have tried to correlate the differences in biological seasons from year to year, with the behavior of zooplankton and herring, to get the whole story from primary production to commercial fish. It seems that the herring distribution is determined by the zooplankton populations during feeding migration, and the zooplankton populations are dependent upon when the phytoplankton start to grow. So, in this way, the distribution of the herring seems to be related to the development of the spring diatom population in the waters.

It is a very complex story and we have not been able to produce any complete picture. But when we finally tried to find the causes of the differences from year to year we had to go back to two main factors. One is the transport of Atlantic water from the North Atlantic across the Shetland-Iceland line, and the other is the whole meteorological situation in the area. So it really comes back to meteorology.

STRICKLAND: Doctor Braarud, what do you think of the possibility of the benthic habitat housing the seed stock of coastal phytoplankton?

BRAARUD: Since the observations made by Ostenfeld,¹⁶² it has been assumed that when *Skeletonema* is not occurring in great numbers it sinks to the bottom, where it may survive, and then, later, either by physical processes like turbulent action, wave action, and so on, or

through change in flotation capacity, it returns again to the planktonic state. But we know very little about this and I think it is essential to get information.

STEEMANN NIELSEN: We have had a single observation related to deep water seeding. During the war, we investigated a Danish fjord and, in May, of one year, there was an enormous burst of *Skeletonema costatum*. The fjord was investigated every two weeks; One week there would be no *Skeletonema* at all, and two weeks later there were 11 million/liter. Of course, it is rather impossible to justify such an enormous population just by dividing.

I am quite sure *Skeletonema* was growing on the bottom. When the temperature increased in May, *Skeletonema* rose from the bottom up into the water and became a planktonic species.

KOCZY: There is one factor Doctor Braarud has mentioned that I have never heard discussed. Two different approaches exist which are called the Eulerian and the Lagrangein. One station or a set of stations can be studied by periodic net hauls, in which case the time variation in population on each station can be studied. The other method we have employed was to place a float in the water and follow it while taking periodic samples. This method permits study of the time variation in the same body of water.

As an example, I can mention the study of the plankton population around Scotland carried out by Peter Rae¹⁶³ a few years ago. He studied the motion of plankton species from the Clyde Sea area north, and around Scotland into the North Sea. The population was rather small in the beginning but grew heavily during its transport into the North Sea. Therefore, we must be careful in our plankton studies and not only study the changes at one point but employ a reasonable dense net of stations.

LUND: In lakes we do have effects of invasions where one lake flows into another, which may flow into a third, and so on. Then there may be changes. Good work has been done on changes in lakes, particularly in Sweden, and I think Doctor Vollenweider probably knows about it.

In our own small lake district, one can certainly see invasions due to large amounts of water being washed out from one lake to the next. The astonishing thing about it, to my mind, is how rarely this seems to have any fundamental effect on the lakes below.

To take an example, Esthwaite Water, a small lake, flows into Windermere (see FIGURE 50, p. 312). In it there is one species of *Anabaena*, and in Windermere another species. This year was wetter than usual even for the English lake district, and very large numbers of the species from Esthwaite came down into Windermere, and the increase in Windermere could be seen quite clearly after these floods.

Just before I left, the weather was quiet, as it often is in the autumn, and there was a water bloom. *Anabaena* floats to the surface—they have gas vacuoles—and I thought: Now is my opportunity, a lot of this species from the small lake has come down and has floated to the surface; I can collect it and do a “Strickland” on it—I can do some analyses. So, I collected this bloom, but what did I find? The bloom consisted of the species characteristic of Windermere, not of Esthwaite. This latter species was present in very small quantities.

This happens again and again. There is a series of three lakes in our district (FIGURE 56) and they are in a line: Lake Buttermere flows into Crummock Water, and Loweswater flows into Crummock Water. The outlet of the latter is very close to the inlet from Loweswater. Buttermere is as close to distilled water as it is possible to get, I think. It is a really oligotrophic lake in a very wet area, with high rainfall, so it gets washed out. Loweswater is a highly productive lake by our standards, so what will thus happen to Crummock? If one collects in it with a net, one finds Crummock so eutrophic, so productive, and so full of blue-green algae. But if, instead, an actual sample of water is taken and the total quantities of all the algae in it are estimated—those that pass through a net and those that do not—then Crummock looks very like Buttermere. So although these invasions are entering Crummock continuously, basically the lake keeps its own character, the character one would expect from chemical analysis of the water, if nothing were known about the algae in the other lakes.

PROVASOLI: Do I understand correctly, that the populations in Crummock are mostly derived from the outflow of Loweswater?

LUND: No, Crummock has a population of its own, which is very similar to Buttermere's, and then it has imposed on it the population that flows in from Loweswater, which never becomes very big. Though the algae are invading, they seem to fail to colonize it satisfactorily.

STEEMANN NIELSEN: How much water a month is flowing in from Loweswater compared with the volume of Crummock?

LUND: I'm sorry, I wouldn't want to try to say. [The drainage area of Loweswater is about one-fifth of that of Crummock, which of course includes that of Buttermere. As the whole area is so small and similar, one may say that this is also approximately the relative volume of water passing from Loweswater to Crummock. However, it must be remembered that the outflow of the latter lake is near the inflow of Loweswater.]

KRAUSS: You say these species have gas vacuoles?

LUND: We are dealing here with species of various kinds, some with gas vacuoles, some without. For example, the diatom *Melosira*, which is very common in Loweswater, is completely absent in Buttermere.

KRAUSS: Does it have a gas vacuole?

LUND: No, it sinks relatively fast, and never produces a crop of a marked size.

KRAUSS: Could you give an example of a species that has a gas vacuole?

LUND: Yes, the commonest blue-green algae in Loweswater is a large one, *Coelosphaerium naegelianum*, which often forms blooms; it has gas vacuoles. It is not found in Buttermere at all. It passes into Crummock, and many specimens of it can be collected in a net. One might think Crummock is a productive lake, but it isn't. This is of interest to me in relation to what Doctor Braarud has said. The thing that strikes me (and there is very little basis for this) is the stability of the lake to hold its own character against these invasions. I don't know whether Doctor Braarud has anything to say about that in relation to the sea.

BRAARUD: Yes, I may say one thing. When the current system brings outside water into another area all the time, such as in the Norwegian Sea, there is a steady introduction of populations from the South, most of which are considered as guests; they are being transported in but they don't reproduce effectively, and they don't really influence the composition of the society to any great extent. On the other hand, some southern species, such as *Coccolithus huxleyi* and the two large diatoms I mentioned, *Thalassiothrix longissima* and *Rhizosolenia styliformis*, grow well after having been introduced and become ordinarily important components of the summer societies of the Norwegian Sea. These invaders may be regarded as exceptions, but very important ones.

STEEMANN NIELSEN: During the war, we investigated the Isefjord for four years. In the inner part of the fjord there is very little water exchange. During the four years, I had expected to find about the same plankton present, but every year it was extremely different. In two of those years, which, according to all the hydrographic and weather observations were very much alike, the species found during the summer were especially different. Throughout the summer there was a plankton of blue-green algae. The following year, under the same hydrographic condition, there was diatom plankton consisting predominantly of *Asterionella japonica*. Never in all the 40 years we had investigated Danish waters had we found this species inside the Skaw, but that year it was the most common species of plankton algae. I can't explain it.

BRAARUD: Did you make observations on the plankton outside of the fjord?

STEEMANN NIELSEN: No, during the war we couldn't go outside of the fjord.

BRAARUD: Then you might have found the solution.

STEEMANN NIELSEN: Of course, the species must have been transported to the fjord from elsewhere, but *Asterionella japonica*, as far as I know, is a spring species, primarily, and it is found off the coast of Iceland at a temperature of 10C. Here it was growing during the summertime at 20C to 22C. It was quite extraordinary.

BRAARUD: It must be a question of initial population.

PROVASOLI: I think we have established quite well the effect of initial populations and of invasions on the succession of forms, so I think we should now explore other factors. Doctor Lund has mentioned that many factors in lakes can be correlated with the growth of certain species. Can he speak of factors related to the succession of forms?

LUND: That is very difficult because anything I say would be supposition. There is an important factor in fresh water, namely, parasitism. There is no question that the effects of parasites are important. For those who may not be familiar with this matter almost every plankton alga in our lakes has a parasite; many have more than one. The parasites, very largely, are fungi, but there are also some animals, very complicated organisms which I am afraid I don't understand at all. The parasites tend to be very specific. They tend to parasitize one alga or a small related group; that is to say, a parasite might infect only one species of diatom, or it might infect three or four species, but if it does this it is probable that it will not affect green algae or blue-green algae.

These parasites have a number of astonishing features in their ecology, most of which we cannot explain. For example, an algae which is not very common, or which occurs only occasionally, may be parasitized by a certain fungus for a period each year. Then the alga disappears, for years, and it cannot be found. Of course, the fact that it cannot be seen does not necessarily mean that it is not there. It probably is there, but its apparent absence for years, despite regular sampling, proves that it is present in very small numbers. When the alga reappears and as soon as it becomes relatively abundant, its own specific parasite will also appear. The problem is: What is this parasite doing in the interim? For reasons I need not go into here, we can be reasonably certain that the obvious explanation, namely, that it is always growing on its host alga, however rare this is, cannot be given as a likely answer to the problem.¹⁶⁴

DROOP: Have you had instances of the parasite being able to wipe out a crop?

LUND: No, I have never seen 100 per cent destruction of a crop.

JOHNSTON: Is it parasitism or symbiosis?

LUND: It is very clear parasitism, as I understand it.

PROVASOLI: Do the blooms appear in regular cycles? When parasites are introduced in new areas to control insects which are detrimental to crops, cycles of parasite or host growth have been observed, especially when the parasite is another insect. Only in a few instances is the parasite able to wipe out the entire population of the obnoxious host; in this case any reinfection of the host has to be checked by reintroduction to parasites. In most cases, however, an almost regular alternation occurs: Years of great abundance of the host are followed by years of high population of the parasite, and so on. Did you find anything similar?

LUND: The extraordinary thing is the irregularity of the whole system. Perhaps I can give a concrete example: Take *Asterionella*, which I am afraid I have mentioned too frequently—

PROVASOLI: As *Skeletonema* has been by the marine biologists. (Laughter).

LUND: It is a sort of fresh water *Skeletonema*. If appreciable numbers of *Asterionella* are observed, some cells that are parasitized will almost certainly be seen. But only occasionally will one see an infection severe enough to enable one to detect decreases in the crop from death of the cells. It is quite impossible to predict when this will occur, or, for me, at any rate, to even suggest a hypothesis on the subject. I am simply baffled.

JONES: Are the parasites inside the cell or on the surface?

LUND: No, outside. The system is normally fairly simple. The parasite has a flagellate stage. This flagellate stage becomes attached to the algal cell. It then throws off its flagella, and small rhizoids grow out and penetrate through the wall of the alga and then ramify inside the cell. In nearly every case, when a parasite is on a cell, it will go on growing until the cell is dead. They are not just sitting on it; they are sitting on it and growing inside it.

OPPENHEIMER: Then you should expect to see some effect in population.

LUND: You do, but it is so irregular.

OPPENHEIMER: I thought you said you didn't.

LUND: No, no.

PROVASOLI: The problem is the irregularity of the phenomenon. Normally the infection should wipe out or periodically reduce the *Asterionella* population. Something is interfering. One example of the fungus attack on organisms has been observed on copepods. In the Bothnian Bay in the northern Baltic Sea, the total population of copepods was wiped out during the summer of 1950.¹⁶⁵ The fishermen couldn't fish any more because their nets were very soon filled with dead copepods. This phenomenon appeared a second time one or two years later. The only correlation that could be found, and it was only

an indication, was the temperature. During these two years the water was extremely warm, about 21C or approximately 4C above normal, and the fungus attack appeared in the warmest period. Finally, the copepods were completely penetrated by the mycelium of the fungus.

BRAARUD: My impression is that we may find parasites in the sea, for instance, in *Thalassiosira* now and then, but we don't pay much attention to them, perhaps because so few cells are affected. But there is one genus, or maybe it is only one of the species *Dactyliosolen*, which has a parasite. I have never understood how these algae are reproduced because I don't recall having seen any cells with ordinary chromatophores. Have you seen them, Doctor Halldal?

HALLDAL: No, I have never seen cells without the parasite.

BRAARUD: When they reach our waters they seem to be so infected that only the parasite is left. The *Chaetoceros* species have small parasites on them but they don't seem to do any harm. Perhaps they are not parasites.

PROVASOLI: Perhaps they are symbions?

BRAARUD: Yes, they may be.

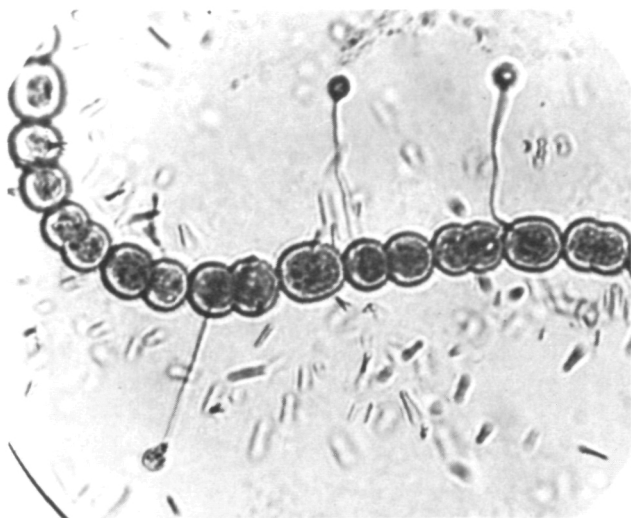
PROVASOLI: Did you observe any symbions in the algae?

LUND: Perhaps we should consider that some of the blue-green algae that we find in the plankton are symbions of blue-green algae and bacteria. In nature, bacteria are invariably found in the sheaths of some of these species, I think. But many of the bacteria are astonishing; they are rather large, and even humble light microscopists can see them.

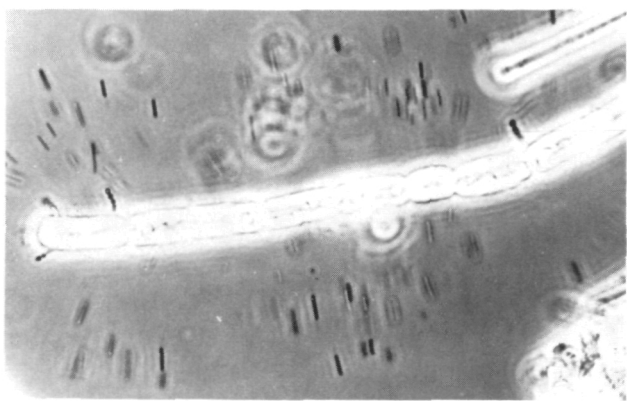
FIGURE 41a shows a planktonic *Anabaena*. The sperm-like bodies are fungal parasites. They are zoospores which have lost their flagella and produced infection tubes that have now reached the cells of the alga. I want to point out the rod-shaped bodies scattered about in the mucilage. These are bacteria and many of them are nearly always found.

I haven't the slightest idea what this means. Are the bacteria anything to the blue-green algae, or are they not? I don't know, but many algae have bacteria on them, as Doctor Oppenheimer has already mentioned. Many of the bacteria are quite considerable in size rather larger perhaps than what one might call the hygienic medical-bacteria, and they have a remarkable structure. They have one other unusual feature, which is very unfortunate, in that nobody has ever managed to grow them. We have a bacteriologist, Miss V. G. Collins, who has tried many times, but so far she hasn't found a medium in which they will grow.

Whether this has any meaning in a symbiotic sense or not, I don't know, but the fact remains that the question of bacteria growing on and around algae is a very important one, about which we ought to know a lot more.

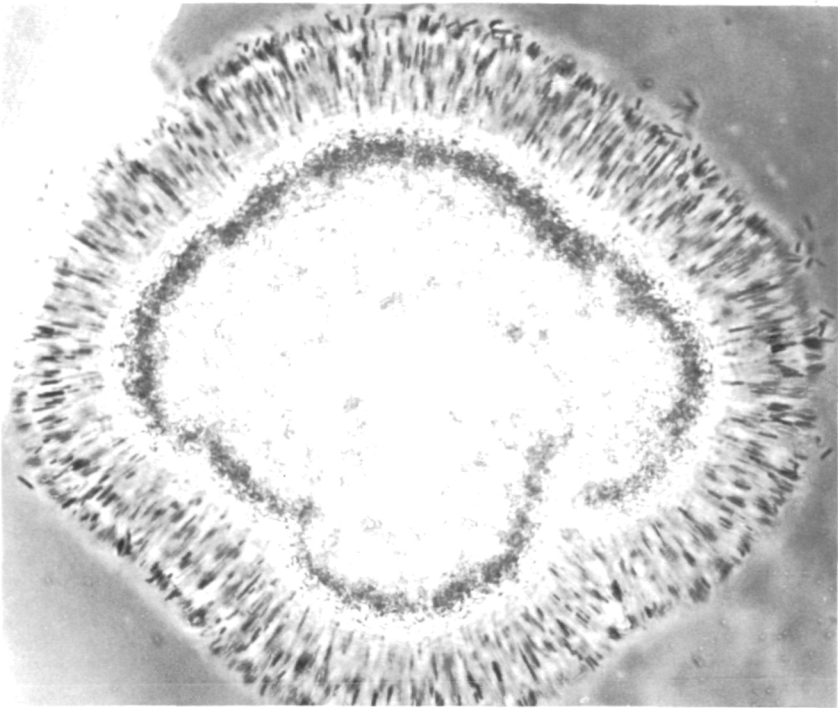


a

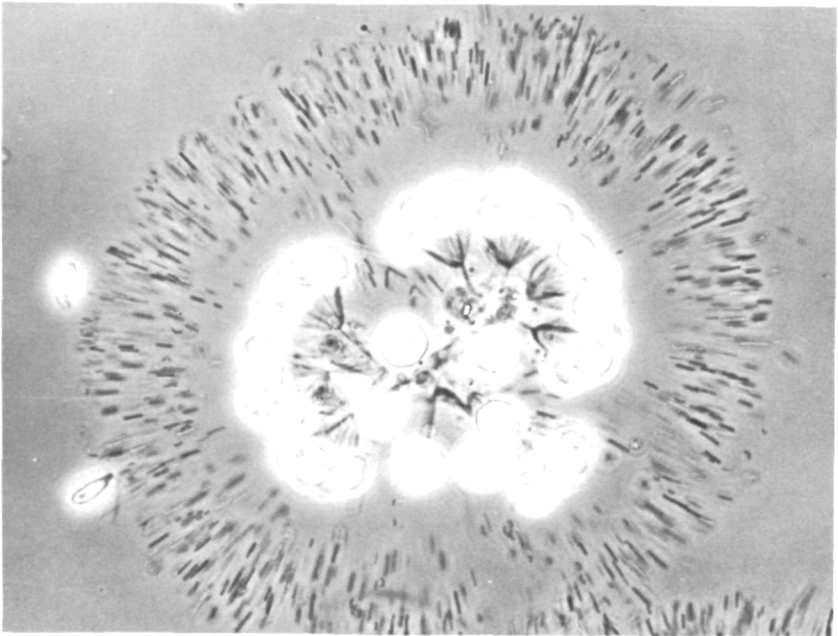


b

FIG. 41. Rod shaped bacteria in the mucilage sheaths of planktonic algae. a. *Anabaena* sp. $\times 1550$ Bright fluid. b. *Geminella* sp. rod bacteria perpendicular to algae. $\times 1000$ Phase. c. *Coelosphaerium naegelianum* $\times 650$ Phase. d. *Dictyosphaerium pulchellum* $\times 550$ Phase. Photographed by Doctor H. M. Carter. (Permission, Annals of Botany, London.¹⁶⁶)



c



d

FREMONT-SMITH: In connection with what Doctor Oppenheimer said before, the physicochemical aspects are very important when we are dealing with this kind of relationship. Shakrum's work with various strains of Flexner bacilli, showed that the virulence is inversely related to the surface charge. This could bear indirectly on the relationship between bacteria and a phytoplankton or zooplankton, or the question of parasitism or symbiosis, I think we are now reaching a level where it becomes necessary to consider the physicochemical aspects of the situation as well as the so-called bacteriological, and the so-called biological aspects.

OPPENHEIMER: Your comments bear out what I mentioned a few minutes ago. Not a single bacterium was attached to the blue-green cells *per se*. On sheathed blue-greens there is apparently a mucoid material which the bacteria like very much, and one can see large numbers of bacteria resting on and in the sheaths.

LUND: What do you mean by a sheath?

OPPENHEIMER: A mucoid membrane which covers the whole aggregate of blue-green cells.

LUND: FIGURE 41a showed the mucilage around the alga.

OPPENHEIMER: Some blue-greens in sediments, seen by phase microscopy, have a much more definite mucoid sheath and look as if the blue-greens were living within a case.

LUND: Well, this blue-green lives within a case of mucilagenous material and that is what the bacteria are living on, inside, not actually attached to the cell within this.

OPPENHEIMER: This discussion on the attachments of bacteria brings up another point. When considering microbial activities, one must separate intracellular from extracellular mechanisms. Theoretically, an extracellular enzyme will supply energy to the bacterium only as far as contact-chemistry will allow. An intracellular enzyme must have a substrate—if it is acting on an external substrate, the bacteria must rest directly on the substrate—before the enzyme can afford the bacteria the result of the reaction. So, one almost always has to consider the contact effect in any microbial activity involving large particles. For example, if the bacteria were not directly in contact with the blue-green, there is some doubt as to whether there is any direct reaction between the algae and the bacterium. If the bacterium was resting directly on the algae, it would be a little different. Proteolytic enzymes, for example, are responsible for breaking down large molecules, some intracellular and some extracellular. One must always visualize unicellular reactions from the viewpoint of looking from inside the organism out, so that one has perspective. Otherwise, one loses sight of many possible explanations.

DROOP: There is a possibility of gas exchange at that sort of distance between bacteria and the photosynthesizing cell.

ALLEN: There is a great deal of variation between blue-greens as to the density of the sheath.

LUND: The outside of the sheath is now shown in FIGURE 41a. The photograph was not made for that purpose. The point that interested me is that, as far as I can make out, all the bacteria are in the jelly material, but I am not convinced that I see any on the actual cell surface. [FIGURES 41b, c, and d which were not shown at the meeting, are added to illustrate Doctor Oppenheimer's remarks about the use of phase contrast for showing the whole mucilage sheath of algae. They also show some more striking examples of the abundance and restricted distribution of rod-shaped bacteria in the mucilage sheaths of planktonic algae than is shown in FIGURE 41a. FIGURE 41b is also of interest in relation to Doctor Oppenheimer's other remarks about the relation of bacteria to surfaces. The rod-shaped bacteria are disposed freely in the mucilage but the small groups of about four bead-like bacteria in chains are attached to the wall of the algae. FIGURES 41b, c, and d show a variation in the orientation of the bacteria to the two species. The flask-shaped cell protruding from the margin of the mucilage at eight o'clock on FIGURE 41d is a *Chlamydomonas* sp. which is attached by its papilla region from which one flagellum can be seen bending backwards.]

FREMONT-SMITH: Are the bacteria inside or on the far surface of the sheath?

LUND: They are inside. In some blue-green algae they tend to be localized as to position. In some of them—this is in the mucilage—there is a layer of bacteria in a certain position here perpendicular (FIGURES 41b, c, d). In fact, I have had them shown to me as mucilage itself, lines of striated mucilage.

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EXPERIMENTS WITHIN NATURAL AND ARTIFICIAL SOCIETIES

Discussion leader:

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STRICKLAND: In opening up the discussion of experiments within natural and artificial societies a quick recapitulation of the factors which cause apparent competition may perhaps be useful. Although we have already mentioned most of them a list can be made:

(1) The effect of the gross nutrient level is not well known, but there does seem to be a tendency for a lot of nitrogen and phosphorus to favor the growth of diatoms.

(2) Of equal importance is the ability of the organism to utilize the nutrients that happen to be available, and in this case I think the ability of certain organisms to utilize organic stocks of nitrogen and phosphorus will be advantageous.

PROVASOLI: Or ammonia.

STRICKLAND: Yes, or ammonia—which may give it an advantage over organisms that cannot.

(3) An organism may have a requirement for a water-soluble growth factor.

(4) There is (and this is another thing we don't know too much about) the effect of the concentration level of the transitional elements in seawater, and their state of chemical combination, which is tied in with the presence of chelating agents and agents which assist the transfer of elements across a cell membrane.

(5) Plant hormones, to use a very generalized term, affect competition, as well as other conditioning agents that may have been produced either by other plants, or by bacteria, or from terrestrial humus.

(6) There is, also, a true induction period which may occur with an organism in the way that Doctor Fogg has pointed out—a sort of autocatalytic effect which may be a function of the organism itself or other plants growing with it.

(7) The adverse effect on one plant of the secretions of the plants and communities that grow with it.

(8) The effect of temperature and light, and, of course, temperature and light and nutrients all acting together in a complex manner. There is the effect of grazing by zooplankton and of the "seeding" of a given area from the benthos.

All these factors have been discussed to some extent and there has been, I think, a general consensus of opinion, with perhaps some dissent, that we must attempt to do a few relatively simple experiments to straighten out our ideas on what are some of the controlling factors in the natural environment. These experiments can be done in two size ranges: One can either use a beaker or a Bay (or an approximation to a Bay) and the great danger of course, is in the extrapolation from beakers to Bays.

I would suggest that there probably is very little point in trying to work on a size-scale intermediate between something that can be handled reasonably well in a laboratory and a very large container. If we are to work in relatively small vessels, it is neater, and probably saves a lot of grief in the long run, to try to work with aseptic conditions and use, if possible, axenic cultures. But this may not be absolutely necessary.

The main reason for using a very large vessel is that the ratio of volume surface area is only a linear function of the dimension. If the surface area of the phytoplankton in seawater is calculated, even allowing quite a reasonable crop, then it is necessary to have a spherical vessel of the order of 20 or 30 feet in diameter before the surface area of this vessel becomes relatively small in comparison with a surface area of plants or detritus present in the water.

The large-scale experiments that have been done to date are of two types. There are bags, and I think the bag situation has been pretty well covered; another, and I think a more elegant technique, is to use a comparatively wide, enclosed column of water, preferably from top to bottom of the euphotic zone. Ideally, work should be done in a shallow lake or an inshore situation, going right to the bottom of the water column.

I would suggest (and I speak with some experience here) that making a 20- or 30-foot diameter sphere should not be undertaken lightly; it is an appalling chore. If you wish to go to a large scale, think very seriously about it before you start, and then if you do it, I suggest that you really go to a sufficiently large container.

The ability to remove a wall effect is obtained by having either a large vessel or by somehow managing to make walls which will support no epigenous communities. This, I think, is pretty difficult unless somebody can produce a plastic containing antibiotics, or be very clever and produce a large, thin film of ice that is continually renewing

itself around the sides of the vessel. This may be quite an engineering feat.

The only point about a bag experiment that hasn't been mentioned is that it is also useful for studying decay processes, which are of some importance. The emphasis on the bag experiments has tended to be on the growth processes. I will not take up time here describing it, but we have obtained some very useful information about decay cycles in a phytoplankton community by using a bag.

I am not quite sure whether anybody else has had the misfortune to have started putting bags of any size on the ocean. If they have, I suggest that they give us an account of their troubles. I know that one or two people have started to put columns in, and this seems to be a very attractive proposition because, with a column, stratification can be obtained immediately and quite a few things can be studied that cannot be studied in a bag.

We have not completely covered, perhaps, the whole question of the excretion of plant hormones and the effect of one plant community upon another. I know that Doctor McLaughlin, at any rate, has actually attempted to imitate a community of plants together in one vessel.

Doctor Johnston may want to mention some other experiments. Then there have been some very interesting experiments done by the people at Woods Hole where they have enriched seawater with various compounds that might be expected to alter the growth of plant organisms, and have studied what happens. Doctor Provasoli, I think, can give us a summary of that work.

Perhaps we might start off by mentioning the question of bags and columns. Has anyone put a fair-sized bag in the water recently?

MCLAUGHLIN: What is a fair-sized bag?

STRICKLAND: One that is several feet in diameter.

MCLAUGHLIN: I haven't put that type of bag in. The type of bag I have used is a two liter plastic bag (Falcon Plastics)—you are probably familiar with it—with gradations on it. We used a commercial Millipore filter and we sterilized the whole business and filled the bag.*

PROVASOLI: It is of the canteen type.

MCLAUGHLIN: Yes. In fact, it is used for picnic liquids. It has gradations on it for the volumes, and air can be excluded from it. Then we obtained from a garden supply store some plastic coated fencing, from which we made a wire cage. We used a large plastic bag as a liner for the cage and put our 2-liter capacity Falcon bags inside this retaining bag.

*This work is being done with Doctor Jan C. Prager, at the new U.S. Fish and Wildlife Marine Laboratory, Sandy Hook, New Jersey.

STRICKLAND: These are bags within bags?

MCLAUGHLIN. Yes. We have also attached the small plastic bags to the wire mesh itself. We had two wooden poles driven into the bottom about 50 feet offshore, just in front of the Marine Laboratory at Sandy Hook, where there is at least eight to 10 ft of water at low tide. We secured the large wire basket to the poles with rope. This pole-positioning permitted great variation in the placement of the basket culture. We could anchor it at the bottom, or at any level up to the surface, depending on where we tied it to the pole.

We have only started with this work. The first thing we tried was growing the organisms in the bag in artificial medium and in enriched seawater, taking the water from the area, much like Doctor Johnston has done. We tried to determine what enrichments were needed for growth in this particular seawater under these *in situ* conditions. We hope ultimately to compare our artificial media with the enriched seawater.

STRICKLAND: The point of having the organisms in a bag is to maintain the same temperature environment?

MCLAUGHLIN. Exactly. And light, too. Because of our ignorance of the amount of light and effects of temperature, and our inability to control laboratory temperatures, light sources, or cycles to any great degree, these *in situ* studies seem mandatory. We hope ultimately to compare the same cultures grown under "natural" conditions with those grown in the laboratory. It is all very preliminary.

We had a bag 10 feet long and about one foot in diameter which was doing very well, then along came a sharp object and punched a hole in the experiment and it went "out to sea." Such sorrows! I'm sure most people have had them with this type of "local" experimentation. A hurricane blew one complete set of bags out to sea last year. But aside from that, it's just another way to find out how our *in vitro* data checks out if the organisms are put into natural seawater. We have used *Isochrysis galbana* and *Melosira* sp. Our work is still very preliminary, as I said, but I hope it will develop into a "farming type" operation. That is the only bagging I've done so far. We believe that there is a future in this type of *in situ* production, especially as regards potential food-chain organisms or others which may require large amounts of cells for some specific chemical studies.

STRICKLAND. Doctor Goldman has started using cylinders of a reasonable-sized diameter in lake water and I believe they have also had some success at Windermere. Perhaps we could hear a little about the philosophy and practical application of cylinders.

GOLDMAN: Some of the problems inherent in oceanographic investigations are also inherent in work with the larger lakes, most notably, Lake Tahoe. It is one thing to put a 100-foot long plastic cylinder in a

small lake (such as Castle Lake, California) that is fairly well protected from the wind and quite another to try to put a bag or cylinder of larger diameter in Lake Tahoe. The latter was attempted this summer and, due to adverse weather conditions at the time, I would say the experiment was not very successful.

A cylinder has the advantage over a bag of isolating a water column, so to speak *in situ*. The thermal stratification is not changed. However, if the bag or the cylinder cannot be anchored and there are subsurface currents, such as develop to a considerable extent in the larger lakes, the bottom of the cylinder bag may be much higher than intended and the stratification may be upset.

STRICKLAND: Could you tell us a little about what you were proposing to do with this type of experiment; the philosophy behind it?

GOLDMAN: The idea is simply to go to a much larger culture container, reducing the wall effects by making it a large cylinder, and, of course, reducing the surface: volume ratio appreciably. The large cylinder is good because it can be sampled very easily. One can remove a reasonably large sample from these cylinders without withdrawing a very significant portion of the total volume.

STRICKLAND: How would you have sampled it?

GOLDMAN: With conventional Van Dorn water samplers. If they are lowered slowly, the stratification within the cylinder is not upset. It is important, of course, to work during a period of good firm thermal stratification, and the cylinder must penetrate the thermocline to achieve this kind of stability.

STRICKLAND: Do the cylinders go right to the bottom of the lake?

GOLDMAN: Almost to the bottom—to the bottom of Castle Lake, but, of course, not to the bottom of Lake Tahoe which is over 500 meters deep at its greatest depth.

PROVASOLI: How far down from the surface does it go?

GOLDMAN: The cylinder is 30 meters long.

PROVASOLI: But it starts off right at the surface?

GOLDMAN: Yes.

PROVASOLI: So that water cannot enter.

GUILLARD: What is the diameter?

GOLDMAN: I have made three different diameters, one slightly over one-half meter, one of $1\frac{1}{10}$ meters, and one in between these two. The construction is quite easy up to a point. It simply requires a sheet of polyethylene of the desired circumference and plastic tape with which to make one seal. That is the easy part. Putting rings on the outside is not particularly difficult but achieving a good anchorage in a lake that has much movement in it is quite difficult.

STRICKLAND: It is possible, then, to study the total community of animals and plants with this cylinder? However, it can't be anchored

to the bottom and filled with filtered water?

GOLDMAN: No, certainly not. However, if a mesh of a particular coarseness is put in, say, to exclude the larger zooplankton, the cylinder with the mesh bottom in it could be lowered and it would filter as it fills.

Another possibility is a method we tried this summer at Lake Tahoe, using sausage-shaped bags which are simply anchored and allowed to drift free with the current. This eliminates many of the problems of structural failure that arise with cylinders, where they are subject to wave action and current action simultaneously.

BRAARUD: Do you have any impression of the effect upon the water quality if you use plastics?

GOLDMAN: No, I don't. Perhaps one of the advantages of a cylinder or a thin film of any kind is that it transmits a lot of the water movement through the skin because it is, of course, very thin and doesn't really provide any structural barrier to the movement. It is just isolating the water contained therein.

PROVASOLI: How about gas diffusion?

GOLDMAN: I have experimented, trying to use C^{14} in polyethylene, and it goes right through it. So, we have a lot of CO_2 permeability. I cannot speak with any authority about other gases.

OPPENHEIMER: Most gases are readily exchanged through polyethylene and it is quite common to keep goldfish in a plastic bag sealed for storage and shipment.

GOLDMAN: Yes. This perhaps would be a real advantage in using a film over using, say, glass or heavy plastic-like Plexiglas. Thomas's "Test Loten" are made of clear Plexiglas, actually screwed together in joints.¹⁶⁷

STRICKLAND: Might it not be an advantage to prevent any horizontal gas transfer?

GOLDMAN: I suppose it depends upon the experimental design. I think if nutrients are simply added to a tube, it would be well to maintain a gas equilibrium within the cylinder and the surrounding water.

STRICKLAND: Doctor Lund, would you like to comment on the Windermere situation?

LUND: As far as what I would call sausages are concerned, that is, polythylene bags shaped like sausages, we have had much the same experience that Doctor Strickland indicated one has to go through before one has a satisfactory container. We have had many difficulties, and in our area one very severe difficulty, which I don't think has been mentioned, is that there are an awful lot of tourists. There are a few things more attractive than an apparent "whale" in a lake. (Laughter).

Our idea behind using these sausages has been to isolate a relatively large amount of water in a large container, to thus reduce the wall effects, and, then, observe the effect of adding various nutrients. We were specifically interested in studying unusual events that take place in the lake.

For example, FIGURE 40 emphasized the regularity of a plankton cycle, but of course it is not absolutely regular. A cycle such as that of *Asterionella*, which I mentioned earlier may take place year after year, but then suddenly there will be a year when things do not go exactly according to plan. It is when a change of this sort takes place that it would be valuable to isolate some water and add some nutrients to see if we can discover what it is that has caused this unusual occurrence.

Unfortunately, the technique has not worked so far. We are still in the stage of going through practical difficulties. As for columns, that is cylindrical tubes placed vertically in the water, the difficulty in lakes is that water inside columns changes its character very rapidly. We have, for example, five columns in a small lake. Each one is three meters wide and about 15 meters in length. Under these conditions one might think that a nice large body of water had been isolated and that one would be able to study some interesting aspects of the biology of the lake.

The remarkable thing, in fact, is the rate at which the water inside these columns changes, so that instead of examining the lake one is examining some ponds which have been made inside the lake. So, for my purpose, in the study of plankton, these columns have not proved very fruitful.

VOLLENWEIDER: What kinds of changes occur?

LUND: The typical plankton algae become much less numerous. Some may even completely disappear, and in their place appear what I would call pond algae. The sides of these containers particularly very rapidly grow a vast mass of filamentous and other algae. Directly after this happens another change takes place, namely an enormous number of animals appear, the same sort of animals, particularly *Crustaceans*, that are found in among tangles of algae around reefs or on the shore. In this short time the situation can become entirely different.

PROVASOLI: It is fantastic.

LUND: Yes, it is extraordinary.

GOLDMAN: What are the time intervals? How long does it take for this to develop.

LUND: It depends on the time of year, of course. I would say, in a month or less.

DROOP: Is it a eutrophic lake?

LUND: Yes.

GOLDMAN: Is there noticeable warming inside the cylinders, as opposed to outside?

LUND: No; if there is stratification it is the same inside as outside, except sometimes under ice cover.

GOLDMAN: In my own experiments, I have never intended to keep cylinders in the water more than, say, a week at a time, at most. Did you find that the changes were very rapid, or was the community built up over a period of time?

LUND: It is quite variable. With some things it is very rapid, with others very slow. I don't think I have enough data at the moment to start making any sort of statements.

PROVASOLI: Did you fertilize the water inside?

LUND: No, I had planned to, but there are only five columns and quite a number of people in our laboratory are interested in them for different reasons, so I could not take all the columns for my own use. Then it turned out, in fact, that this biological change took place relatively rapidly and, when it was also found, quite clearly, that they could be used for certain chemical investigations with great success, quite naturally, I rather stepped out of the picture.

STRICKLAND: What are the chemical investigations?

LUND: The effects of stratification on, for example, the development of anaerobic conditions.

STRICKLAND: This is relevant to the population growing in them, surely.

LUND: Yes, it is. But of these columns, one of them was, as it were, an extra, just a column open at the bottom, while the other four were in pairs. The bottom of one of each pair was open so that the bottom of the tube was actually in the surface of the mud. The bottom of the other member of the pair was in touch with the mud, but closed with polyethylene. So in one tube mud is in contact with the water above, and in the other it isn't.

It was discovered very rapidly that a rather surprising thing takes place with these columns. When the lake becomes anaerobic in the lower layers, shortly after stratification develops, the water inside the columns does not become anaerobic, which is roughly the exact opposite of what I would have suspected, since we have isolated the column and reduced the movement inside it. This was true whether or not the column was aerated.

That was one of the reasons why it was much more profitable for the chemists to take over; so I am not sure, from the point of view of our present discussion, that I have very much of value to say. Perhaps I should add that the reason for this curious performance is not understood, although you will yourselves understand that now I am talking

not about my work at all; I am talking about F. J. M. Mackereth's work.¹⁶⁸

It is just possible that in the development of anaerobic conditions in this lake, and very probably in many others, what actually happens is this: In the spring there is a big crop of algae, such as was shown in FIGURES 39 and 40, and when this crop dies and descends to the bottom, decomposing, there is a large utilization of oxygen and up near the surface a deoxygenated layer is built up, which, in part, is flowing downward. Consequently, in the center of the lake there are very anaerobic conditions, or more than, in fact, there would be if there wasn't this flow. It is a matter of flow. When the tubes were put in the water, which was partially anaerobic and becoming more so all the time, it flowed down the slopes and came to form a pool around these tubes, but it could not get into them. Consequently, it was more anaerobic outside than it was inside. There was a lag before the anaerobic conditions began to be marked inside the tubes. This is, of course, a purely tentative explanation and an alternative one could be imagined.

Even more astonishing things have happened in these tubes. When I left Windermere recently, the two tubes which were being aerated and mixed by having compressed air blown up from the bottom contained less oxygen than the two that were not aerated.

JOHNSTON: By less oxygen, do you mean less integrated throughout the column?

LUND: Yes. That is very difficult to understand. That also includes a tube which was not in contact with the mud.

JOHNSTON: Could this be due to washing the attached algae from the walls—that is, adding to the production of anaerobic conditions?

LUND: That is something we must look into next year. We don't know. At the moment, we have no explanation for this curious result. There is one difficulty, incidentally, in putting polyethylene tubes in lakes: If the lake freezes, the polyethylene at the top will be ripped to pieces, and also become very brittle. You probably have experienced this.

It was essential, especially for my purpose, to put the tubes in before the spring growth period started, so that I would be ready. That was a great mistake. We had unending difficulties; in fact, we had to sink the whole lot well below the surface to prevent their being torn to pieces by freezing and thawing.

STRICKLAND: Has anyone actually tried to fill a tube with filtered lake water?

LUND: No, we have not.

STRICKLAND: Had this been done, the inoculum of some of this wall material might have been removed.

GOLDMAN: This can be done too, by simply filling it as it is sunk to the bottom.

LUND: A rather large amount of water would have to be filtered. I can't say how many tons are involved, but our tubes are three meters wide and 15 meters deep.

STRICKLAND: Oh, yes, one would need a big plant. I think this is very interesting. Has anybody else had any nice, crazy-looking equipment on a large scale?

BRAARUD: I think we should hear a little more about the Strickland bag. We haven't heard anything about the bag, itself.

STRICKLAND: The actual construction?

BRAARUD: Yes, and your experiences.

STRICKLAND: I will send you a reprint.¹⁶⁹ One of the troubles with cylinders in sea water is the effect of tides; people working in lakes are pretty lucky. A 15-foot tide, with a cylinder, requires some sort of gasometer device or bellows.

An additional problem near Nanaimo, British Columbia, is that there are large changes of salinity from the Fraser River outflow, so that a 20-foot diameter sphere containing water of salinity 29 parts per thousand may become surrounded overnight with water of salinity 23 parts per thousand and sink like a stone and, alternatively, rise like a whale unless there is a very large compensatory flotation buoy. This was one trouble we did anticipate, fortunately. The worst problem was that the bag had a very long narrow neck. (The thin neck was desirable so that one had the minimum surface area of atmosphere.) Some rather nasty density changes can occur between the water outside and the water in the bag, and this produces a sphincter in the plastic of the neck. After about one week of operation, we found it was impossible to get anything into the bag! This desperate situation was relieved by pouring fresh water in the top. Of course, boats were always hitting the assembly and the stirrer would break down, and so on.

FOGG: How did the stirrer operate?

STRICKLAND: In the old days in America, washing machines were stirred by water pumps and we found one of these in a junk yard. The water that was used to fill the bag was diverted into the washing machine and it lifted a piston up and down. We found that if we had two inverted parachutes going up and down with a stroke of about two feet, the forced circulation became absolutely enormous. One or two strokes would have no effect, but eventually an entire column of water was set in motion and the result was probably better stirring than could be done with any paddle wheel arrangement.

DROOP: What was the frequency of the stirring?

STRICKLAND: I know it too desperately well—up in eight seconds and down in eight seconds. It is a very efficient method of stirring; when carried on long enough the whole system becomes resonant.

The third bag we put in the water lasted for 100 days. The phytoplankton crop grew up, depleted the nitrate and phosphate in about 20 or 30 days, and then began to die and to decay. The decay was, of course, in an illuminated sphere, so in order to imitate what might happen to plant cells sinking beneath the euphotic zone we blackened the top half of the sphere with large sheets of black plastic. We didn't cover the bottom half, but the amount of back-scattered radiation in sea water as densely populated as our coastal water is very small and radiometers indicated that there was very little light entering the system.

We measured the production of mineralized nutrients for about 80 days. We did not, however, stir all this time. We were hoping that we would see ammonia, nitrite and nitrate produced in nice regular succession. When absolutely nothing had happened after about 54 days, we stopped the stirring and tried to compact the mass of detritus on the bottom to encourage the right bacteria to develop; but still nothing happened.

The regeneration cycle is, I think, rather interesting (FIGURE 42). It starts at 30 days as arbitrary zero, when the large diatom crop had

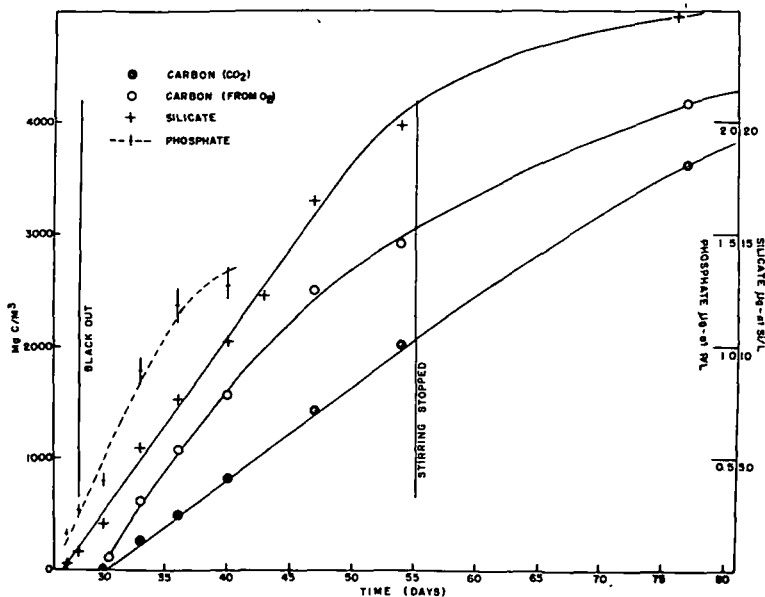


FIG 42. Remineralization of a diatom crop in coastal sea water. (Permission Limnology and Oceanography).

begun to decay. The phosphorus regeneration was very rapid, as everybody expects, and, in a little over one week, one-half had remineralized, and then we had a very severe contamination. I have not yet found out what is was; I suspect a fisherman threw in a dead fish! The phosphorus concentration went up ridiculously, so I am afraid we can't give much credence to the phosphorus curve after the first week or so.

The silicon regeneration until day 55, when we stopped the stirring, is a zero order reaction kinetically and is what would be expected from an approximately uniform surface undergoing dissolution at an approximately constant velocity per unit area. After compacting, of course, the velocity is less, but something like half the silicon had returned to solution in a matter of only about 25 days. This makes me wonder how a diatom ever reaches the bottom of the ocean at 5,000 feet unless the temperature effect is very appreciable. The mean temperature was about 17C.

The most interesting thing is the regeneration of carbon dioxide which is linear and, remembering that the stirring stopped at day 55, the regeneration is remarkably linear through the entire period. At the end of the regeneration period, about 4,000 mg C/m³, as carbon dioxide, had appeared, which was considerably more than the particulate carbon that was produced initially by photosynthesis. In view of the fact that most of the particulate detritus was still visible, I am led to suppose that in the sea oxygen depletion is not due to the direct oxidation of much of the particulate matter, but that it is the large reserve of organic material that is consuming oxygen with the detritus material acting largely as a substrate for the bacteria which "catalyse" this reaction. Of course, some of the particulate material may well have disappeared, but not a very large amount.

The corresponding plot calculated from the decrease of oxygen is most definitely not a straight and it appears as if the respiratory quotient of the process changes the whole time. Water immediately after a phytoplankton bloom uses up very much more oxygen per unit of CO₂ produced than is used up by comparatively old water.

STEEMANN NIELSEN: What about the silicon? Diatoms are able to sink to the bottom, but many diatom species are not able to reach the bottom because all the silicon is dissolved before they can do so. There are two forms of silicon in the diatom; one is easily dissolved and the other dissolves very slowly depending on species.

STRICKLAND: That is what Jorgensen says.¹⁷⁰ The easily dissolved portion has certainly to be over 50 per cent because there is no kink in the curve in FIGURE 42. I wonder if some of the diatom frustules found on the sea bottom in the deeper parts of the ocean are not from diatoms growing heterotrophically on the bottom.

STEEMANN NIELSEN: I would suppose so.

STRICKLAND: One doesn't have to use enormous bags or columns, and one obviously can still produce valuable information with beakers. I have been very impressed recently by a real induction period in our cultures of *Skeletonema*, and Doctor Fogg has already described the glycolic acid experiments. Has anybody any evidence that there is such a thing as true induction periods in the growth of phytoplankters in the sea itself?

FOGG: A lag phase has been described for several different species of alga. I don't think any of them are glycotopic. For instance, Doctor Spencer has described a clearly marked lag phase in *Phaeodactylum*.²¹³

SPENCER: *Phaeodactylum* will show a lag phase. Apparently from its kinetic characteristics, it is basically similar to the one Doctor Fogg has been talking about for *Chlorella*. Whether there is a similar production of glycolic acid, I don't know.

DROOP: In *Nannochloris oculata* a very similar phenomenon occurs. Cultures set to pH 7 had an induction period that varied with the size of the inoculum, just as Doctor Fogg was describing, and it could be spared, if that is the right word, by various organic acids, notably by glutamic acid and, as it proved later, by bicarbonate. Then I found that when pH was set to 8 instead of 7 there was no induction period, and I concluded that my supposed "diffusible intermediary" was merely the hydroxyl ion.

KRAUSS: We had a *Chlamydomonas* that showed a rather interesting type of lag phase. A few years ago, we were irradiating with X rays to see what mutants we could get, and one of them showed a lag phenomenon which I think is of some interest.

FOGG: Lag phase and induction phase are synonymous, I take it.

KRAUSS: They may be called synonymous. Wild-type *Chlamydomonas* in a test-tube batch culture shows a typical sigmoid curve. Presumably, the early part of the curve is exponential and then tapers off as things become limiting and the population becomes fairly large.

When we inoculated one of our mutants to a minimal medium, the lag phase was very, very long, a matter of many days. Then, after approximately 10 days, the mutant began growing and the exponential part of the curve showed exactly the same rate as the exponential part of the growth of the typical wild type.¹⁷¹

This was very disappointing because it wasn't the kind of mutant we were looking for. We hoped that it would turn out to be a mutant for amino acids or something that was absolute. What appeared to be happening was that the organism had a weakened capacity for the synthesis of something, such as a vitamin or an amino acid that had to be built up to a certain concentration in the water before the organism could begin to grow. If one took a small inoculum during ex-

ponential growth and placed it back into another fresh medium, exactly the same very long lag phase took place. This is evidence something like that mentioned by Doctor Fogg earlier, that things are secreted into the solution, particularly in small test-tube type experiments, that are required to maintain exponential growth.

GOLDMAN: Could the long lag phase of the mutant type be removed by using the preconditioned medium of the wild type?

KRAUSS: Yes, we could add a complex medium enriched with vitamins and various things to overcome this lag phase. So it appeared to be a secretion.

OPPENHEIMER: Speaking of long lag phases, some time ago, Doctor S. C. Rittenberg, of the Department of Bacteriology, University of Southern California, showed me some experiments that were quite revealing. He had set samples of seawater aside for periods of as long as nine months, and, at the end of this time, they showed a growth of sulphate-reducing bacteria. One would say this would be a lag phase of nine months. It would be rather interesting to look at some of your resting cells with this in mind, Doctor Krauss, because if they do fall to the bottom and, at some later time, reinoculate the surface water, maybe they would have similar exceptionally long lag phases. What are they doing during this period of time? Are they just resting, do they have to metabolize, are they building the concentration of some chemical? It is a rather curious phenomenon.

STRICKLAND: If everything was as nice and neat as Doctor Fogg's glycolic acid work, then we could presumably expect that there would be enough glycolic in the sea, directly after the spring bloom started, to set the others off; but if we have to presuppose that *Thalassiosira rotula* has to produce its own particular compounds, then we get quite a different order of complexity. I suppose the answer is that none of us know at the moment.

JONES: Isn't it also possible that certain substances in solution, perhaps toxic substances, could result in the development of a lag phase for other organisms?

STRICKLAND: This subject of toxic materials has brought me to another point. There is a considerable amount of literature on the subject of the excretion of a substance by one alga which is inhibitory or occasionally enhances the growth of another, but most of the work has been centered around the fresh water Chlorophyta and Cyanophyta, and not too much has been done with axenic cultures.

The evidence is quite conflicting and rather rudimentary. The question of one marine phytoplankter producing an auxin-like material which stimulates another has, I think, never been completely substantiated. Is anyone aware of, or working on anything related to, the question of mutual antagonism, preferably, of marine phyto-

plankton? Doctor Guillard, would you like to pass on any words of wisdom on the "croaking factor" for diatoms, as I believe you call it?

GUILLARD: The only thing I can say about it is this: we all have observed that if many cultures are in the light in a nutrient medium, they do not all attain the same final density; some become terminal when they are very dense, and others when they are less dense, so something obviously stops some of them from growing. I think Doctor Droop mentioned to me at one time that *Skeletonema* will bleach rapidly in strong light when the culture gets old. I think you said that the bleaching actually spreads; that you can see it start at some point in the flask and then spread.

DROOP: The experiments are done in a flat-bottomed flask with light from beneath. Bleaching always starts at one little point and spreads out. It is like a forest fire.

GUILLARD: I have not observed that, but in the case of a culture of one particular diatom that bleaches very rapidly, I have found if I filter off the cells and reinoculate the water, neither that same species nor certain other ones will grow. But if I first treat the water with charcoal and then inoculate, all species will grow in the water again. Obviously, some toxic organic matter has been removed.

MCLAUGHLIN: We were interested in whether, first of all, we could develop a common synthetic medium which would grow the organisms *Gyrodinium* sp. and *Peridinium* sp., equally well. We then determined the growth of each organism independently in this medium (TABLE 7). Equal numbers of both organisms were placed in this common medium and subjected to various light intensities, both organisms singly, grew well in this common medium. FIGURE 43 shows that at the higher light intensities the *Gyrodinium* will grow about one-fourth of the density of *Peridinium*. At all light intensities tried, it appears that the *Peridinium* will grow to greater cell concentrations than the *Gyrodinium*.

STRICKLAND: In a given time?

MCLAUGHLIN: In a given time. If they are mixed, starting out with equal numbers of each cell, *Gyrodinium* overgrows the other organisms.

At 250 ft-c, the *Gyrodinium* in the same time period will only grow about half that cell number, but the *Peridinium* still grows rather well; but once again, when the *Gyrodinium* and the *Peridinium* are mixed in one culture tube, the result is all *Gyrodinium*.

At 125 ft-c, the *Gyrodinium* alone is just barely growing; three or four divisions here in a week. The *Peridinium* does rather well alone, and in mixture all *Peridinium* survive.

STRICKLAND: By "all," do you mean literally all?

MCLAUGHLIN: Yes, the entire 10 ml population.

TABLE 7
ARTIFICIAL SEA WATER BASE*

NaCl	2.5%
MgSO ₄ ·7H ₂ O	0.9%
KCl	0.07%
Ca(as Cl)	0.03%
NaNO ₃	0.005%
Na ₂ glycerol PO ₄	0.001 - 0.002%
Na ₂ SiO ₃ ·9H ₂ O	0.003 mg %
*P II Metals	0.5 - 1.0 ml/100
NTA	0.003%
B ₁₂	0.01%
Thiamine HCl	0.0001 - 0.0005%
Biotin	0.001 - 0.01%
TRIS (Buffer)	0.01 - 0.05%
NaHCO ₃	0.005%
pH 7.8 - 8.2	
*PII Metals 1 ml =	
Na EDTA	1.0 mg.
Fe(elect)	0.01 mg.
Boron	0.2 mg.
Mn(Cl)	0.04 mg.
Zn(Cl)	0.001 mg.
Autoclave 1) 15 lbs	10 min
2) 5 lbs	1 hour

*This base has proved successful in growing diatoms, chrysomonads, cryptomonads, euglenoid and dinoflagellates from the nentic zone (From Burke, 1962¹⁷²)

STRICKLAND: Have the other cells lysed?

MCLAUGHLIN. Yes, most of them have lysed. If the *Peridinium* are filtered off and the medium is inoculated with *Gyrodinium*, the *Gyrodinium* will not grow. The reverse is also true.

If the medium is enriched with some limiting agent—nitrates, phosphate, trace elements, vitamins—one still cannot overcome the disadvantage of first having grown *Gyrodinium* in the medium.

DROOP: May I give an example of the opposite effect? *Monochrysis* requires vitamin B₁₂ and thiamine and it cannot be maintained in a medium which does not have these vitamins. *Nannochloris*, on the other hand, has no vitamin requirements. Now the interesting thing is that *Monochrysis* can be grown in a vitamin-free medium if *Nannochloris* is grown with it. So it would appear that *Nannochloris* produces vitamins for *Monochrysis* to use. Doctor Fogg, didn't you show some years ago that *Chlorella* produced vitamin B₁₂?

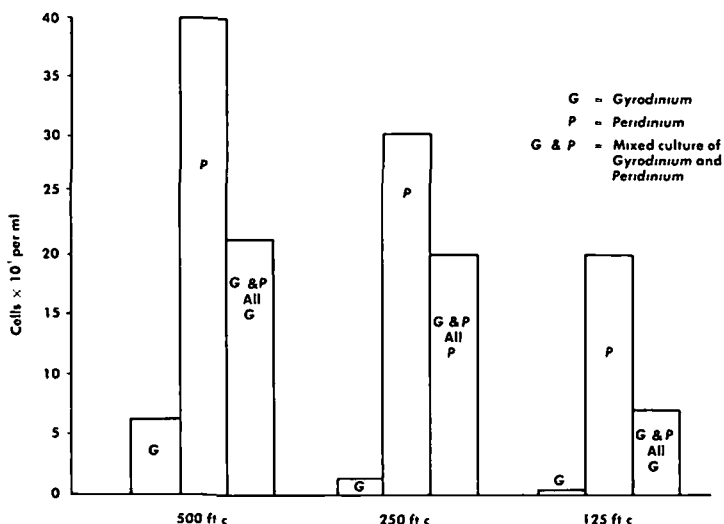


FIG 43. Competitive growth in mixed cultures under continuous light for seven weeks.

FOGG. Yes, not extracellular, but the cells contain it. We did not look for it in the medium.¹⁷³

MCLAUGHLIN: Both of these organisms I refer to have identical vitamin requirements. I don't know why the *Gyrodinium* wins out at those light intensities or temperatures.

STRICKLAND: Are you in a position to say anything about the work on hormones at Aberdeen, Doctor Johnston?

JOHNSTON: The Aberdeen work on growth hormones* is not related to competition in any way. It is purely a matter of tracing plant hormones in marine algae, seaweeds, phytoplankton net collections, and culture species. The work is mainly analytical, looking for growth hormones which give an auxin response by means of the *Avena* assay, and for gibberellin-type hormones using a variety of different assays. I am not competent to give you very much detail.

STRICKLAND: I believe Doctor Provasoli would like to report on some quite interesting work relative to this which has been done at Woods Hole. We would appreciate some of his views on the subject of mutual inhibition.

PROVASOLI: This work has nothing to do with mutual inhibition, really. It is being done on the Sargasso Sea by Doctor John Ryther

*This work on growth hormones is carried out by Doctor Joyce Mowat (nee Bentley) who has recently published several papers on it.²⁶¹

and his group,* and I feel it is important. I did not intent to talk about it myself, because Doctor Ryther should have been with us, but at the last moment he could not come. But I will try to do the best job I can under the circumstances.

*Editors note: Dr. Ryther, who could not attend the conference, submitted the following statement:

RYTHER We have studied the role of iron in two ways: (a) by measuring its concentration and distribution with season and depth in the Sargasso Sea, and (b) by studying the effects of artificial enrichment of Sargasso Sea water with iron on C^{14} -uptake. The results of the latter approach showed that iron consistently enhanced carbon assimilation. This was not an experimental artifact caused by C^{14} adsorption to iron particles, as has been suggested, since enhanced growth could also be demonstrated by cell counts and chlorophyll content.

The stimulating effect of iron was transitory when iron alone was added. That is, C^{14} -uptake would approximately double in 24 hours compared to the unenriched control, but not increase thereafter. However, if iron, nitrogen and phosphorus were added in combination, the same brief enhancement in the first 24 hours occurred but was followed, after a one day lag, by a very pronounced increase which reached its peak at the end of the third day. Uptake at that point was as much as 100 times that of the unenriched control, and the carboys in which the experiments were carried out were turbid and colored with phytoplankton.

We have interpreted the initial stimulation (during the first 24 hours, obtained with iron alone or in combination with nitrogen and phosphorus) as enhancement of photosynthesis by the existing population. The much greater stimulation after three days was obviously due to rapid growth of the population after a two-day lag period. The latter occurred only when iron, nitrogen and phosphorus were added together.

Thus, photosynthetic stimulation was caused by iron alone, but substantial growth required nitrogen and phosphorus in addition. After the third day, the population began to die off and by the end of a week was reduced nearly to its initial level.

The significant feature in the above experiment which has not yet been mentioned is that nitrogen and phosphorus (the two nutrients most frequently referred to as "the limiting factors" in the sea) did not increase C^{14} -uptake or population growth when added by themselves, within the five-day period of our experiments. Thus we attached great significance to iron as the "missing link." This was hard to explain ecologically, however, since the concentration of dissolved and/or particulate iron does not vary with depth in the Sargasso Sea, as does nitrogen, phosphorus, and silicon.¹⁷⁸ We had already demonstrated that winter mixing, by bringing these nutrients up into the euphotic zone, set the stage for the spring phytoplankton bloom.¹⁷⁹ How could this be so if iron were the critical limiting factor but was no more abundant in the deep water than at the surface?

Our second disillusionment with iron came about when we inadvertently let one of our enrichment experiments go for an entire week, due to a lazy dishwasher. At the end of the fifth day, the carboy enriched with only nitrogen and phosphorus (no iron) suddenly showed signs of life and after seven days reached the same peak of relative growth as achieved in a shorter time in the carboy enriched with N, P, and Fe. The same results were observed on several subsequent occasions, but not consistently. The long lag period before growth occurred in these experiments, and the fact that it sometimes did not occur at all, led us to the tentative conclusion that organisms, initially very scarce, had been favored by "unnatural selection." Unfortunately, the organisms were not examined on this occasion to see whether the different types of enrichment

The work is the result of a concerted attack on an ecological location and combines the data obtained from the chemical analysis of the waters, the species composition and seasonal variations in phytoplankton, *in vitro* nutritional studies, and enrichment experiments on natural samples of waters. It is a prototype of what can be done by collaboration of different specialists, because the data fit in a picture which, although still sketchy, permits a good insight into the ecological events.

The Sargasso Sea presents some interesting features: (a) Light intensity varies seasonally but the water temperature is almost constant the year around; (b) iron is present in extremely small quantities and, apparently, its concentration varies very little both seasonally and with depth;^{261,175} (c) The quantity of B₁₂ in the upper 50 m layer fluctuates from undetectable to 0.03 $\mu\text{g/liter}$ from May to November. Menzel and Spaeth⁹⁶ measured the monthly content of vitamin B₁₂ by employing the bioassay with *Cyclotella nana* developed by Ryther and Guillard (FIGURE 44).¹⁰⁸ This paucity of B₁₂ seems to control the species composition of the phytoplankton, as is indicated by the seasonal cycles in the species composition and by the nutritional data.

The seasonal variations in phytoplankton show that many species of diatoms are present throughout the year, but at extremely low levels, and that *Coccolithus huxleyi* is the dominant species throughout the year.¹⁷⁶ A diatom bloom—a very small one as compared to our

produced different populations. However, later work²⁶² did show that even slight variations in the artificial enrichment produced quite distinct populations. In that connection, it was interesting to observe that a five-gallon sample contains practically all the members of an annual phytoplankton cycle, many extremely scarce and missed by most counting techniques (perhaps present in undistinguishable resting stages), but ready to grow when exposed to the proper environment.

By this time we had serious doubts about our earlier conclusions on the importance of iron, and the following experiments provided the finishing touch. We had tried to measure iron uptake using Fe⁵⁹, and this failed utterly because within 24 hours the iron precipitated quantitatively (by our methods of detection) from whatever form it was added, including Fe-EDTA, and we could not separate the cells from the precipitate. Obviously, then, the cells in our 5-day enrichment experiments were using particulate iron, as Harvey showed back in 1935²¹⁴ and Goldberg¹⁸⁰ most recently confirmed. This then led us to wonder, in view of the puzzling ecological evidence referred to above, if the precipitate rather than the iron *per se* was providing the stimulatory effect. To test this we added, instead of iron, aluminum which has no known nutritional significance but does form an insoluble precipitate in sea water. The addition of nitrogen, phosphorus and aluminum caused a growth stimulation comparable in time and magnitude to that produced by N, P, and Fe. Again the results were not consistent enough to be conclusive, but they were repeated several times.

The problem now rests at this unsatisfactory stage until we have the courage (and assistance) to tackle the more sophisticated problems of trace metal chemistry and nutrition.

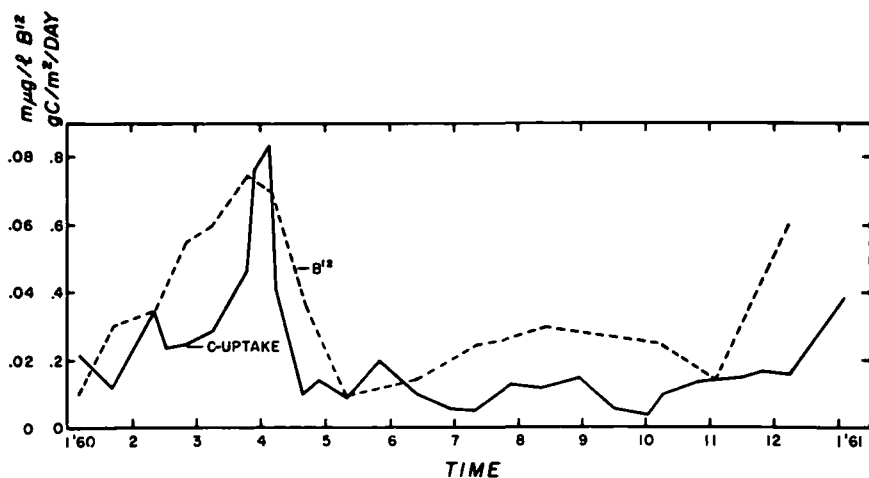


FIG. 44. Vitamin B_{12} in the Sargasso Sea. Relation between primary production and the mean concentration of vitamin B_{12} in the euphotic zone

waters—develops in March and April after B_{12} has increased to $0.06 \mu\text{g/liter}$, and, similarly, B_{12} drops to $0.01 \mu\text{g/liter}$ during the course of the diatom bloom. This apparent relationship between diatom growth and presence of sufficient B_{12} is confirmed by the *in vitro* data of Guillard and Ryther,⁴⁸ and Guillard and Cassie.¹⁰⁹ Most of the diatoms isolated from the Sargasso Sea, including some bloom species, require B_{12} for growth. The seeming mystery of the dominance of *Coccolithus huxleyi* throughout the year is again explained by the nutritional data: *C. huxleyi* needs thiamine but not vitamin B_{12} .¹⁷⁷ The data from field observations, the vitamin content of waters, and the nutritional requirements of the organisms all fit beautifully together in the hypothesis that the level of vitamin B_{12} is one of the factors governing the diatom bloom and that thiamine is probably present in the water at a level permitting a small permanent population of *C. huxleyi*.

Another puzzle remained: The extremely small quantity of iron in the waters. An analysis of the iron requirements of coastal species such as *Skeletonema*, oceanic species like *Coccolithus*, and Sargasso diatoms, shows a clear differential.²⁶⁰ The requirements of the oceanic species is so small that it can not be demonstrated even after depletion (*Coccolithus*); In other species, it can be demonstrated only after depletion; For some species, such as *Coccolithus* and *Cyclotella*, more than $10 \mu\text{gFe/liter}$ becomes inhibitory. In contrast, deficiency in iron can easily be demonstrated in *Isochrysis* and other littoral and supralittoral species: some species require more iron than others.

DROOP: The response of *Skeletonema* and *Phaeodactylum* to iron in the presence of N, N-dihydroxyethyl-glycine showed that all the supralittoral species I tested didn't seem to need as much iron as *Skeletonema*.

PROVASOLI: I am afraid I don't remember offhand all the species tested by Ryther, so I cannot say whether his data are in accord with yours. By the way, what do you consider *Isochrysis* to be?

DROOP: *Isochrysis*, like *Skeletonema*, is neritic. I should say; *Monochrysis* and *Phaeodactylum* are supralittoral.

PROVASOLI: I would like also to comment on Ryther's results with enrichments, because I feel that this type of experiment can clarify and confirm whether the *in vitro* results are applicable to the natural situation. For instance, in the discussion of Doctor Johnston's enrichments an important thing came up. Using phosphate, nitrate, chelated materials, and vitamins, he enriched summer waters from the North Sea, which, at the moment of enrichment, contained a mixed population of *Rhizosolenia* and dinoflagellates. This enrichment favored the growth of the spring diatoms, and the dinoflagellates disappeared. What does this mean? Obviously, the spring diatoms are favored—there is an enrichment for them—but what favors the dinoflagellates? Yet artificial media containing the same enrichment permit good growth of single species of dinoflagellates and diatoms alike. We need to know more about the differentials between the two algal groups, then we should be able to favor diatoms or dinoflagellates at will.

JOHNSTON: That might mean that instead of enrichment, there has to be a reduction in what is already there.

PROVASOLI: That might be the secret.

GUILLARD: Doctor Provasoli, is silicate included in that enrichment?

JOHNSTON: Practically, yes. It is not really added however, because the work is being done in glass tubes.

GUILLARD: Doctor E. M. Hulburt of the Woods Hole Oceanographic Institution, found something of the same sort. When he made enrichment studies in the southern Sargasso Sea using polyethylene vessels with no added silicate but with a small enrichment of other nutrients, he frequently found almost only flagellates, but when he included silicate in the enrichment, the diatoms often competed successfully.

PROVASOLI: Samples of the same water with their natural populations were variously enriched and dispensed in two sets of containers; glass carboys and polyethylene bottles. The addition of nitrate and phosphate in glass carboys caused dominance of *Skeletonema* (75 per cent) and the growth of three species of *Chaetoceros* (25 per cent). When Menzel, *et al.*¹⁸⁹ added nitrate and phosphate to the same waters

in a polyethylene bottle, a short-lived bloom of *Skeletonema* appeared, followed by a large *Coccolithus* bloom.

The addition of nitrogen, phosphorus, and iron in glass bottles induced a short bloom of *Chaetoceros simplex*, later supplanted by an unidentified flagellate. When the same enrichment was added to polyethylene bottles, a large *Nitzschia closterium* bloom, concomitant with a small *Coccolithus* bloom, appeared. But when silica, iron, nitrogen, and phosphorus were added in a polyethylene bottle, a very dense population of *C. simplex* came up. The results are similar to those for the glass bottles; i.e., silica leaching from glass favors diatoms.

These experiments show clear differentials, giving us some insight into the effect of nutrients on the competition of species in quasi-natural populations. Since the work was done in containers, the effect of bacterial growth cannot be discounted. Even so, this type of experimentation approaches the natural situation and it makes it possible for us to tell whether the data acquired *in vitro* and with bacteria-free cultures—an unnatural situation at best—can be applied to the natural events.

STRICKLAND. Doctor Johnston, would you like to comment your work on mutual enhancement?

JOHNSTON: Yes, this is fairly new. It is a continuation of the work I reported at the 1959 International Oceanographic Congress. I had just started examining the sensitivity of marine algae to antimetabolites. At the time, I was foolish enough to believe that I could draw the conclusion that sensitivity was connected with the vitamin requirements of the species. I was put right on that. I still saw differences in these sensitivities and I wanted to try to interpret them, so I carried on more experiments using the enrichment technique and using natural phytoplankton populations.

Before beginning, a series of enrichments containing phosphate, nitrate, silicate, and chelated trace metals is prepared and, in the same medium, graded amounts of an antimetabolite are also prepared. These are all sterilized very briefly and prepared sets of tubes are taken out to sea. Then a sample of seawater with an unknown phytoplankton population is put in and the cultures are illuminated. At the beginning, one does not know too much about the species one has or whether they will grow, so this process has to be repeated many times—at least 10 times. Upon returning to the laboratory ashore, an analysis is made for the total growth; that is simple enough. Then as good an analysis as possible has to be made of the species present and their abundance. There are controls without antimetabolites, and also a graded treatment. I have used a wide range of antimetabolites and, of course, it is not possible to avoid meeting a very wide range

of species. Full details of the antimetabolites and the species encountered are reported in my paper.¹⁸¹

Instead of trying to translate sensitivity into some measure of particular details of metabolism, it is much more useful to consider *relative* sensitivities. I use *Skeletonema* as the reference organism because sea water results can be related, at least indirectly, to pure cultures and to vitamin requirements, and so on.

I found that different antimetabolites produced different amounts of inhibition in different species, so one could almost formulate a code for the reactions to antimetabolites and say: "That must be the species." Nevertheless, from the sensitivities it is possible to distinguish two main groups of species with rather similar responses. These groups turn out to be spring diatoms characterized by high sensitivity to all the antimetabolites tested relative to the late summer diatoms which are rather resistant. I have a feeling that this ties up with the sort of vitamin requirements that Doctor Provasoli has just described.

PROVASOLI: Or to the succession of species.

JOHNSTON: I don't want to make this too specific, in translating it back in terms of metabolites. I would rather leave it at the moment as sensitivity.

PROVASOLI: Did you determine the species composition of the original population by fixing a sample and counting it?

JOHNSTON: No, I haven't tried that.

PROVASOLI: I think you should follow the successful procedure of the Woods Hole group. The species composition of the initial population should be determined before the addition of the enrichment and of the graded quantities of antimetabolites. Then as the samples grow, the changes in species composition should be followed by sampling. It will then be possible to detect the species that have been favored and those that have been excluded.

JOHNSTON: But I feel that if I were to take this 10 cc sample and filter off the organisms, the results wouldn't give me a clue as to what I started off with.

PROVASOLI: No, you are enriching a natural population and adding the antimetabolite.

JOHNSTON: Yes, but I am not sure whether I could recognize in the original inoculum anything like the species that I get out.

PROVASOLI: This is the point.

JOHNSTON: One would have to do this, I know.

BRAARUD: What we have heard has been very illuminating. I was especially glad that we received some real information on metabolites and their effect on other species because we haven't really touched

on that before, although it has been in the background of our discussions on succession for a very long time.

Enrichment experiments seem to give an explanation, in some waters, at least, of the succession of taxonomic groups or ecologic groups, so I think we are really getting down to something. But we also have to take into account temperature and light properties, and I would like to mention one very clear example of temperature effect upon succession in the Oslofjord. In May of this year (1962), we made some observations in stratified waters, where there was a winter temperature of just a few degrees at a depth of five meters. In this very narrow cold layer, there was a rather dense vegetation of *Nitzschia frigida*, a rather pronounced Arctic form which has been recorded from various localities at low temperatures.¹⁸² Of course, this is an extreme case, but within the various groups we may have definite temperature requirements in many species.

If we could get down to the main factors, which of course would change a diatom to a dinoflagellate population, then we would have reached one of the most important goals. At this point I would like to bring an obscure detail concerning the nutritional pattern of *Dinophysis*. I suppose you have not been able to culture any species of this dinoflagellate genus, Doctor Provasoli.

PROVASOLI: No. Is that the one which has a "collar"?

BRAARUD: This genus may have very large populations in the euphotic zone. It uses light and it appears to thrive best in the summer season. There may be a combination of a special nutritional factor conditioned by populations of diatoms and temperature which decides its seasonal distribution. In other dinoflagellates, like *Peridinium triquetrum* and *P. trochoideum*, we find in Norwegian coastal waters distribution patterns which indicate that temperature is not the only factor which governs their seasonal distribution patterns.

STEEMANN NIELSEN: May I call attention to a very simple example of succession in two Danish eutrophic lakes studied by Doctor Jørgensen.¹⁸³ During the spring, diatoms were found in great quantities and, then, mostly in the beginning of April, the concentration of silicon decreased to about 30 mg/m³. From culture experiments we know that most diatoms stop growing at this silicon concentration, and this is what happened in these two eutrophic lakes. Then the silicon deficiency caused quite another plankton, the green algae, to develop. During the autumn, as soon as vertical mixing took place large quantities of silicon were again found in the water and the diatoms started to grow again. Doctor Lund has told us that in the English lakes, for the most part, there won't be such a clear correlation between the concentration of silicon and the occurrence of diatoms

as in the eutrophic Danish lakes. Very often in the sea perhaps we have the same situation. For instance, I would like very much to know whether any modern determinations have been made of the silicon concentration in the Sargasso Sea.

GUILLARD: I don't know if they have been made there. It may have been done on some of the cruises from Woods Hole to Bermuda.

STEEMANN NIELSEN: Perhaps the silicon concentration there does not decrease to the very low concentration found in eutrophic lakes when the diatoms begin to stop growing. It may be the same as in the English lakes, where the diatoms disappear at a rather high concentration of silicon. It would be interesting to know much more about the silicon in the sea. In recent years we have developed a really good technique for measuring very small concentrations of silicon. Most of the older determinations don't tell us anything.

STRICKLAND: One of the many remaining possibilities which we haven't discussed, which could possibly give one species some advantage over another, is the ability for either complete heterotrophic growth or some rather speculative type of mixed heterotrophic-phototrophic growth. I believe there is evidence, although it is rather tenuous, that *Thalassiosira rotula* and perhaps *Skeletonema* are capable of heterotrophic growth. But these organisms cannot be made to grow if they are put on glucose in the dark. Of course, glucose is probably the wrong substrate and a large number of substrates should be tested.

It is possible, too, I think, that with heterotrophism a little bit of light is required, either to enable cell division to occur or perhaps to couple respiratory processes with certain critical growth processes. The amount of positive evidence for heterotrophism occurring in the sea, other than on the sea bottom, is fairly tenuous. We have reports of large numbers of coccolithophores living at great depth in the Mediterranean, and we have a certain amount of data from limnologists which indicates that there is heterotrophic growth of flagellates. Doctor Vollenweider, I wonder if you have anything to report at this stage?

VOLLENWEIDER. Our common experience is that very often there is a second growth peak in lakes in which light intensity is always so much reduced that, from a classical point of view, one shouldn't expect any further growth. For a long time this has given us the idea that we must expect some kind of more or less intermediate activity, such as heterotrophic growth.

I have tried, then, to test this idea in the laboratory. Unfortunately, I am not sure about my results. There seem to be certain populations that, when they get old, have a much higher CO₂ uptake in the dark. If we conduct photosynthetic experiments under light with a dark control, then we find that the uptake in the dark in old cultures is

much higher than in young ones. This is a fact which I cannot understand. Perhaps it points to a possible change to heterotrophic activity.

I have been impressed by the last reports on *Hunderina*. If one makes an experiment with very low illumination, *Hunderina* very often has a very low photosynthetic activity. In fact, the dark uptake of CO_2 is rather high.

PROVASOLI: Was this done in crude culture or water which had contained a great majority of *Hunderina*?

VOLLENWEIDER: No, it was made in crude cultures.

PROVASOLI: You studied only one species?

VOLLENWEIDER: Yes.

PROVASOLI: A great many of the green algae are able to grow in the dark.

DROOP: Can you clarify the relation of uptake of CO_2 to heterotrophy?

VOLLENWEIDER: No, I can't.

DROOP: I thought heterotrophic growth would not involve CO_2 uptake.

OPPENHEIMER: Carbon dioxide is taken up in Wood-Werkman cycle during heterotrophic activity.

ALLEN: And several other cycles.

STEEMANN NIELSEN: Very often, the rate of uptake of C^{14} compared with the rate of respiration is about 10 per cent in heterotrophic plants.

STRICKLAND: I can confirm from work in our plastic bag that there is very definite photosynthesis (as measured by both C^{14} uptake and oxygen evolution) in a "light" bottle as compared with a blackened bottle when the illumination is so low that the radiometer recorded nothing (say less than 0.002 ly/min).

GOLDMAN: Yes, I have a very unusual situation that I think may bear on this, and that is the C^{14} uptake under some four meters of ice in Lake Vanda in the Antarctic. It is one of those things I wasn't able to repeat by the time I got the data worked up. The unusual thing was a tremendously high, dark uptake of C^{14} as compared to the light uptake. On the second day, it was not so striking except at depth. I went back and used formalin in some of the samples and included more dark bottles. The dark uptake was still fairly high but not very exciting, and then, at the very end of December, we established what I would consider a more normal situation, with the light uptake being appreciably higher than the dark.^{107,185}

This might be explained on the basis of a light injury to the organisms which simply stopped their metabolism. This is a situation I mentioned earlier concerning samples exposed at the surface, and those that had the protection of black bags before being put to their

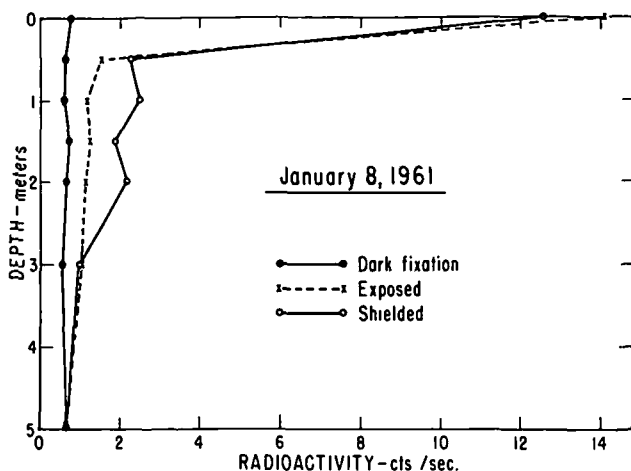


FIG 45. Inhibition of carbon fixation in samples exposed to direct sunlight before a four hour *in situ* incubation under ice cover in Castle Lake, California. (Goldman, C.R. 1963). The measurement of primary productivity and limiting factors in freshwater with carbon.¹⁴ Symposia on Marine Productivity in the Pacific, 10th Pac. Sci. Cong. Honolulu (Ref. 185).

respective sampling depths.¹⁸⁶ The ones that were shielded from this brief exposure to incident light had an appreciably higher carbon uptake during the subsequent four-hour *in situ* incubation (FIGURE 45). That certainly suggests either injury to the organisms or something that is not well understood concerning dark fixation of CO_2 .

PROVASOLI: Before we call on Doctor Lund for the summary, I would like to remark that I have profited and learned a great deal from this conference, especially because the ecologists have given us a wealth of information and have brought into the open a number of interesting puzzles. I am sure that when the proceedings are printed, a number of researchers may pick up some of these leads. But we can do something more. Many times the ecologists have some very good hints on correlations between the physical and chemical factors and phytoplankton growth, or succession of species. These hypotheses are often buried in taxonomic or ecological publications which are not read by algal physiologists or nutritionists. This information could be the object of short notes with a self-explanatory title easily found in the literature. In this way, laboratories lacking a complete staff, could recruit distant collaborators and create groups similar to the one of Woods Hole.

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SUMMATION

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There are five preliminary matters which must be mentioned.

First, in commenting on various of the points raised, I shall often illustrate my own understanding of the situation or my own viewpoint by referring to what is known or what is believed to be known (which may not be the same thing) about the freshwater environment or freshwater organisms. Yet, further, I shall refer most frequently to those aspects of freshwater ecology with which I have had closest contact. This may seem to be reprehensible but the fact that freshwater biologists were invited to attend this Conference on Marine Biology is an index of the amount of common ground between investigations on the plankton of fresh and salt water.

Second, I have written the summation in a manner which would not be acceptable for the usual printed paper but which is, I hope, more in the spirit in which the discussions were carried out.

Third, I am neither treating the proceedings in the order in which the various topics were raised, nor always keeping to one topic at a time. In this way a precis is partially avoided. However, it is scarcely possible to write a coherent account of what was said. Intentionally, and rightly, the participants were encouraged to pursue any line of thought which they considered profitable, irrespective of whether it was strictly concerned with synecology or did not impinge on matters discussed in previous sessions.

Fourth, Doctor Provasoli said, at one point: "We would like to have, at the end of today, a list of the factors which have been supposed or demonstrated to show a correlation existing between events, the succession of forms and other factors." TABLE 8 is an attempt to do this. It includes some factors which were only mentioned in passing or were discussed in earlier sessions. The list does not include all the factors of importance for we are only concerned with what was said on October 24th, 1962. A summary is given of what was said about some of these factors. In this way, the views expressed about the approach to ecological problems and the interpretation of data can be considered. Again and again it was pointed out that the truth may be

TABLE 8
LIST OF FACTORS MENTIONED IN RELATION TO
SYNECOLOGICAL PROBLEMS

I Physical

1. Temperature. Range within which growth is possible, effect on rate of growth, on nutrient demands and on enzymatic processes; thermal stratification.
 2. Light. Length and brightness of day; spectral composition; light saturation, inhibitory or lethal intensities; I. R. absorption
 3. Water Movements. Horizontal and vertical transport into and out of an area or depth zone; invasions; eddy diffusion.
 4. Density distribution; effects of salinity, temperature metabolism or gas production, in relation to the sinking or rising rate of organisms.
-

II Chemical.

1. Inorganic substances. Iron, nitrogen compound; phosphates; silicon; sulfides; trace elements; oxygen; ionic ratios and salinity; redox-potentials; pH.
 2. Organic substances. Vitamins (B₁₂ and thiamine), acids (glycolic, glutamic), chelates, unknown or imperfectly known compounds such as 'humus,' natural chelates and most extracellular compounds.
-

III Biological

1. Inhibitory or stimulatory substances; through the activities of previous populations or the organisms own extracellular products (e.g. lag phases)
 2. 'Clock' mechanisms.
 3. Life histories, changes in the response of an alga to a given environment in relation to.—growth; sexual recombinations; morphology and habit; resting stages
 4. Symbiosis *sensu lato* (a living together). Bacteria on algal cells or in their mucilage.
 5. Grazing; qualitative and quantitative effects.
 6. Parasitism.
 7. Rate of sinking, variations in weight of organisms in water.
-

IV Human

1. Sampling and experimental techniques. Imperfections in sampling, identification of species; area sampled and frequency of sampling, uses of bacterized or axenic cultures, or enrichment experiments; effect of sterilization or filtration or of the walls of a vessel on the medium (e.g. adsorption of phosphates); size, shape and location of experimental containers.
 2. Interpretation of the data obtained.
-

very different from what we believe it to be; that our 'simple' answers may be deceptive. These are matters that I have loosely termed "human" factors in ecology.

Fifth, if occasionally it seems that I am being critical or have superior information, let it be remembered that I have the advantage of being able to think over the day's proceedings and to discuss one or two matters with my colleagues Mr. F. J. H. Mackereth and Doctor J. F. Talling. My comments, therefore, are never to be interpreted as criticisms of the speakers. It is just as easy to be wise after the event as it is to forget something or to overstate one's case in the cut and thrust of discussion and argument. If I have misinterpreted anyone's remarks I hope he or she will forgive me. The summation, moreover, is based mainly on the verbatim report and cannot, therefore, take account of all the alterations or additions made by the speakers to the original transcript.

Temperature

Riley and Braarud gave accounts of changes in seasonal succession or annual events which could be related to fluctuations of the water temperature at different times or at different depths. Guillard gave an example of an alga which does not multiply in warm water in the laboratory or in nature. In the laboratory, when the temperature is raised, resting spores are formed and this appears to be the case in nature, too, for it is then that they are found. It was pointed out that some apparent relationships of the type described by Riley and Braarud could be the result of other factors, temperature simply being an index of change. There did not seem to be any objection to this reasonable view, but Guillard, Strickland and Riley were of the opinion, with which I agree, that the criticism that simple explanations are to be mistrusted because they are simple, can be carried too far. What can equally be carried too far is the belief that the succession of species or their periodicity is the result of the action of one or two factors. As Johnston said "I don't know, really, of any good example of succession where we can trace it back to single factors."

In an area studied by Riley, if the temperature of the water is below 2C in late winter, *Thalassiosira nordenskiöldii* is the dominant diatom, but, if it is above this, *Skeletonema costatum* replaces it. Other participants said they had found it difficult or impossible to grow *Skeletonema* at very low temperatures. The previous temperature at which it has been growing is important as a preconditioning factor. Thus, it may be easier to obtain growth at low temperatures if the population is grown through many generations at progressively reduced temperatures; i.e. a few degrees at a time. Clearly, this is equiv-

alent to what commonly happens in nature, though relatively rapid changes may occur in an area in relation to invasions of species from outside (see later). Guillard, however, quoted a case where Ryther transferred *Skeletonema* from 20 to 4C and obtained a good culture. There was, however, a gap of a few weeks before growth restarted. Guillard himself found it grew very poorly even when the temperature was reduced step by step.

Similar examples to all those quoted could probably be given for freshwater algae, but, because so many freshwaters are so small, there can be little doubt that the ability to tolerate sudden temperature fluctuations and a wide range of temperature is common to many algae. In lakes, diatoms also usually predominate at low temperatures, but I cannot recall any correlation similar to that described by Riley. The freshwater diatom *Asterionella formosa*, which, like *Skeletonema*, was frequently mentioned in this session, shows no ill effects from sudden temperature changes within the range of temperature to which it is exposed in nature, apart from the expected alteration in the rate of growth (e.g.¹⁸⁶).

McLaughlin and Droop gave examples of increases in nutrient demands at high and low temperatures (see also⁷³), but the reasons for these changes, if they are known, were not given. There is evidence that *Asterionella* needs more phosphorus as the temperature rises (FIGURE 4 in²²⁷) but the hypothetical explanation of this is that, since a rise in temperature is likely to increase the rate of metabolic processes, so will there be an increased demand for such a nutrient in unit time. McLaughlin finds that the viability of some cultures after freezing may depend on the medium into which they are put. For example, they may not live in a medium which previously supported excellent growth.

There was considerable discussion of the growth of algae at temperatures close to freezing. Oppenheimer and Goldman gave striking examples of the massive development of algae in the Antarctic. There seemed to be some surprise that such populations should exist. Goldman wondered if it is possible that a cell would be at a higher temperature internally than externally, but Oppenheimer felt that heat transport is so rapid in such cold water that this is improbable. It is known that parts of land plants can be at a higher temperature than the surrounding air. Thus, the inner parts of the catkins of arctic willows may be, as it were, miniature greenhouses, thanks to the hairs surrounding them. It did occur to me that something analagous might be present in these arctic algae, for example, very wide mucilage envelopes or an equivalent situation produced by their massing together and producing moderate amounts of mucilage. In fact, however, we are concerned almost wholly with diatoms, and some

of these have little or no mucilage around them. In freshwater, some of the commonest diatoms growing under ice, such as species of *Melosira*, *Asterionella* and *Stephanodiscus*, have equally insignificant mucilage envelopes and are not in contact with one another. The same is true of the dinoflagellates which may form blooms directly under the ice sheet.

There is no doubt that many algae can grow at 0C, or in the sea at below 0C. Some algae seem to carry out the whole of their growth at 0C. A striking example is the *Chlamydomonas nivalis* aggregate of species or forms. These algae live on or in snow. In Northern Sweden, where I made some casual observations on red snow many years ago, I found the cells to be more numerous under the surface of the snow than on top of it. They were, therefore, often enclosed in frozen snow and ice at night and washed by melt water by day. Since this melt water was percolating through the snow it must have been at 0C or, if sufficient dissolved salts were present, at slightly below 0C. Many of these algae were parasitized by a chytridiaceous fungus. This, in part, answers Doctor Provasoli's question as to whether organisms embedded in ice are viable. Braarud said they were and it may be mentioned that there are a whole range of algae and fungi known from fresh water snow and ice (cf. ¹⁸⁷ ¹⁸⁹ and references therein). It appears, from Braarud's information, that some in glaciers are in resting stages but this is not true of most of those studied by Kol.

Goldman tried to cultivate algae thawed out from ice but obtained no carbon uptake at 0C. It is possible that, in this case, the algae were dead. It is known that algae show very varied abilities to withstand temperatures below the freezing point of fresh or salt water. These differences depend largely on the inherent ability of the algae to live at such temperatures, their physiological condition at the time when they are frozen, the temperature to which the ice falls, and the rate at which the temperature falls (see various authors in ¹⁹⁰ pp. 543, 761, 805, 820).

Light and related matters

A supposedly simple ecological relationship which was quoted, was that the start of the spring increase of *Asterionella* in certain lakes is determined by the synchronous increase in the length of day and intensity of the daylight. There is much evidence to support this belief (e.g. ¹⁸⁶ ¹⁹¹ ²²⁸ and in FIGURE 55). It has been suggested that this is only a specific example of the general situation in the more northerly or southerly freshwaters of the world¹⁴⁴ and, no doubt, in the oceans.¹⁹² It is, however, in determining such a relationship that, as Braarud said, "we must admit that in the sea we have more complications,"

while Johnston's work shows that the scarcity in winter of a diatom such as *Skeletonema* may not be simply a reflection of the paucity of light or the lowness of the temperature. In many lakes we can make general observations on the phytoplankton from one or a few fixed points and can exclude or determine the effects of other water masses. As Braarud and Riley explained, in the sea the influx or efflux of large masses of water may mean that what may appear to be a relationship between the activity of an alga and the climatic conditions is, in fact, largely or wholly a reflection of conditions operating elsewhere beforehand. Koczy put it thus: "The point in space which is studied is just one patch which has moved." Of course, the larger the lake the more this situation is approached, so that in studying the Great Lakes of America, for example, an almost oceanographic outlook is necessary.

The discussion on heterotrophy (see later) touched on matters discussed at an earlier session. The reasons for abnormally low carbon fixation under high light intensities, and abnormally high fixation at low intensities may, in part at least, be related respectively to light injury, as Goldman tentatively suggested, and to the ability of some algae to photosynthesize at light intensities below the level normally considered possible. These are matters on which there is at present much uncertainty. In natural environments it is not always clear from the accounts how much carbon has been fixed per unit mass of algae at photosynthetically great depths. There are also technical difficulties in assuring that the algae in the experimental bottles are not exposed to significant amounts of light before reaching great depths.

Water Movements

From his long experience, Braarud gave examples of the complications arising from ocean currents which carry great masses of water from one area to another. Invasions of species from a distant area into the region investigated are possible, together with removal of part of the 'normal' population. As a result, at times, algae characteristic of oceanic regions may be brought inshore and those of coastal regions carried far out to sea. Moreover the same exchanges of water do not necessarily occur each year or over the same period of the year. Even when the water movements are understood, the changes that will take place in the production and succession of species in the area concerned are often difficult to predict. Most of the species from outside may, perhaps, in Braarud's words, be looked upon as guests, but some do at times arrive in sufficient quantity at a time favorable for their multiplication; for example, *Coccolithus huxleyi*, a regular member of the plankton of the Norwegian Sea and sometimes abundant in the Oslo-fjord. Johnston said *Skeletonema* was present in the area of open

ocean he studied. Though he had not detected it in samples collected by net he had found it in enrichment cultures. Braarud said that coastal forms can be found far out to sea in and around the ice on the outskirts of the polar current. Such masses of ice may, perhaps, be considered as parts of the coast which have grown or floated outwards.

Some examples of resistance to invasions in freshwater were given. In one case (see FIGURE 56) where a lake receives the outflow of two other lakes, one of which is extremely oligotrophic and the other moderately eutrophic, the predominance of the oligotrophic flora may be ascribed to the relative volumes of the inflows. Nevertheless, the failure of any of the algae from the moderately eutrophic lake to multiply significantly is surprising. Invasions in freshwater are a simpler problem than in the sea because, as Braarud emphasized, movements of water masses are less complex. In general, the chief variations in the effects of the inflows are quantitative rather than qualitative. This, however, does not mean that the results of invasions are often predictable, and very complex situations may arise in areas where influxes of brackish water can occur (e.g. ¹⁹¹).

In the sea, as Koczy said, a choice has to be made between studying events at one or several stations in an area of limited size, or following a population as it is transported. Johnston gave an elegant example of the use of *Skeletonema* to give a measure of the variability of the seawater in a given area and the use of the water itself to determine its suitability from time to time for the growth of different types of phytoplankton. In some areas there is sufficient information about the movements of water masses horizontally and vertically to make this a method of alleviating the difficulties outlined by Koczy. Braarud supported Johnston's approach but added that there remained the difficulty of there being such a varied spectrum of ecological types in the sea. This is true, and, as in freshwater, some species are extraordinarily sensitive to small changes in the environmental conditions and, so, to being exposed to life in bottles and other small containers. Striking examples in freshwater are the reactions of some parasitic fungi to laboratory conditions. When *Asterionella* is severely infected by a parasite, the infestation can be stopped in a matter of days by filling a bottle with the lake water. On the other hand certain other fungi (biflagellate Phycomycetes) which parasitize desmids will flourish in bottles so that infection increases after collection. It may be that the use of large containers such as those described by Strickland and Goldman could be combined with Johnston's techniques to overcome some of the difficulties outlined by Braarud.

The predictability of events in freshwater may be mentioned here since it is, partly, at least, related to the small size of most lakes relative to the seas. Examples were given for Windermere (FIGURE 39),

and probably could be discovered for many other lakes of moderate size, but it is well to emphasize again that I was only speaking from one small experience. Examples could also be given of unpredictability, for example, in ponds. Incidentally, in England a pond is a very small body of water, much smaller for example than the famous Linsley Pond in Connecticut. The effort put into studies of the plankton of ponds is rarely commensurate with the results obtained. This is largely because the bottom, sides, and open water are so close to one another, while water movements are so small and capricious that varied, minute aquatic worlds can develop in close proximity to one another and suddenly be altered. Such a viewpoint should not, of course, be interpreted as a suggestion that ponds should not be studied. It is still impossible to know how far our knowledge of Windermere is peculiar, in that the lake has been studied so much and is of such a convenient size for limnological studies.

Braarud referred to the general agreement on the importance of vertical mixing, for example, in relation to the start of the vernal increase in plankton, and to the absence of detailed knowledge of some other possible effects of what he so graphically described as "vertical churning" of the water. He cited examples from a fjord which is very deep in one part and relatively shallow in another, and from the Norwegian Sea. There are unsolved questions concerning what happens to species which are carried deep down into the water, notably during winter, and the great dilution in numbers that must follow from this. At a later point in the discussion, the possibility of some ability for heterotrophic growth was discussed but, as Braarud said, there is a paucity of information about the distribution of many plankton species at great depths, especially in winter.

Chemistry—Inorganic

Droop showed how *Skeletonema*, in a defined medium, grows better if inoculation takes place from 1 to 3 days after autoclaving, than after several days. This effect can be removed if small amounts of sodium sulfide are added, so altering the redox potential momentarily. This is a reminder that inorganic substances may act in other ways than as nutrients or poisons. This result is obtained with small or large initial inocula. Droop's view is that, although the system as a whole is reduced momentarily, there is in it some component which once reduced is only slowly reoxidized by molecular oxygen. An attractive hypothesis is that it is ferric iron, and the equilibrium between ferrous and ferric iron, which is important (for details see²⁰¹). Strickland remarked that sodium thiosulfate will also be produced but, Droop replied that the amount would be minute. Provasoli mentioned

that if species of the freshwater diatoms *Asterionella* and *Fragilaria* are aerated shortly after inoculation they will not grow but, after the growth phase has continued for a time, aeration is beneficial. McLaughlin gave an example of the improvement of a medium with time, after autoclaving and before inoculation. Droop used cotton wool plugs and McLaughlin screw caps.

Strickland described the mineralization of a plankton crop in his large translucent sphere (circa 6m diam), the upper part of which was shaded by black plastic for this experiment so that only back-scattered radiation was received. The expected set of ammonia, nitrite and nitrate curves was not obtained. The amount of carbon evolved as CO_2 was much greater than that in the particulate matter produced. As the detritus appeared still to be present, he concluded that the dissolved organic matter believed to be excreted by the algae was responsible for this apparent excess of CO_2 . It is, therefore, he said, unwise to attribute oxygen minima in the oceans to a slow combustion of small particles of detritus. It would, clearly, be of value to carry out a similar experiment in freshwater.

The regeneration of silicon in Strickland's experiment was approximately what would be expected from the dissolution of a uniform surface at an approximately constant velocity. Steemann Nielsen referred to Jørgensen's¹⁷⁰ work showing that the silicon in diatoms is in two forms, one which dissolves relatively easily and rapidly and the other which does not do so. Strickland found that about half the silicon had reappeared in solution in 25 days and pointed out that, from his curve of regeneration, the soluble silica would have to be over 50 per cent of the total. Steemann Nielsen said that it was also true that the ease of regeneration of silicon varied from diatom to diatom.

Strickland's remark that from his results it is remarkable that a diatom ever reaches great depths in the ocean is also pertinent. His suggestion that the answer may be that there are heterotrophic diatom populations growing on the ocean floor may be correct, but we have no knowledge of such populations. When the fossil remains are examined I think it is true to say that species known to grow in the upper layers of the sea predominate. Kolbe's^{174 195} view is that diatoms are generally relatively resistant and that a physicochemical selection takes place as they sink. He agrees with Steemann Nielsen and Jørgensen that they vary among themselves in the ease of solution. The deep sea 'fossil' assemblages lack the delicate pelagic forms but are relatively rich in coarse species. Judging from the results of Strickland's group when they were measuring primary production in their plastic sphere,¹⁰⁵ the predominant forms were thin-walled species. It is, therefore, perhaps premature to invoke heterotrophy. It is also

true that our knowledge of the biochemistry of silicon is still unsatisfactory.

In many freshwaters, mineral solution is likely to be small because the pH is relatively neutral. In Windermere (pH approx. 7), no solution has been detected.²²⁰ The statement¹⁹⁶ that silica originally available is recycled seven times during the spring phase of growth is incorrect. This situation is in contrast to lakes in which, unlike Windermere, the hypolimnion becomes anaerobic in summer. Then significant amounts of silicon are liberated from the sediments,^{197a, b} but it is not known to what extent this comes from the frustules of diatoms. They show no clear visible signs of solution in cores until material is reached which was deposited some 8,000 to 10,000 years ago. This is so in Windermere and in Esthwaite Water. The latter is weakly eutrophic with anaerobic conditions near the bottom every summer, a condition which analyses of the cores suggest has existed for most of post-glacial time. Even in alkaline lakes (e.g. the lake described in¹⁹⁸) richly diatomaceous cores may be obtained.

Provasoli referred to the work on iron as a limiting factor in the sea (e.g. ^{17, 200, 201}). Marked differences may exist in the requirement for iron. The few species so far investigated could be divided into oceanic ones growing in media containing very little or no added iron, and coastal ones demanding relatively large amounts. Droop's remarks show that care must be taken not to believe that this is a universal situation. The supralittoral species he tested needed less iron than *Skeletonema*. In view of the difficulty of determining the nature and amounts of the various forms of iron in water, the experimental approach is the only one available. Many sweeping statements have been made about the importance and availability of iron in freshwater but little factual evidence is available.¹⁹⁹ Even the iron relationships in the Sargasso Sea are not clear. For example, somewhat similar results were obtained by enriching seawater with aluminium.^{175, 200} The authors point out that in view of the importance of vertical mixing, there should be evidence of the utilization of iron in the form of a reservoir of this element below the euphotic zone. However, the question is how much of the iron they estimated is available to or utilized by the algae. The actual amount incorporated into the relatively thin populations may be so small a part of the whole that its fluctuations are masked by those of forms of iron which are not metabolized.

Strickland found confirmation in the experiments with a plastic sphere (see¹⁰⁵) that the spring crop in the area concerned was limited by nitrogen. Some reference was made to the amounts of nitrogen in snowfall and in different areas. (Concerning CO₂ uptake in the dark see the section on heterotrophy.)

Chemistry—organic. A. excluding extra-cellular products, anti-metabolites, etc.)

Droop finds that humus may enhance the growth of *Skeletonema*. Depending on the state of the inoculum "one may get an all or nothing effect." A possibly similar effect occurs with the use of soil extract. Below a favorable range of concentration the growth of *Asterionella* is erratic, so that one gets a diversity of results for the addition of the same amount of soil extract.

Johnston mentioned that the carbon-nitrogen ratio in the organic matter of the sea is higher in coastal than in oceanic or deep waters because the inshore waters are rich in plants, and the others are rich, by comparison, in animals. He suggested the possibility that there could be an important difference between the animal manure of deep waters and the vegetable compost of shallow ones. Extracts which can replace that from soil for the culture of *Asterionella* can be produced from a variety of sources. Some years ago I tested extracts made from the muds of all the lakes in the English Lake District, in some of which *Asterionella* occurs while in others it does not; from recent and old *Sphagnum* peat; from freshly fallen, and live leaves of deciduous trees; from leaf compost; from fresh and well-rotted dung and urine. Only fresh leaves, fresh dung, and urine were ineffective but, after allowing the two former forms to rot and 'humify' they became good alternatives to soil, but urine never did so.

Oppenheimer said that the water and sediments in certain areas of the Antarctic are very low in organic matter, but the ice, when melted, contains up to 18 mg/liter (the sediments have less than 0.5 per cent organic carbon). The tentative conclusion from this is that the organic matter is mineralized in the upper layers of the water where great bacterial activity can occur.

Provasoli mentioned vitamin B₁₂ in relation to the instructive work done on the succession of algae in the Sargasso Sea. This is a model example of how synecological problems can be elucidated by the combined expertise and knowledge of a group of workers whose main interests and specialties may differ quite markedly. The small plankton pulse early in the year in this sea reduces the B₁₂ concentration to levels which may even be below the limits of detection. Guillard found that all the diatoms he isolated from this community required B₁₂ but, of the flagellates which usually predominate in the succeeding period, *Coccolithus huxleyi* does not. Menzel & Spaeth¹⁷ do not think that B₁₂ limits production in the Sargasso Sea but that it has an important qualitative effect on the composition of the plankton. This is a stimulating viewpoint and it leads to a very interesting question, namely, why the species not requiring B₁₂ (e.g. *Coccolithus huxleyi*) fail to flour-

ish during the period dominated by those which do require this vitamin.

A side issue of this discussion was Provasoli's reference to Johnston's enrichment experiments, to point out that, in general, we do not know what enrichments should be added to make dinoflagellates grow. When, from laboratory studies, there is more knowledge of their nutrition, it will be possible to design better enrichment experiments. Johnston made the comment that what in fact may be necessary is not an enrichment but a reduction of what is already there. This is the same view which was discussed in a previous session about certain freshwater flagellates¹⁵⁷ in relation to the influence of the concentration of $\text{PO}_4\text{-P}$ on their seasonal cycle. Provasoli did not specify what flagellates he was referring to but, probably, in the main he was thinking about small Chrysophyceae since a number of dinoflagellates are cultivated in his own laboratory. A considerable number of these can be grown in undefined media, such as Erdschreiber mixtures, but some freshwater ones have still to be cultivated successfully even in bacterized media containing soil extract. For example, Rodhe¹⁸⁴ only had limited, temporary success with a very common and widespread *Uroglena*.

Chemistry—Organic: B, and Biological Factors
(Other than II, 4, 5 and 6 in TABLE 8)

We still know little about the organic chemistry of natural waters beyond the fact that the compounds that ought to be there are there. What we do not know is the composition of the remaining organic matter and what are the variations in the concentrations of various known compounds. The unknown material can be the major portion of the whole in many lakes and, no doubt, in coastal areas of the sea. This is the realm of humus and yellow substances. There is also a minor portion of the organic matter which we believe to be of major importance. This is the world of inhibitory, poisonous, and stimulatory compounds—of extracellular substances—a view of which is appearing slowly through the mists of supposition. Since we are still mainly in the stage of measuring the effects of these substances and, therefore, cannot satisfy the overwhelming human desire to classify, this vital region of hydrobiology would have to be considered in a somewhat disorderly manner even in a formal review.

Shortly after some appropriate introductory remarks by Riley, Braarud, and Johnston, Johnston himself described his very interesting experiments with *Skeletonema* and, later on, further work on enriched seawater which has been referred to earlier. He used *Skeletonema* as a test organism because . . . "in a very broad way I

think *Skeletonema* betrays the usual pattern of succession in the water." He collected seawater from different areas and at different seasons and enriched it so that it should contain all the necessary nutrients. He then inoculated it with *Skeletonema* and exposed the cultures to a standard light intensity and temperature. (Fremont-Smith, early on, raised the question that a biological clock mechanism might be involved in the behaviour of the diatom, but Johnston doubted this unless it was some kind of chemical clock.) *Skeletonema*'s growth showed that there were variations in the quality of the water, but a dinoflagellate he tested grew much the same whatever kind of water was used.

Johnston found, presumably for one area, that there was little growth of *Skeletonema* in enriched seawater in winter, better growth in spring, very poor growth in summer and an improvement in autumn. It is of interest to compare this result with what is known about the freshwater diatom *Asterionella*. No such elegant work as Johnston's has been done with *Asterionella*. Nevertheless, in the lakes in which it is at times very abundant it shows one very clear difference from *Skeletonema*. It is that, in winter, the water is potentially most favorable for its growth and, as has been mentioned earlier, the reason for the small size of the populations at this time of year is the lack of light. Even at other times of year, good growth can be obtained if it is inoculated into the right media (e.g. Lund¹⁰⁵). Johnston's work shows the presence of some inhibitory or stimulatory substances over and above all the things we know to be necessary for growth. He pointed out that this can be shown in relation to spatial as well as chronological differences in the nature of the seawater, for example, in relation to the timing of the spring diatom pulse in inshore and open waters. Even when the spring pulse has started in the sea, the water is very patchy in quality.

Provasoli remarked that whatever the limitation of Johnston's approach might be said to be, "up to now we have never done any experimentation to really try and find out what makes a water good or bad." However, the words 'good' and 'bad' are often misinterpreted. We must add good or bad for what, because all waters support some life.

Johnston also showed, for mixed plankton, how enrichments generally bring the seawater to a state favorable for the early spring diatoms. He did not think that this was because the other forms were killed but because faster growing species outgrew them. This situation applies to a large extent to freshwater, with the added complexity that, for some unknown reason in such experiments in bottles, sooner or later it is not the characteristic spring diatoms which predominate but small green algae and small nitzschoid and naviculoid diatoms,

including species which are never abundant in plankton. In the general discussion, Johnston mentioned that, for his experiments, zooplankton must be removed. It is possible that it is their removal which leads to the predominance of nannoplankton in the freshwater experiments, yet my own experience, imperfect though it is, makes me doubt whether this is more than a partial explanation of the mystery. Oppenheimer said that a precaution necessary for this type of work is to avoid heat sterilization, citing as an example how this destroys the inhibitory effect of seawater on *E. coli*.

There was some argument as to whether Johnston was measuring different degrees of inhibition with *Skeletonema* or of enhancement. It is, of course, difficult to do all that one might wish and, as Johnston mentioned, his work involved a very large number of assays. The impression I gained was that Johnston was, as he said, measuring inhibitory effects in one type of experiment and enhancement in the other. In relation to testing two or more algae at a time, he pointed out that another difficulty may be that they grow at such different rates that there cannot be a standard exposure time for all of them.

Fogg gave a description of a clear relationship between an extra-cellular substance and the lag (induction) phase in a *Chlorella* sp. During photosynthesis, glycolic acid passes from the cells into the medium and, when a certain concentration has been built up, it may be reabsorbed. If a large inoculum is used there is no lag phase, if a very small one is used, there is. If, however, 1 mg of glycolic acid is added to the medium there is no lag phase even with a small inoculum. Strickland said he had found a maximum of 0.2 mg/liter glycolic acid in seawater and Fogg said that as this is about the upper limit found in freshwater, it is, therefore, important to do experiments with additions of less than 1 mg/liter of the acid. Fogg has published a valuable review²⁰¹ of what is known about glycolic acid in natural waters and its release into the medium from algal cells. Droop's account of the effect of sodium sulfide on lag phases in *Skeletonema* has been considered earlier. Strickland also said he had observed a lag phase with *Skeletonema*. Krauss found a long lag phase in a mutant *Chlorella* which could be removed by using a complex enriched medium. Oppenheimer referred to the delayed appearance of sulfate reducing bacteria in some stored samples of seawater. Spencer said that the lag phase he finds in *Phaeodactylon* seems to be similar in nature to that in Fogg's *Chlorella*, though it is not known whether glycolic acid is the key substance. A *Nannochloris* studied by Droop shows a lag phase whose length varies with the size of the inoculum. This can be reduced or eliminated by various things, including setting the pH at 8, so that the metabolite may simply be the hydroxyl ion. Jones pointed out that, besides the necessity for an organism to produce its own particular compound

before multiplication can start, it is also possible that substances originally in the medium can produce a lag phase and that he had noted that this often happens when an addition is made to a given medium. This led to a discussion of inhibitory substances, but it may first be mentioned that it does not seem to be known how important lag phases are in natural situations. The varied experiences described here suggest that we should expect lag phases to be of importance in determining natural events. Using *Asterionella* as an example again, it seems unlikely that a lag phase is involved in the change from low or decreasing numbers in winter to the great increase in spring. We have seen that this change is explicable on the basis that increased light is responsible. It also seems unlikely that glycolic acid can play a major role at this time of year because, during winter, there is very little algal activity and the products of metabolism of the previous summer's crops are so much diluted by the inflows. In the north basin of Windermere (FIGURE 39 [See page 170]) numbers of *Asterionella* may fall to less than 1 cell/ml or even to 1 cell/10 ml, the absolute minimum being unknown. The populations of most other algae fall to much lower levels.

Strickland considered that much of the evidence for inhibitory substances is rudimentary or conflicting and wondered how many carefully substantiated examples are known. McLaughlin described a good example of the interaction between a *Gymnodinium* and a *Peridinium*, which can be grown in the same medium separately but not necessarily together, and how their interaction fluctuates with the illumination. Droop added that the opposite can happen. *Monochrysis* can grow in a medium which is originally free of vitamin B₁₂ and thiamine, if *Nannochloris* is added. *Nannochloris* has no vitamin requirements but presumably produces them, and the extracellular moiety is used by *Monochrysis*. Organisms producing vitamins will not, however, necessarily release them into the medium, an example being the *Chlorella* studied by Fogg. In McLaughlin's example, vitamin requirements are not involved, as the needs of both dinoflagellates are the same. Droop described how it is possible to see bleaching of the cells in a culture of *Skeletonema* start in one small area and then spread rapidly outwards. Guillard found that if the medium from the bleached cultures of a diatom is reinoculated with that diatom, or with other species, there is no growth. If the water is treated with charcoal the inhibitory substance is removed. Johnston investigated the sensitivity of some marine algae to certain antimetabolites. The interesting result was that, using enriched seawater and adding graded amounts of the antimetabolites, he found that the spring species are most sensitive to antimetabolites, and those coming in later in summer are least sensitive.

One may conclude that inhibitors are not uncommon, that they are important ecological factors, and that determining their effect in nature is a hard task beset with pitfalls.

Heterotrophic growth has been mentioned earlier, but several accounts of high uptake of CO_2 in the dark were given after Strickland had said there was some slight evidence that *Thalassiosira nordenskiöldii* and *Skeletonema costatum* are capable of heterotrophic growth. He referred also to the extraordinarily large numbers of coccolithophorids at great depths. Vollenweider discussed evidence for heterotrophic growth, perhaps with some slight photosynthesis, in freshwaters. He had obtained some remarkably high results for the uptake of CO_2 in the dark. Droop raised a doubt as to whether there is such CO_2 uptake in heterotrophic growth and Oppenheimer said there could be. Steemann Nielsen said that, using C^{14} , the uptake in heterotrophic growth was about 10 per cent compared with respiratory relationships. Vollenweider said the cycle in the dark could be very curious in that it paralleled the photic cycle. Strickland and Goldman also described further singular results they had obtained. Although all the speakers were cautious in simply saying that such results are not clearly explicable, it seems that the question of limited amounts of heterotrophic growth is just as open as that about the utilization of light at intensities commonly considered to be too low for photosynthesis (cf.^{106 279}).

Biological Factors

Braarud introduced the subject of grazing by saying that though there were many statements about the inability of zooplankton to ingest different kinds of algae, there was not, in fact, much definite information. After he had given an example showing that grazing may be selective, Fogg referred to Professor Edmondson's work on the food of rotifers. Professor Edmondson has developed a technique for estimating the growth rate of certain species.²⁰¹ In Windermere, and two other lakes in the English Lake District (FIGURE 56) he finds a high degree of correlation between the abundance of certain very small algae (e.g. *Chrysochromulina parva*) and the temperature of the water, on the one hand, and the growth rate of these rotifers, on the other hand. His direct observations on the feeding of rotifers support his statistical correlations. They are clearly selective feeders. Vollenweider and Oppenheimer supported this view, quoting other investigations. Droop found that a rotifer he studied did not ingest about 99.9 per cent of the material coming into the vortex. This, he suggested, might have something to do with the compactness of the organisms. Oppenheimer pointed out that colloidal aspects and surface charges become

important when such small particles are involved, a matter discussed further in relation to bacteria on and around algae. He had had the same experience as Droop, and considers that it is possible for an organism to distinguish differences in the surface charges on the potential prey. Braarud referred to the importance of such problems in relation to the herring fisheries. The distribution of the herring seems to be determined by that of the zooplankton which, in turn, is dependent on the phytoplankton. Herring breeding seems to be related directly to the development of the spring increase of the algae. This brings us back to the patchiness of seawater as far as the growth of plankton is concerned and, so, to the movement of water masses. It is, as Braarud said, a very complex story.

Koczy raised another aspect of the herring story when describing the effect of a severe epidemic of parasitism of copepods. Braarud said that, though it was realized that fungal parasites of marine plankters existed, the information about them is rather small (see²⁰⁴). In freshwater, fungal parasites are very common and rather more is known about them. Many are restricted to one or a few near-related algae. It was explained that despite all the information available very little was understood about the reasons for the fluctuations in the degree of infection of populations. The fungi might have little effect on the host alga for long periods and then rapidly increase, producing "epidemics," so that there was a marked decline in the abundance of the alga. Such changes cannot be correlated with other known fluctuations in the environment. It should also be mentioned that there are algae whose populations are almost always parasitized to a small extent, but practically never severely enough to lead to measurable decreases in their numbers. The diatom *Melosira italica* subsp. *subarctica* furnishes a good example. In the 8 years depicted in FIGURE 5b only once in three bodies of water was there a serious infestation of the alga by its special parasite. Data for the succeeding 10 years have confirmed this relationship. In all 18 years, only one epidemic has occurred (25 per cent or more of the algal cells infected). Three epidemics have, however, been seen in other lakes of the English Lake District. For further details about these fungi the papers by Canter, Canter & Lund, and Sparrow²⁰⁵ can be consulted.

Freshwater algae are also commonly "infested" by bacteria though these are not parasites (FIGURES 41a, b, c and d). The characteristic forms concerned have not been cultivated, so their biology is not understood. It would be unjustified, therefore, to believe that they are symbionts. There does, however, often seem to be a community composed of an alga and its attendant bacteria. Oppenheimer again referred to the importance of surface charges and the polar nature of many such bacteria, and Fremont-Smith gave an example from medi-

cal science. Oppenheimer referred to the remarkable facts that the living diatoms in the sedimentary environment he studied were free of bacteria but, as soon as they died, large bacterial populations developed on them. This, he suggested, could be related to the changes in the surface potentials of dead and live algae. This situation exists also, to some extent, in freshwater plankton diatoms, but I am not convinced that live diatoms do not have bacteria attached to them sometimes. This is merely from rather casual observations. *Asterionella* often shows what I may call "the Oppenheimer situation," actively growing populations in nature or in bacterized cultures being free of attached bacteria. When, however, the catastrophic decline starts at the end of the spring maximum, the dead or dying cells are colonized by large numbers of bacteria. These, like those described by Oppenheimer, are polar located. However, identical bacteria may be found on some, but not all, of the other diatoms whose populations may still be increasing. A good example is *Tabellaria flocculosa* var. *asterionelloides* which almost invariably accompanies *Asterionella* during the spring bloom and may continue to increase for a time after the beginning of the decline of the population of *Asterionella* (FIGURE 39).

This brings up the question as to why are healthy diatoms so commonly free of bacteria? Goldman asked whether it had not been postulated that the production of a metabolite kept diatoms free of bacteria. Oppenheimer said that was so but when the sedimentary environment is studied carefully, as he has done, so many diverse organisms are seen moving about and growing in such close proximity to one another that these antimetabolites can hardly be expected to exist. He also added that the enzymological possibilities are fundamentally different when a bacterium is living on a cell than when it is at some distance from it (e.g. in mucilage).

Certain ecological aspects of the life histories of algae were raised. Riley and Braarud gave examples of the importance of resting stages. These have long been considered to be of fundamental importance in relation to season cycles. Braarud stressed their frequency in coastal forms and rarity in oceanic ones. Riley made the good point that the apparent failure to find a species may be simply that we do not look for it quite as thoroughly as we might. It is, of course, often a physical impossibility to do so.

While agreeing with what was said, I think that the importance of resting-stages can be over-estimated. Apart from the many species which have no known resting stage, such as some of the commonest freshwater diatoms, the production of resting stages by algae which possess them may not be essential to their success, though they may be helpful. A striking example within my own experience is the very common blue-green alga *Aphanizomenon flosaquae* f. *gracile*. Accord-

ing to Komárek,²⁰⁶ this alga overwinters as spores, though he quotes instances of what appear to be examples of the perennial presence of threads. In Esthwaite Water, and in the south basin of Windermere, which receives its outflow (FIGURE 56), spores have only been seen in 2 out of 18 years and were common only for a time during one of those years. For the remaining 16 years none were seen, despite weekly sampling. In Esthwaite Water the alga is always present and overwinters as isolated threads. In Blelham Tarn (FIGURE 56) no spores have been found during the 12 years the alga has been seen in the lake. Other freshwater algae almost invariably produce spores at certain seasons that in some, though not all, lakes may be found throughout the year (e.g. *Ceratium hirundinella*). The plankton desmids of our lakes, mentioned in an earlier session, may be able to produce spores (zygotes) but have never been seen to do so in these lakes. Lastly, there are species which generally produce spores in abundance and cannot be found for long periods each year (e.g. *Dinobryon divergens*, *Uroglena americana*).

In freshwater, therefore, and maybe in the sea, there are, I believe, three main types of species. First there are those in which spores must be produced in one year in order to assure success in the next. Second, those which can succeed without possessing any resting stage (cf. oceanic diatoms). Third, those which can and do produce resting spores, albeit perhaps at irregular intervals, but for which the importance of these spores depends on the type of environment in which they live. Thus an alga may over-winter in the form of isolated cells, etc., but, if resting spores have also been produced and will germinate in a period favorable for the growth of the alga, then the sparse population already present will be rapidly augmented at the right moment. The same arguments apply to algae which do not produce spores but can pass into what may be termed a physiological resting stage (e.g. *Melosira* sp.)

Strickland mentioned in passing that algae may exist in another form, not a resting stage, between planktonic phases, citing coccolithophorids. For some of these it is clear that there is a benthic phase in their life histories,²⁰⁷⁻²⁰⁹ a phase which may already be known under a different generic name. This was such an unexpected discovery that we must be prepared for others, though not, I think, among diatoms. Even for coccolithophorids there must be some question as to how general such life cycles can be far from the shore and, therefore, how significant they are in the ecology of oceanic species.

In commenting on Guillard's difficulty in growing *Skeletonema* at low temperatures, Droop quoted Von Stosch's belief that much of the variable behavior of this and other diatoms is because of recombinations from one cycle to another.²⁵⁹ Since the progeny of a single cell of

Skeletonema will be able to develop both sexes, this raises the difficulty, referred to in an earlier session, of how far contradictory results at different times and places are caused by differences in what are alleged to be identical organisms. To the changes which may take place after relatively long periods of cultivation must be added those arising from sexual recombination. There may, however, be a danger of over-emphasizing such difficulties. It depends on the organism and the problem to be solved. It is perfectly possible to obtain valuable ecological information when observing or experimenting with many sexual organisms. It would be unfortunate if the study of so important an organism as *Skeletonema* were to be avoided because of such doubt. Almost anything we do is imperfect, the question is how great does an imperfection have to be before it makes it impossible to obtain a significant result. This viewpoint leads into the next section.

The Human Approach—Techniques

Techniques were mentioned frequently, because in this type of meeting it is possible to cross-question or interrupt the speaker. There is one technique which merits special consideration because most of the last part of the session was devoted to it. This is the use of plastic containers to isolate masses of water which are far larger than can be manipulated in the laboratory, and to keep them under more natural conditions. These may be spherical or cylindrical, floating or attached, and placed vertically or horizontally. They offer us a further method of testing our theories experimentally and of making observations, the results of which can be compared with those using small containers, axenic cultures, and special assay organisms. Ecology, some say, is not a science at all, and, so far as erecting and testing theories is concerned, it does not, as yet, offer the same opportunities for precision in thought and deed as do physics and chemistry. At the moment we must depend on what may be termed statistical evidence. If, for example, the results of observation, analysis, and experiments with diverse equipment and methods all lead to the same conclusion, then there is a reasonable prospect that this is the correct answer.

Strickland and Goldman, two pioneers in using plastic spheres and tubes, and I all agreed that there are a number of practical difficulties in making and maintaining these "sea-monsters." Strickland emphasized that if this approach is to be tried, there is little point in covering the size scale intermediate between something which can be handled in the laboratory and that represented by a very large vessel. No material is absolutely inert biologically. The greater the surface to volume ratio, the greater the influence of the attached population, and the possibility of large losses of substances on to the walls (e.g.

Koczy's information about phosphates). It is in using natural mixed populations that the larger vessels are especially valuable. The inclusion or exclusion of a number of unusual or relatively large organisms (e.g. zooplankters) is not fatal to the success of the experiment. When using small containers, some pretreatment is inevitable, and the greater this is, the more artificial the world inside the container will be. It is also possible to take frequent samples of adequate size from large vessels for all kinds of analyses without altering the volume inside significantly. They offer, therefore, new opportunities for research but do not replace the other experimental methods.

McLaughlin described a good arrangement for using several 2-liter polyethylene containers filled with axenic cultures. The advantage of this relatively small scale technique is that comparisons can be made between axenic cultures in the laboratory under fixed light and temperature conditions, and those exposed to natural variations of the underwater "climate." To do such pure culture experiments in spheres the size of Strickland's would be a formidable task. Apart from bags and spheres, Goldman and I discussed the use of cylinders. With these the natural "climate" in depth can be obtained. They can be let down onto or into the mud surface so that comparisons can be made between the influence of the water alone or the water with the mud surface on chemical changes near or at the bottom of a lake. It is relatively easy to arrange for occasional aeration or mixing and, in small lakes, for continuous aeration. Though polyethylene is permeable to oxygen, the rate of exchange is slow enough to permit different concentrations to develop inside and outside the cylinder.

The populations in a large container may change considerably in a month or so, largely because of the ever-increasing growth of algae and other organisms on the walls. A cylinder holding over 400 m³ of water has been found to produce floras similar to those in littoral regions of the lake concerned or in ponds in such periods of time during summer. The changes are naturally slower in a more oligotrophic lake and in winter. It is also to be expected that they will be less marked the farther the water used to fill them comes from the shores. Therefore, in big lakes and in the sea these difficulties should be less, or delayed in time for so long that this exceeds the period of the experiment or observation.

When you listen to people whose abilities you respect and whose work you admire, and hear them suggesting various approaches to problems which all agree are very complex, you are bound to come to some conclusions of your own. The summation offers an opportunity to air one's opinions and a strong temptation to propagate one's prejudices. In yielding to temptation—often a delightful process—my excuse is the hope that, in so doing, I am carrying out the instructions

to summarizers. The session was characterized, in part, by what might almost be termed the exchanging of anecdotes about the mysterious behavior of algae in nature and in laboratory cultures. In this, the wishes of the organizers of the conference were fulfilled. There was also valuable information on techniques and the results obtained when they were used. There was some divergence of view about the use of simple approaches in studying synecology. I favor the simple approach, but it is quite true that what one believes to be simple may be very complex; the simplicity is in one's own mind. Yet, in fact, everyone favored the experimental approach and no one has found an experimental method that does not involve the study of seminatural and very artificial situations. I would suggest, therefore, that, in truth, we all favored the simple approach and that the real disagreement among us was how much simplification can be introduced before we are studying environments which are too far removed from the natural one to have much value in synecological work, and whether we have understood the real nature of our man-made or man-altered environments.

What then is to be said for or against the various types of investigation described in this session? In my view there is nothing to be said against them. If there were to be criticisms they would be that some approaches in certain circumstances were better than others. To rephrase a well-known statement: all investigations are valuable but some are more valuable than others. There are three other good reasons for not making criticisms. First, that most of the discussion was about marine plankton and, without first-hand experience in this field, criticism might well be based on faulty understanding. Second, everyone has to adapt his expenditure of effort and money according to the time and resources at his disposal. Third, unless a plan for an investigation can be shown to be bad before it is put into action, no one should be prevented from doing what he wishes to do. This is, in any case, unlikely to be what he would wish to do in an ideal world. No one is really free. The most he can hope for is to do what he can within the limitation of his own ecological situation.

The third reason may well be considered scarcely worth mentioning. It is only an insipid restatement of one of the principles of fundamental science. Yet we should try to keep it in mind always or we may fall all too easily into the pit of mental pride; into the error of deriding theories or experiments because they seem to us to be fantastic or exotic. This is indeed a great danger. Thus, once some new method has been proved to represent an advance in a certain direction the use of the old method is likely to be derided, sometimes just because it is old, without realizing that it may still have its uses. Many examples could be given but two methods of proven importance in elucidating our own

problems may be mentioned, namely, the use of cultures derived from one cell and those free of all other organisms. If you do not use clonal and, preferably axenic, cultures, you are almost certain to feel inferior whether you admit to such feelings or not. This is because impure or nonclonal cultures cannot be used to elucidate some problems, have led to fallacious conclusions in the past, and are commonly not the best methods to use. However, since, in nature, such single populations if they do exist are extremely rare, it is always possible that nonclonal and impure cultures can be of some value even today, limited though this may be.

If there is one approach to synecological problems which I would consider to be specially valuable, it is what I will call generalship. By that I mean an analogy to the preparation of a general for an approaching battle. He will ask for all the material and human resources he believes to be necessary, knowing that he will not receive them. He will then consider, after careful preparation, how, when and where to dispose of the insufficient resources he has been given. Lastly, he will imagine himself to be the enemy's general and consider what resources he would have then, and what he would do. The ability to do this last is, perhaps, the decisive test of generalship. It is analagous to what Kekulé meant when he said: "Let us learn to dream dreams, then, perhaps, we shall find the truth" (quoted from²¹⁰). Any attempt to project oneself, as it were, into the world of plankton is bound to have pitfalls, but, equally, if we cannot to some extent envisage this aquatic world, our attempts to understand it are likely to be illusory. Indeed, it would be possible to quote examples of statements that certain things do not take place in nature when all the evidence of our senses suggests that they do so. It seems to me that a sensible and indeed simple approach is to start from the viewpoint that our senses are giving us correct information. If we, at the same time, remember the rest of Kekulé's remarks—"But let us beware of publishing our dreams before they have been put to the proof by our waking understanding"—then we shall not forget that our senses may mislead us.

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III. INTEGRATIVE IN VIVO AND IN VITRO ASPECTS

NUTRITIONAL PROBLEMS

Discussion leader:

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DROOP: To continue with the discussion on nutrition, so far, we have not discussed inorganic nutrients or trace elements and iron. Personally, I have always tended to take the major inorganics for granted, but a number of others present have not. I don't feel competent, for instance, to say very much about phosphorus, but I think we could, for a start, discuss the effects of putting too much of these nutrients in our culture media. In order to get any reasonable amount of growth in a culture, if it is a batch system, rather a lot of nutrient has to be put in, more than a natural amount. This applies especially to those of our so-called defined media that are designed expressly to avoid precipitating calcium and phosphorus. In the old days, there was a culture medium called Miquel.^{211,212} By means of a rather complicated sort of ritual, a precipitate was obtained, very important precipitate. I forget now whether it had to be left in the medium or taken out; I think that varied from laboratory to laboratory. In my laboratory, it was taken out. I heard recently that, at Wood's Hole, at least in some of his media, Doctor Guillard gets precipitates and leaves them in the medium. I know that he can cultivate a great many diatoms that I can't, and I am wondering whether the secret of this is not a matter of concentration of such substances as phosphorus which may be toxic. In other words, is the precipitate an effective way to avoid high instantaneous concentrations of phosphorus and, yet, at the same time, provide a large reserve? "Biphasic" culture media have been very successful in the past, for some purposes.⁷⁶ Doctor Spencer, can you comment on this?

SPENCER: I can't say anything about precipitates in culture medium, as such. If, however, one carefully follows the kinetics of

growth, with even such a robust diatom as *Phaeodactylum*, one can see quite easily the markedly adverse effects on the pattern of growth caused by changes in the initial phosphate concentration of the medium.²¹³ These effects show up in various ways. Increases in the initial concentration of phosphate (in medium where phosphate is the crop-limiting nutrient) sometimes produce slight symptoms of deadadaptation when exponentially growing cells are used as the inoculum. If phosphate deficient cells taken from the stationary phase are used as an inoculum, much more marked effects which include prolonged initial lag phase, are produced.

I always look at the results in this way: *Phaeodactylum* is a pretty sturdy diatom, and if *Phaeodactylum* shows these effects even slightly, there seems to be a chance for the more delicate organisms to show them very much more acutely. In fact, taking an organism on initial isolation out of the sea, and putting it straight into the typical sort of initial phosphate concentrations that we use, may involve a very severe physiological shock that many of them may not get over. Hence, the rational step I believe many people employ on isolation is to use considerably diluted medium.

HUTCHINSON: "Is there any case known in the sea, like that of *Dinobryon* in freshwater, which appears to be, at least in nature, unable to live with more than 5 mg/m³ of phosphate phosphorus?"

STEEMANN NIELSEN: I would just like to stress the importance of using very dilute suspensions when growing algae. In our laboratory, we always use very dilute suspensions, being sure first of all that we are really able to give the whole suspension the same illumination. With a dense culture, hardly anything specific is known about the illumination of the single algae. In many other respects, a dilute culture is of importance. For instance, if just ordinary air is bubbled through the culture, the algae will get a sufficient concentration of carbon dioxide. When ordinary air is blown through a dense culture, the carbon dioxide supply is insufficient for photosynthesis. Therefore, in more dense cultures, air containing, say five per cent carbon dioxide has to be used and that is not at all a natural condition.

It is as easy to work with very thin suspensions of an alga. If the culture is to be used for measuring the rate of photosynthesis, and if the Carbon¹⁴ technique is employed, it is not necessary to have a lot of algae. It is very often important to have a little more natural condition than when a real dense culture is used.

PROVASOLI: Don't you think the five per cent carbon dioxide is going to be harmful, also, because it might bring down the pH if the medium is not well buffered?

STEEMANN NIELSEN: No. One can always compensate for pH change.

PROVASOLI: But that in itself is an unnatural condition.

STEEMANN NIELSEN: Yes. If algae are grown using a concentration of carbon dioxide, as in atmospheric air, and then transferred to five per cent carbon dioxide, there may be a narcotic effect for some time; but, of course, after a while the algae will adapt to the higher concentration of carbon dioxide. We have to try to get natural conditions in our cultures.

DROOP: Yes. In the case of *Skeletonema*, 25 cells/mm³ out in the sea is a good spring maximum, whereas one deals with nearly 25,000 cells as a maximum in the usual culture tube.

STRICKLAND: One can measure growth kinetics with cell concentrations ranging from seawater concentrations up to about 50 times this level without any marked differences, so it does not follow that using rather high cell densities is bad.

DROOP: That is not necessarily bad, only more difficult to control.

GUILLARD: In media in which a precipitate forms upon sterilization, the concentration of soluble phosphate remaining in the medium is not actually high. If the medium is membrane-filtered, most of the phosphorus is recovered on the filter, and only a few microgram atoms per liter are left in the medium.

DROOP: Do you know if that is replaced from the precipitate reserve as it is used up?

GUILLARD: Yes. First, some of the precipitate disappears as the culture grows. Further, the amount of phosphate in solution isn't enough to produce the final crop of algae. About 3 μ g atoms/liter remain in solution after autoclaving, and somewhat more than this is required to produce a dense culture, so it must come from the precipitate.

PROVASOLI: Since many of us employ different methods in media making, I think this is the time to discuss the various units and their equivalents.

GUILLARD: One μ g atom/liter of phosphate equals about 31 μ g phosphorus/liter, corresponding to 10^{-6} M of KH_2PO_4 , for example. One can't speak of a mol of an element; it is a gram-atom. Usual enrichments call for about 10 mg/liter of some phosphorus salt, yielding about 70 μ g atoms of phosphorus/liter, which is about 30 times what one would expect to find in the sea. If seawater media are not buffered, a somewhat yellow precipitate forms upon autoclaving, which contains all but about 3 μ g atoms/liter of the phosphorus. It probably contains much of the iron, too.

EPPLEY: Does it matter whether the cells are using the soluble or the precipitated phosphate?

GUILLARD: I don't think there is evidence that they use the visible precipitate directly. If one makes a bioassay for phosphate similar to

the vitamin bioassays described previously, the algae will grow if the phosphate is autoclaved in the medium either with or without a buffer, or added after autoclaving. Some phosphorus remains soluble, at least in such a form that it passes through a membrane filter.

GOLDMAN: Is it possible that there is a dynamic equilibrium in the culture between the precipitate and the growing culture? This condition could make the precipitate a beneficial part of the culture medium.

PROVASOLI: I think that precipitates may be an excellent reservoir of nontoxic nutrients if their solubility constant provides soluble ions at a rate and quantity compatible with the need of the organisms.

HUTCHINSON: Is there any good work on physical chemistry of phosphate in sea water?

GOLDBERG: No.

HUTCHINSON: It seems to me to be an extremely important thing, although very difficult.

JOHNSTON: As a rule, if phosphate is added to natural seawater, it doesn't improve the C^{14} uptake at all. The algae won't grow any more because more phosphate is put in.

MCLAUGHLIN: It depends on how much phosphate is present in the original water.

JOHNSTON: I am thinking of the midsummer North Sea concentrations of phosphate, which are seldom less than 0.1 μg atoms/liter. That is never limiting, I should say.

MCLAUGHLIN: The idea of having precipitates is something I have tried to avoid. Unlike Doctor Droop, I am more concerned with precipitates. A good number of the organisms I have tried to isolate, and those that I have isolated, will neither take on isolation nor grow in a precipitated media. In our experimental bases, we work out the minimal phosphate requirements and those concentrations which are inhibitory to growth of the algae.

EPPLEY: My point was not that precipitates are good. I had some rather questionable evidence that *Porphyra* cells (which so far as being a seaweed is not perhaps quite cricket here), were using phosphate directly from the precipitate at a greater rate than they were using the dissolved phosphate.

MCLAUGHLIN: You mean the phosphate disappeared?

KOCZY: How?

EPPLEY: I was trying to measure phosphate uptake as a function of pH, and, of course, at the higher pH's much phosphate precipitated. But I ignored it. Measuring the time course of radio-phosphate uptake, taking samples every 10 minutes or so for an hour, I found that, at neutral pH, where the phosphate is soluble, there is a fairly slow and linear uptake of phosphate by pieces of the seaweed. At higher

pH, where the precipitate forms, there is a large initial absorption which probably doesn't represent phosphate entering the cells. Following this, there is a linear phase in which the phosphate uptake continues to increase, and it does so at a greater rate than in any of the treatments in which the phosphate was dissolved. I am still perplexed by this.

GOLDBERG: This might be explained by increasing the amount of the more highly ionized forms of the phosphate. That is, making for available the univalent, divalent, and possibly even the trivalent ions as the pH increases.

SPENCER: What was the pH range?

EPPLEY: The precipitate was most obvious at pH 8.5 and 9, and it was at the higher pH that the maximum linear rates of phosphate uptake took place.

MCLAUGHLIN: Was the medium buffered?

EPPLEY: It only went down to pH 7.

STRICKLAND: Perhaps the plant likes to work at the higher pH.

HUTCHINSON: Were the algal cells in contact with the precipitate?

EPPLEY: Yes. The pieces of seaweed were shaken in flasks containing the precipitate.

HUTCHINSON: Is there a possibility that there is an ionic atmosphere around the algae at a lower pH so that at the contact between algal cell and precipitate, the latter may be able to dissolve?

EPPLEY: I have no way of knowing.

DROOP: That is Harvey's idea.²¹⁴

HUTCHINSON: Yes, and I don't see any reason to abandon it, do you?

DROOP: No, not at all.

MYERS: I have a rather simple-minded notion about the problem of ion provision and uptake from complex media. It is based on the idea that the total reservoir for any one ion is provided by three components: (a) the free ion concentration, (b) the concentration of any insoluble or colloidal precipitate which ties up the ion by virtue of a low solubility product, (c) the concentration of any chelate which ties up the ion in an un-ionized complex.

In a medium without chelate, one can observe an iron deficiency develop with time as it sits on the shelf. There is no visible precipitate. One infers the formation of a colloidal phosphate or hydroxide. But when a chelating agent is added initially, then an iron deficiency never develops. It must be that the chelate lowers the ionic concentration to a level less than that of the solubility product of the insoluble phosphate or hydroxide. Apparently, the chelate is readily reversible; it provides a useful reservoir for ions even though the ionic concentration is held very low.

A precipitate also can provide an ion reservoir, but its usefulness depends on the rate at which it may redissolve, on rates rather than on equilibria. This is a rather messy problem, as we are all aware from the simple experiences with precipitates in analytical chemistry. However, the two-phase system provided by an insoluble salt certainly provides a possible approach to the creation of ionic reservoirs for culture media.

I understand Doctor Steemann Nielsen's desire, and the desire of you all, to work at low concentrations in order to mimic natural conditions. But, sooner or later, you are going to be faced with the problem of obtaining quantities of plankton to work with in order to find out what there is. When that happens, then, in marine media, there will be an awkwardness of low limits of stability for many ionic concentrations. The device of a two-phase system, if it can be made rapidly utilizable, is certainly one possible answer.

STRICKLAND: Ion exchange has been used for this purpose.²¹⁵ Has anyone here ever tried using ion exchange resins?

JOHNSTON: Yes, I have tried that. If, instead of adding EDTA to a culture medium, one or two of a chelating- or ion-exchange resin are put in, mixed diatom populations will grow quite happily in this two-phase system.

I think I can help Doctor Myers a little about the failure of this stored ion solution. Commonly accepted values for the solubility of ferric hydroxide (about 10^{-7} M) have recently been shown by Lengweiler²¹⁶ to refer to a colloidal dispersion rather than a true solution. Freshly precipitated ferric hydroxide transforms itself into crystalline -FeOOH which is at least 100 or even 1,000 times less soluble than the freshly prepared ferric hydroxide.

DROOP: Which of those two states is described by the solubility constant 10^{-39} , I believe that one has learned?

JOHNSTON: I refer to the solubility of $\text{Fe}(\text{OH})_3$. A freshly prepared solution is said to be about 3×10^{-7} M. This is quoted from recent studies by Stumm and Lee, and Hem and others.²¹⁷⁻²¹⁹ Lengweiler has shown this so-called solution transforms into -FeOOH, which may be as much as 1,000 times less soluble.

DROOP: There probably isn't an answer to my question, because the solubility product is a question of ions, and we are talking about solubility of an un-ionized substance, aren't we?

JOHNSTON: Yes. I am a little confused here. It is a matter of sorting it out. [In addition to airing the problem of precipitates from iron solutions, Doctor Myers desired information about the rate of solution of the precipitate formed. Lengweiler²¹⁶ also studied the rate of solution of ferric hydroxide at different pH's using Fe^{59} . He found the rate of

resolution was immeasurably slow except at very high or very low hydrogen ion concentration.

Regarding the solubility constant (product) of ferric hydroxide mentioned by Doctor Droop, the value 10^{-39} referred to is the product of the activities of the "theoretical" ferric and hydroxyl ions, thus:

$$[\text{Fe}^{3+}] [\text{OH}^-]^3 = 10^{-39} \quad (1)$$

This, in theory, establishes the limit for ferric ion in a pure salt medium. For practical purposes, in culture work involving complex media, what is really required is greater knowledge of the nature of the iron ions, molecules, or particles a diatom can utilize. I know of no experimental evidence that can answer this directly, but some points in this connection are examined in detail in my forthcoming paper.^{220]}

GOLDBERG: Has anyone ever analyzed any of the precipitates that come in a culture medium.

MCLAUGHLIN: Yes. Most of it is iron. It depends on what is put in to start with.

GOLDBERG: Is this yellow material iron phosphate?

MCLAUGHLIN: Essentially, yes.

GOLDBERG: How about the white phases?

MCLAUGHLIN: That is calcium. It depends, again, on such variables as how long it is autoclaved, what the pH is to start with, what the redox is before autoclaving, and how well it is buffered. If a very weak buffer is used and the pH drifts acid and then goes alkaline, as the solubility of certain ions is exceeded, they precipitate out and don't go back into solution.

One can autoclave or inoculate 10 tubes with the same organism and five tubes will grow and five tubes won't, and it will be found that someone forgot to clean the glassware of the five tubes that don't grow; there was a little soap on them, a little detergent that caused a precipitate. These are variables which one must take into account when constructing artificial media. The phosphate concentration is very important in a synthetic base, as it forms precipitates with other components quite easily. Our bases could be better constructed if we had more information on phosphate concentrations in the biotope and some knowledge of the types of natural chelators found in the oceans. We could then adjust our medium to reflect the *in vivo* conditions. As to forming reservoirs in the bases, one can usually add 10 times as much PO_4^{-3} as the minimum concentration to be found, say, in the North Sea. The medium will not precipitate providing the iron is in a chelated form and the buffer is strong enough to hold the pH during autoclaving. These are some of the variables one is faced with in designing synthetic media.

GOLDBERG. With regard to the iron problem, there is work going on now by Professor Sillen and Doctor T. Chow in Sweden.* They are trying to ascertain the types of soluble complexes of iron, and whether they are charged or not, in order to account for the soluble iron that many investigators report. We define soluble iron as that which goes through a membrane filter, and the particulate iron as that which is retained. Enough laboratories now have found that seawater containing iron can be repeatedly filtered through rather small pore-sized membranes, and one still finds iron going through at levels of around 3 $\mu\text{g/liter}$. Apparently there are undissociated iron complexes, perhaps soluble $\text{Fe}(\text{OOH})$.²²¹

PROVASOLI: One has to take into consideration that there are also organic chelators in natural seawater.

GOLDBERG: Tell me one organic compound that will chelate iron in natural seawater.

KOCZY: The yellow substances are supposed to do that.²²²

GOLDBERG: From the experiments of Laevastu and Thompson,²²³ who observed high amounts of soluble iron in coastal waters, the results have to be attributed to chelation or organic complexing of some type. In the open sea, considering the total amounts of organic matter that are measured, it is difficult to find enough that can possibly be chelated with iron.

JONES: May I ask, what is yellow substance?

HUTCHINSON: It has apparently a quite low molecular weight, but is otherwise like what used to be called humic acid.²²²

JONES: Are we talking about a peptide now, an amino acid, something like that?

HUTCHINSON: Yes, presumably nonnitrogenous.

DROOP: It is a polyphenol affair.

HUTCHINSON: I believe it is now thought to be.

GOLDBERG: Peter Williams²²⁴ isolated a dicarboxylic acid from seawater that had a molecular weight of around 395, and an equivalent weight of 208. It is apparently fairly abundant, that is, of the order of 1 or 2 mg/liter.

HUTCHINSON: Is it yellow?

GOLDBERG: Yes.

JOHNSTON: Does it contain sulphur?

GOLDBERG: No, sulphur, nitrogen, halides, phosphorus, boron or iron. No unsaturation or aromaticity based on infrared spectra were detected.

* L. G. Sillen, Royal Institute of Technology, Stockholm, Sweden, and T. J. Chow, Scripps Institution of Oceanography, La. Jolla, Calif.

HUTCHINSON: Does the net carbon content give an inconsistency?

GOLDBERG: The trouble is that there have to be ether linkages to account for the oxygen. If a carbon, hydrogen, oxygen analysis is made, the resulting structure must show ether linkage to make sense. This worries us.

HUTCHINSON: I think there is something queer about the structure.

BRAARUD: The problem of organic matters is a very interesting one, but since there are limnologists present, we should also mention silica before we leave the subject of the nutritional factors. In fresh water, silica is shown to be definitely a limiting factor for diatom growth, but in seawater, it is a little bit of an enigma. What about silica concentration and its effect upon growth?

My own impression, from studying populations in the sea, is that the various species adapt themselves to the actual concentration or supply of silica and produce thick walls if there is ample supply, and increasingly thinner walls according to the lower concentration. But this is only an impression. In some species, such as *Skeletonema*, the morphological changes tend to a great extent to be due to a lower supply of silica which induces the cell not to be as extravagant as it usually is in producing long spines, and only the essential part of the cell is left.²²⁵

That is the general impression based on populations in the sea. There is very little experimental data on this subject. I would like to ask Doctor Lund, who knows a great deal about the fresh water situation, if he has any idea about the situation in the sea.

LUND: I don't know anything about it.

DROOP: Tell us about silica in fresh water.

LUND: In fresh water some diatoms²²⁶ can be grown that can and do reduce the amount of silica they have per cell. There are other diatoms which so far nobody has been able to grow in such a way as to reduce significantly the amount of silica per unit surface area. So there do seem to be marked differences between them. Still other diatoms can be grown that eventually will become completely naked, with no silica wall at all.

BRAARUD: Are these planktonic?

LUND: No; so far as I know, none of them are planktonic.

KRAUSS: Do I understand you to say that some diatoms will grow and continue to grow in the absence of silicon?

LUND: Yes, this is being done for soil diatoms, if I remember correctly. [Now that I have been able to refresh my memory, I am not convinced that any diatom has been grown in the absence of silicon, nor does the appearance of cells without the normal silica wall seem to depend particularly on the supply of silica. Reductions in the amount of silica per cell can sometimes be related to decreases in the

amount of silica available. The so-called naked cells represent a sudden change, what might reasonable be called an abnormality.]

PROVASOLI: Was this work done in glassware or in polyethylene containers?

LUND: The work I am thinking of was done some time ago and I am sure it was not done in polyethylene.

DROOP: Could you say something about the phosphorus/silicon interaction and growth limitation? You did find some interaction?

LUND: This is very difficult because I really don't know that I understand what I found out. It is hard, therefore, to know where to start.

With regard to one diatom, *Asterionella*, in some lakes it has been shown that, at certain times, there is not enough silica for the population as a whole to go on dividing; that is, there is not enough for a further cell division. When that happens, one can be quite sure that there will not be further multiplication. In fact, before the spring cycle ever starts, it is always possible to predict that the crop will not be above a certain level.

A very curious thing happens, in that nearly always when the concentration of silica dissolved in water reaches half a milligram, 500 μg /liter, it has reached the minimum after which the cells begin to die, although some are still utilizing silica. This looks like a limiting concentration. There are a lot of data—for example, in one lake I now have data for 18 consecutive years, and over those years this system has worked without fail. It is so extraordinary that it doesn't seem natural to me; natural systems are not usually as simple as this.

On the other hand, there is little doubt that this isn't really an absolute limiting concentration, because if experiments are done in the right way, these diatoms can be made to utilize all the silica they are given, if natural lake water is used. This is done by taking lake water, enriching it with a little phosphorus, and allowing the diatoms to grow slowly.²²⁷ Then the 500 μg /liter concentration will be passed and, eventually if the bottle is given to a chemist with a request to try to find some silica in solution he won't be able to. What does all this mean, exactly? That is where I am in difficulty. I don't really know; and the phosphorus situation troubles me just as much. By using different methods, it is possible to show that this diatom, *Asterionella*, needs 1 mg. of phosphorus/liter to grow. It is also possible to show that it only needs 1 μg or less/liter to grow. The results depend entirely on the experimental technique. Where is the truth in all this? What, really, are the phosphorus needs in nature? That is where I would like some help and advice from people here.

I find it so difficult to know the truth of what is happening in nature. What are these controlling factors, and how are they working

in nature? The great problem is that without meaning to, it is all too easy to arrange experiments so that one gets the results one expected to get.

PROVASOLI: Regarding silica, when you say that the diatoms cannot utilize it below 500 $\mu\text{g/liter}$, isn't that due to a concomitant phosphorus deficiency, since, experimentally, the consumption of silica is reprimed by addition of phosphorus? Is the lake at that time depleted of phosphorus and growth reduced by a double limiting factor?

LUND: Yes, I think so. The phosphorus is very low; it will be 1 $\mu\text{g/liter}$ or less, even as low as 0.1.

PROVASOLI: Then there is probably the combined action of limiting phosphorus and sublimiting silica. But you said that, depending upon the experimental conditions, it is possible to grow the diatom with 1 mg or 1 μg of phosphorus. This is quite a range! What are the experimental conditions needed for growth at low phosphorus concentrations. Low light intensity, low temperature?

LUND: The vital thing seems to me that they must not be given too much (if I may use a phrase which may be quite forbidden) of an energy push. I am sure many people must have observed this in culturing, as it is quite general, I believe. If cultures are left in the laboratory for some time in rather low light conditions, very large crops can be obtained relatively easily; but to make very large crops in bright light requires all sorts of effort. I think this gives us a hint of what might be happening in nature.

What I think we are seeing, in fact, is the reaction between the rate of supply and demand. There are no absolute limiting factors.

PROVASOLI: This is extremely interesting!

GUILLARD: At this time should we consider the question of the concentration level at which algal cells can take up nutrients? We have been talking about the growth in nature of *Asterionella* and the fact that one anticipates that it will not grow any more when the silica has been reduced to a certain level.

LUND: I don't want to go on talking indefinitely, but that is not quite true. Some of this was published a long time ago,^{229,230} and I didn't want to bring up old material.

It is not true to say they stop growing. They don't. What happens is that the peak of the population is reached at 1/2 mg silicon dioxide/liter, and some are still getting enough nutrients to grow while some are dying; it is rather like a sort of atomic half-life system. There is a definite and usually increasing percentage dying all the time, so the crop decreases.

VOLLENWEIDER: Did you find this limiting concentration in several lakes?

LUND Yes, although not every year. Windermere is a particularly favorable lake. I know of five lakes for certain, and some quite good evidence for three or four more, but there are many in which this doesn't happen at all.

GUILLARD One gets the impression that phosphorus can be present in nature in such small quantities that the size of the algal crop is limited. I would like to hear a discussion of whether or not, in nature, the concentration of any element becomes so low that it actually prevents some organisms from growing. We expect that in the case of *Dinobryon* the concentration may be so high that it inhibits growth, but I am curious to know if the concentration can ever be so low that it really prevents growth. I wonder about this because in laboratory experiments, using P^{32} as a tracer, the algae will reduce the concentration of phosphorus to two or three orders of magnitude lower than accepted values for low concentrations in nature.

PROVASOLI: What do you mean by that?

GUILLARD: If one takes some seawater and measures the phosphorus concentration, and then puts in a small amount of P^{32} and some algae and later measures the partition of the P^{32} between the algae and the water, practically all of the phosphorus is in the algae. In short, they have taken out almost all of the phosphorus from the water and reduced the concentration to perhaps $0.0005 \mu\text{g atoms/liter}$. In short, the algae are capable of stripping the water of phosphorus. Yet in natural sea water some phosphate is always found to be present.

LUND: That is not so in fresh water, where one finds very low levels, less than $0.1 \mu\text{g}$ of phosphate phosphorus/liter.

STRICKLAND: As far as the major metabolite-forming nutrients in the sea are concerned, a more simple situation exists than in some lakes, because there is never a shortage of sulfur or carbon. The general tendency is for surface waters to have rather less nitrogen in comparison with phosphorus than is represented by the mean composition of plant cells, so that the nitrogen in the water becomes depleted first.

I have found no evidence of much effect on cell division kinetics when nitrogen first becomes depleted, and I would suggest that perhaps the tendency to maintain a constant rate of cell division is very strong in marine phytoplankton. When the environment becomes depleted of certain elements, the cells continue to divide but will change their composition.

FIGURE 46 shows what happened in the sea bag (and we have had other evidence in the laboratory). The interesting thing to note is that when the nitrogen becomes depleted, which is somewhere just after day 14, the rate of C^{-14} uptake and the rate of oxygen evolution

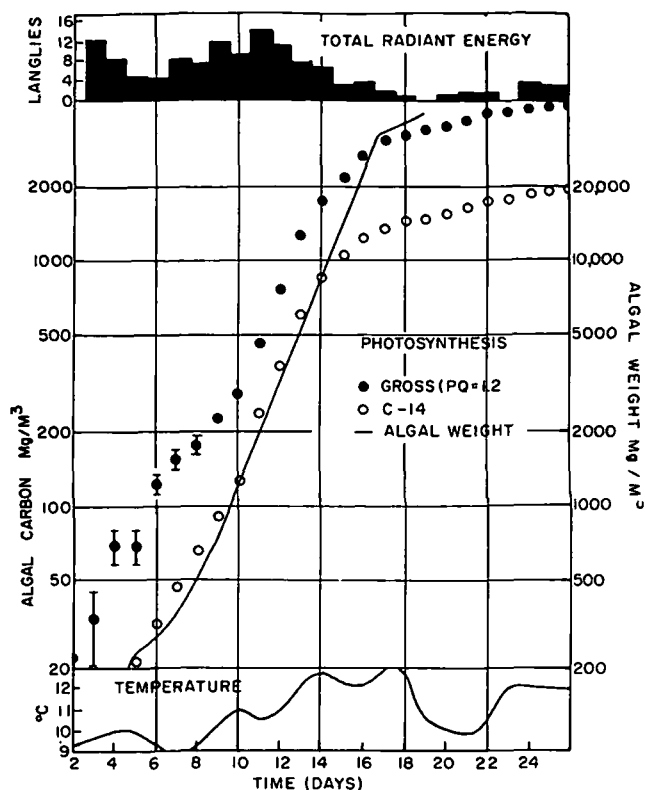


FIG. 46. Photosynthesis and cell division (increase of algal weight) of a diatom bloom in a large plastic bag. (Permission, Limnology and Oceanography)

changed, but there is no indication (and this is a log scale, don't forget) that the cell division rate changed very significantly.

I haven't put the points along the line showing cell division because it confuses the diagram, but the experimental points were quite close to that line. The "crop" in the bag was a mixture of about seven or eight diatoms—there were lots of other species but the main fraction of the biomass resided in seven or eight species and they continued to divide at practically the same rate after nitrate had been used up from solution. The phosphorus never did disappear. There is every indication, and we have confirmed this in work with pure cultures, that when the nitrate disappears the cell goes on dividing, sometimes at a decreased rate, but sometimes with no decrease at all in mean division time for the first division.

This can only occur if the mean composition of the cell changes and

that is, in fact, what happens. It is quite interesting to see what changes do occur.

GUILLARD: Are you sure the phosphorus doesn't disappear?

STRICKLAND: Yes.

GUILLARD: Does it go down to a lower limit?

STRICKLAND: It goes down to about $0.1 \mu\text{g-at/liter}$.

GUILLARD: I just wondered if that isn't an artifact.

STRICKLAND: No. The graphs in FIGURE 47 show the changes of percentage composition. Cell weights were estimated from the cell numbers and a pretty crude geometrical approximation of the cell volumes, multiplied by a density of unity. All of this, I know, is open to heavy criticism, but I still think that values are approximately of the right order. Before day 14, when the nitrogen was depleted, the protein per cell began to drop slightly. The nitrate concentration, when this drop became apparent was about $5 \mu\text{g-at/liter}$. It was originally $25 \mu\text{g-at/liter}$. At day 15, all nitrate had disappeared.

The protein per cell decreased to one-third of its maximum value. The silicon stayed practically constant, as if cells like to keep their

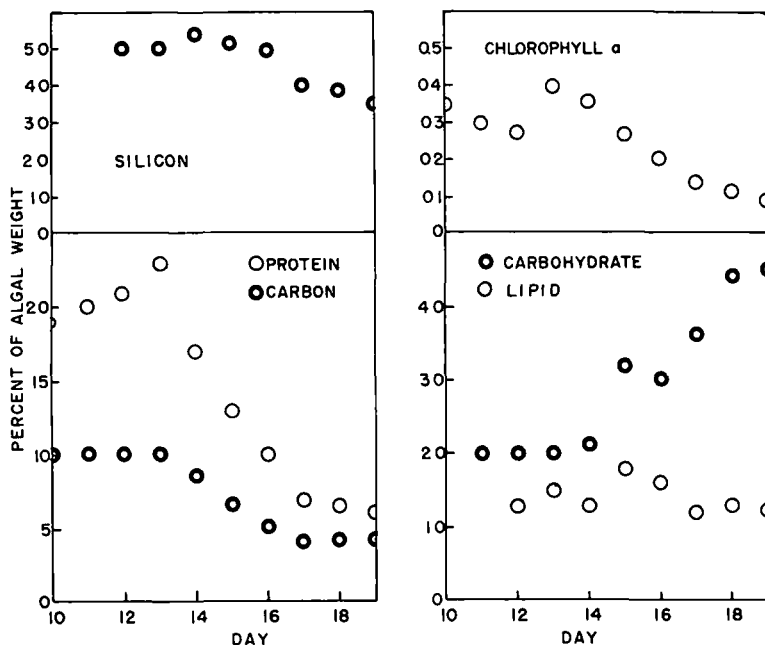


FIG 47. Change of mean cell composition with time during depletion of nitrate from sea water containing a crop of diatoms. (Permission, Limnology and Oceanography).

silicon skins, but they can't get any more nitrogen so their protein has to decrease and, of course, the total carbon per cell decreases. The cells presumably try to make up their protoplasm by increasing their carbohydrate content. But what surprises me is that they apparently make little or no more fat per cell, in the first few days of nitrogen starvation. The chlorophyll per cell goes down, of course. The general picture is not new, in the sense that such effects have been shown many times with Chlorophyceae but it is interesting to see the effects with marine phytoplankton growing in the sea.

OPPENHEIMER: Are the values for wet weight or dry weight?

STRICKLAND: This is simply algal volume.

KRAUSS: How many cell divisions took place after the nitrogen was exhausted?

STRICKLAND: This whole history corresponds to about four cell divisions.

PROVASOLI: I don't think so. It seems to me that in FIGURE 47 it was one and one-half cell divisions after the fourteenth day.

STRICKLAND: No, I mean about four divisions for the whole period shown.

PROVASOLI: But after the fourteenth day you had only one and a half divisions.

STRICKLAND: Yes.

KRAUSS: This corresponds very nicely with the data we have taken on *Scenedesmus*, which will also continue to divide after nitrogen has been removed from the medium. There are usually about three further divisions and the N content drops to about one-quarter of the normal.²³¹

STRICKLAND: A very little change in rate.

KRAUSS: There is a change in rate but it does continue to divide.

STRICKLAND: We made our measurements only to day 19 because we counted only cells that were obviously whole and healthy. The phosphorus per cell, which is not shown in FIGURE 47, gave a very nice linear decrease. I think we must revise any ideas that the concentration of nutrients has any clear-cut effect on kinetics. Nutrient concentrations are much more important because of the effect they may have on the composition of a crop.

JONES: Since you have expressed your values in FIGURE 47 on a percentage basis, could not the fall in percentage protein be simply due to the fact that the carbohydrate has increased and, therefore, that a decrease in protein is not necessarily true? The amount of protein could be the same all the time, but because the carbohydrate concentration goes up with time as a result of a percentage basis now, the protein value would appear to go down.

STRICKLAND: Data were obtained by dividing the total amount of protein, carbohydrate, etc., in suspension by the cell number multiplied by the specific gravity, multiplied by a factor to give a volume. The volumes are not necessarily correct. They could be getting progressively smaller than shown, but there is evidence from work on *Chlorella* and other species that a drop in protein content of this magnitude is reasonable.

JONES: In other words, does the data obtained, expressed on this basis, follow exactly the same type of graph that would result if it were plotted on the basis of per cell?

STRICKLAND: This is virtually per mean cell volume.

JONES: Is the weight constant throughout?

STRICKLAND: No, the weight goes up, as does the number of cells. This is a mean composition per unit cell volume, not per unit cell, because it is a mixed species.

HUTCHINSON: Most of the material is water?

STRICKLAND: Yes.

OPPENHEIMER: I think it is time to request that whenever nutrients and growth are being discussed people do not simply state that something grows at a given level of nutrient. It is important to report all growth response in terms of the cell mass per unit of nutrient being studied. If the nutrient is phosphate, it is very important to know how many atoms of phosphate are necessary for one division because, essentially, one division is growth, even though we normally determine growth by culture response.

Once nutrient requirement is placed on a unit per mass basis, that is, dry weight or number of cells per unit of phosphorus or atoms of phosphorus, we can begin to look more quantitatively at the results for comparative purposes, and, in any natural environment, be able to relate this to types of environment where the conditions may be somewhat different. Thus, we can visualize what is happening and determine what growth really means.

STRICKLAND: Yes, we have to distinguish between division and growth. The tendency here is for division to continue, but the velocity constants for any of the metabolites will all be changing. This has been known about *Chlorella* for quite some time.

GOLDMAN: This presents a severe limitation of counting cell divisions for assay of culture growth.

KOCZY: About 30 per cent has been accounted for.

STRICKLAND: No, 95 per cent of the biomass is included in FIGURE 47 in wet weight values. There were about seven species.

FOGG: I would like to endorse Doctor Krauss' statement about cell division continuing after nitrogen is exhausted. The results in

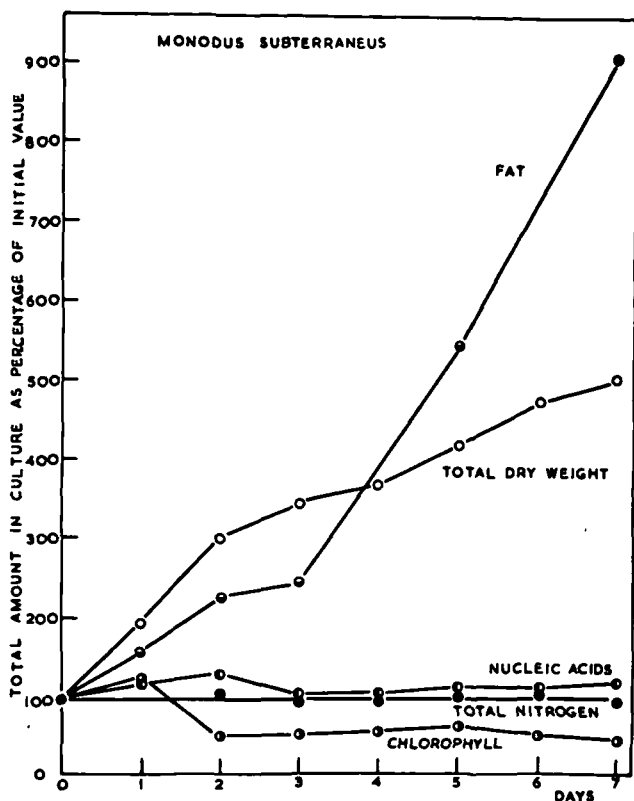


FIG 48. Changes in amounts of various components of actively-growing *Monodus subterraneus* following transfer to a medium containing no source of nitrogen and incubation in the light. Amounts are expressed as percentages of those initially present in the cell suspension. After Fogg (Ref 232).

FIGURE 48 were obtained with *Monodus*, transferred from a nitrate-rich medium to one which contained no combined nitrogen sources.¹⁵⁹ The amounts of different components are plotted as a percentage of the amounts initially present in the material. Total nitrogen in the cells, of course, remained constant, but nucleic acids increased somewhat and this evidently took place at the expense of chlorophyll and other nitrogenous components.

Dry weight increased as photosynthesis went on. At first the product was largely carbohydrate but, after three days, cell disorganization began and then fat content went up enormously.

In this experiment, about two cell divisions took place. The point seems to be that cell division is determined by the capacity of the cell to synthesize deoxyribonucleic acid, DNA (I'm sorry—I think

I'm the first to have mentioned those particular letters in this conference). Cells can apparently synthesize DNA to a certain extent at the expense of other nitrogenous components, and this allows for about two cell divisions after the supply of nitrogen is exhausted. In experiments such as this, we have observed that the nitrogen content on a percentage dry weight basis can fall from about 6 to 1.5 per cent, but, once it is down to 2.5 per cent, no further cell division can take place.

MCLAUGHLIN: Has either Doctor Strickland or Doctor Fogg taken into account the possibility of material exuding from the cell as a function of being in this semimoribund condition and, if so, where would this material be? You say that as a function of phosphate limiting nucleic acid production, cell division is stopped. This would correspond to some point at the end of the stationary growth phase.

DROOP: But it is not necessarily moribund.

MCLAUGHLIN: Not necessarily, but at stationary phase these organisms are exuding something into the environment.

FOGG: In the experiment I just described, there was probably a slight loss, becoming appreciable after three days when the fat increase started. At that point, organization of the protoplasts began to break down, as evidenced by a climactic increase in respiration rate, and one would expect soluble matter to start escaping in quantity from the cells.

MCLAUGHLIN: Is there any production of exogenous material to elicit the continuing growth?

FOGG: Oh, yes, but quantitatively slight with this particular form.

MYERS: May I comment on this problem? I interpret Doctor Fogg's data differently from the way everyone else seems to be interpreting them. These are elegant data in demonstrating a general phenomenon: The development of a nitrogen deficiency. There is a considerable knowledge of nitrogen deficiency since it has been used as a tool in studying the biochemistry of nitrogen assimilation.²³³

In nitrogen deficiency the cells change in composition, diluting themselves out of nitrogen. They reduce the amount of protein and cellular machinery per cell. What is it we want to know? Do we want to know how many cells there are, or do we want to know how much working machinery there is?

I wish to take the unpopular view that we are most interested in knowing how much working machinery there is. Talking about how many cells there are seems to me rather incidental. What is going to be the productivity of the system? That is going to depend upon the amount of cellular machinery, mostly protein.

DROOP: Are there any data on composition from steady-state experiments? In FIGURE 48 though the cells may not be moribund, their composition isn't constant and there is a lowering of cell nitrogen

during the time growth is taking place. What happens? How does one get the thing to work when its machinery is being whittled down all the time?

MYERS: *Chlorella* will easily dilute itself by a factor 2, will it not, with respect to nitrogen? It will go from eight to 10 per cent down to four per cent and still be dividing culture.

DROOP: And can be maintained indefinitely at that?

MYERS: No, not forever, obviously. But we are talking about something with an interest rate that is growing, that has a first order reaction constant. With respect to what does it have the reaction constant? With respect to the amount of working machinery. I think, for example, if we were to talk about increase in growth rate, one might discover that it maintained a specific growth rate with respect to nitrogen even when the nitrogen content of the cells was going down.

STRICKLAND: I don't think its composition would change if it did that. These are the results of a set of first order reactions, the growth constants of all of which, except the cell division and the silica growth constants, are tending to change.

MYERS: But was the growth really logarithmic?

STRICKLAND: Oh yes. That was a semilog plot for the cell growth in FIGURE 47.

KRAUSS: It was semilogarithmic, but also fairly slow. He mentioned that there were only four divisions, over the whole period of about eight or 10 days, which in our experience is relatively slow even for the constant, but it is.

STRICKLAND: By marine standards, this is normal.

BRAARUD: I want to make a remark in connection with the question of whether we are interested in machinery or in number of cells. The point of view may be somewhat different depending on whether one is working on experiments or working in the sea. Experimental work may be said to be one huge machine.

If in the sea there is the same amount of machinery but divided in numerous cells, the possibility exists of dispersal and of seeding or exploiting growth conditions in very many different water masses. So, from a field ecological point of view, both of these factors must be taken into account because, for the productivity in the sea, the question of the number of cells is rather essential. Their effectiveness as machines, may differ very much, but if conditions for growth improve, then the machinery very quickly may become more effective.

STRICKLAND: Would it be reasonable to assume that it is advantageous to phytoplankton systems to divide as much as possible, even if the resulting cells are in metabolically poor shape, because the greater the number of cells of a species the greater the possibility

that some cells will survive adverse conditions?

RILEY. I have noticed a situation following spring flowerings in Long Island Sound which is qualitatively quite similar to what Doctor Strickland has described in his bag. That is, the cell division overshoots after the nitrogen has been used up and there is some decrease in the nitrogen content of the cell.

However, this nitrogen content never falls to the low level that he describes in his bag experiments, nor does it ever fall nearly as low as one finds in senescent cultures in the laboratory. What happens is that the phytoplankton begins to sink out of the water and is found only as a slurry on the bottom.

A point I would like to make is that I think this essential machinery, as we call it, is necessary in order to keep the phytoplankton in the water column in an active state of growth. Once the organism gets beyond this active point, the chances of its maintaining any sort of active pelagic existence are rather slight, and, furthermore, if the nitrogen content is followed on a year-round basis, it is very similar at all times to that found in cultures in a log state of growth.

From an ecological situation, I think a cell that is overloaded with carbohydrates is a moribund cell, even though it may be quite viable under laboratory conditions.

KRAUSS: We have been talking about the cell's ability to dilute itself with regard to nitrogen. The cell's ability to do this is, to a degree, limited. However, such is not the case with all elements. We have been looking at phosphorus recently, and my colleague, Dr. Galloway, has come up with some very interesting curves showing the ability of *Chlorella* to store large amounts of polyphosphate.²³⁴ One can grow *Chlorella* in a medium containing an ample amount of phosphate, wash it well, and place it in a medium containing no phosphate. The growth rate, somewhere between three and four divisions per day, will continue for almost a full day without any diminution whatever. These data are given in FIGURE 49. The decline in growth rate on P-free media is very gradual. The algae will continue to produce additional cells for days after the phosphorus has been removed. We could not hope to get as much continued growth if we did a similar experiment putting the cells into a nitrogen-deficient medium. The cell has a greater capacity to store phosphorus than it has to store nitrogen. So, when we consider the ability of the cell to dilute itself, we find that the ability is different with regard to different elements. An organism may perpetuate itself in a condition of deficiency a great deal longer if it happens to be a deficiency of an element which it can store.

SPENCER: I would like to confirm Doctor Krauss' comments concerning the ability of algae to store phosphorus with some results obtained using *Paedactylum* grown in a chemostat. When phosphate is

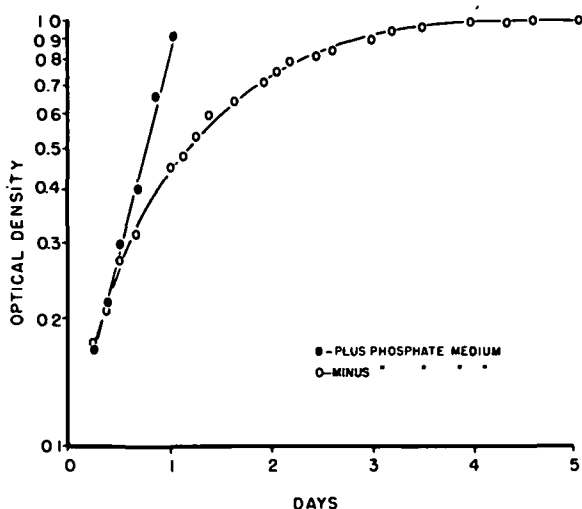


FIG 49. The growth of *Chlorella pyrenoidosa*, van Niel's strain on a medium free of phosphate (open circles) and on a medium with phosphate (solid circles) after being cultured previously on a phosphate rich medium (from Krauss and Galloway, 1963.²¹⁴)

used as a limiting nutrient, certain apparent anomalies show up in the magnitude of the steady-state population.

Briefly, what happens is that, relative to the maximum cell concentration achieved in a batch culture, the range of steady-state cell concentrations which can be obtained with a constant concentration of phosphate as limiting nutrient in the inflowing medium shows a greater variation with dilution rate than would be expected on the basis of simple theory. One explanation, although others are also possible, is that in a chemostat one is dealing with equilibrium cells, whereas in a batch culture for, perhaps, the last half of experimental growth, the cells are growing at the expense of phosphorus which has been acquired previously and is stored in the cell.

There is another point I might mention with respect to the chemostat and phosphorus. It is possible to get *Phaeodactylum* to grow in a steady state at about half the growth rate appropriate to the physical and other nutrient conditions (in this case, the maximum growth rate would be slightly over two divisions a day) when the equilibrium steady-state concentration of phosphate in the medium is analytically undetectable. I don't think these figures for the phosphate concentration as measured mean very much, because, frankly, I just don't know what one is measuring with the phosphate methods under these conditions. As regards the figures that would be returned by a

chemical oceanographer, the phosphate concentration is zero. What we have here is the cell mobilizing phosphate at an ample rate from a steady-state concentration which is analytically undetectable, but is nevertheless a constant concentration.

RILEY: What is the phosphorus content of the cells?

SPENCER: It varies depending upon the flow rate and the growth rates, of the cells. With very low growth rates, the figures work out around the generally accepted ones. When growth rates approach the maximum in a batch culture, under the same conditions, then the amount of phosphate per cell presumably increases.

LUND: What do oceanographers mean by analytically undetectable? What sorts of levels are we talking about?

SPENCER: I would say less than about $0.01\mu\text{g-at/liter}$. I would like to emphasize that I don't believe that measured amounts of phosphate in these ranges of concentration can be relied upon because of the anomalous behavior of all the usual methods of analysis, particularly in culture filtrates at these levels of phosphate concentration.²³⁵

STRICKLAND: It seems to me impossible, with our present state of knowledge, to set up any kinetic model using nutrient concentrations in the sea. I think matters are much too complex for this. We cannot say that a nitrate concentration is below some critical value, therefore, the growth of a crop is nutrient limited. It may be, but we still cannot predict matters. The crop is also changing in chemical composition.

McLAUGHLIN: I do think this lends itself to laboratory studies, because one of the premises one could work on is that the organisms first take up phosphate, then wait around for proper salinity, or proper concentration of vitamin, or some change in the environment and when the time becomes ripe they go into an explosive, even logarithmic, growth phase. Instead of dividing every 48 hours (or as some cultures do, every 96 hours), suddenly everything is right: They have a package of energy, and material; the machinery is all set. Only one variable had to be wrong—temperature, salinity, or what have you. It should be worthwhile, I think, to precondition a culture with phosphate, then eliminate the phosphate, and add back phosphate in various concentrations and combine this with a similar nitrogen study; We might get some useful results as to the phosphate-nitrate inter-relationships.

SPENCER: In a chemostat, in a flowing system, with growth rate limiting phosphate concentrations, one can only tend towards or approach the maximum growth rate achieved under the same conditions in a batch culture. Nevertheless, the presence of excess phosphate in the cells of a batch culture does not make it grow appreciably quicker. The stored phosphate will only enable it to grow for

longer periods in the absence of an external supply of phosphate. The stored phosphate must, in some way, be biologically unavailable.

The chemostat is set up using a flow of medium through a constant-volume culture vessel, such that the washout rate is less than the rate of increase of the cell concentration. The population in the culture vessel, therefore, continues to increase to the point where it starts to reduce the concentration of a chosen nutrient to within the range where it is growth-rate limiting. The growth rate of the culture then starts to fall until it reaches a value which is equal to the washout rate. Since new medium is continually being added at a constant rate, equilibrium conditions will then be set up. The culture in the reaction vessel will now maintain a constant cell concentration and a constant growth rate which is fixed by the equilibrium concentration of the rate-limiting nutrient in the reaction vessel. The system is self-regulating and stable. The growth rate and the equilibrium concentration of the limiting nutrient are then controlled only by the flow rate and can be fixed at any chosen value by choosing a suitable flow rate. The cell concentration in the reaction vessel is fixed by the concentration of the limiting nutrient in the medium flowing into the system.

The principal difference between a turbidostat and a chemostat, therefore, lies in the physiological state of the cells in the culture. In a chemostat, the cells are always in a state of nutrient-limited growth rate. In a turbidostat, one is working with a culture, the cell concentration of which is not controlled by the nutrients. Usually, in this apparatus excess nutrients are present and the cells are growing at the exponential rate appropriate to the physical conditions. Since the system is controlled by the cell concentration in the reaction vessel via some external device, it is possible to work at any desired constant cell concentration.

MCLAUGHLIN: It may grow quicker if the other physiological circumstances at that time keep the cell from tapping this energy—except at a small constant rate. Raise the temperature 4C and the culture takes off. At least the phosphate was there.

SPENCER: We are talking about conditions when phosphate is growth-rate limiting.

KOCZY: I am surprised, because if there is a phosphate solution in a glass vessel, about 10 to 20 per cent of the phosphate content is extracted from the solution and adsorbed on the glass wall.

STRICKLAND: No.

KOCZY: Yes, With plastic, it is more. From glass, adsorbed phosphate can be recovered rather easily. From plastic, it cannot even be recovered.

GUILLARD: Algae can remove phosphate from glass surface.

KOCZY: Yes, algae will. So, for that reason, if there is a reduction in phosphate concentration, I don't know what happens to the system; I agree with Doctor Spencer that I don't know what we are talking about any more, because there is a relationship of phosphate to the glassware surface. That is difficult to control.

MCLAUGHLIN: A lot depends on the way it is being handled.

LUND: I think this is in direct contradiction with what has been found in studies with phosphorus in fresh water: In a recent paper, Heron²³⁶ found that if bacteria are eliminated, then the phosphorus loss is reduced enormously.

OPPENHEIMER: Sorption should occur as a rate reaction. I think Doctor Koczy refers to the phosphate adsorbed almost immediately on the walls after the water is placed in the glass.

JONES: What about the release of phosphate from a medium when it is aerated. Phosphate will be associated with the surface of bubbles and, when they break, the phosphate is lost from the surface and falls into the glass vessel above the level of the liquid. This is another problem with aerating cultures.

PROVASOLI: The important point brought up by Doctor Lund and Doctor Myers fits in quite well with what Doctor Braarud has contributed. It seems to me that the primary business of organisms is to remain alive and to do so they take any risk. They modify their morphology, if necessary; they change their composition and, as Doctor Myers said, they strip down the machinery to the point where survival is still possible.

Doctor Riley brought out the point that when cells may no longer have buoyancy, and this is especially true of diatoms, they may have to be considered moribund from an ecological point of view; and they might then sink and disappear from the photic zone. This is somewhat in contrast with Doctor Braarud's viewpoint that in the ecological situation, survival is the most important thing, and in a flagellate, an extra cell division may mean the possibility of covering more ground and reaching a better environment.

This can apply to the diatoms also. It is true that they may sink, but again in many ecological situations there may be turbulence which will bring them back to the surface or somewhere else before reaching physiological death.

MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSE OF ORGANISMS DURING LIFE CYCLES

Discussion leader:

G. E. FOGG

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FOGG: It seems to me that so far we have been talking about responses and that, possibly, we should clarify our ideas on this somewhat. It is obviously important to know how an organism will respond in a given set of circumstances, whether we want to be able to recognize it and put a name to it if we meet it again, to predict to what extent it will grow, or to say what its value as a food for other organisms will be.

For purposes of discussion, I would like to suggest that physiological and also, I think, morphological responses, can take place on one or more of several levels. An organism, at any point of time, has a certain enzymic make-up, a certain balance of metabolic reactions, and if conditions change, it can only respond immediately in a way which is dictated by this enzymic balance.

The sort of thing that I have in mind can be illustrated by the effects of the deprivation of nitrogen, which I mentioned a short time ago. If a cell is deprived of nitrogen abruptly, the metabolic paths along which the material being assimilated is flowing will alter. If the organism is actively growing, protein synthesis will then predominate. But if the nitrogen source is removed, then the carbon which is still being made available by photosynthesis must now flow along other paths. These new paths will be determined by the relative activities of the different enzyme systems present and some new product will accumulate instead of protein. Morphologically, this sort of change results in cell expansion and visible accumulation of material in cell wall or preserve products.

If the organism is exposed to a changed condition for some little time, then the next possibility is that the enzymic balance may change. What I have in mind here is the fat accumulation which occurs when an algal cell is deprived of nitrogen. Immediately following the deprivation of nitrogen, the carbon fixed in photosynthesis seems to go to carbohydrate. The carbohydrate enzyme system is the

most active in competing for the intermediate products of the photosynthesis. But if the nitrogen starvation is prolonged, the activities of the enzyme systems will alter and, in most algal cells, the fat synthesizing enzymes become dominant.

I am not very sure what the mechanisms of this alteration in enzymic balance may be. With the algae we have studied, this change seems to require one- or two-cell generations. I think there is a possibility that it may represent simply the stage in cell development at which the deficiency, whatever it is, halts the machinery. Tamiya and his colleagues²³⁷ in Japan have found evidence that the metabolism of *Chlorella* varies according to the stage in the life cycle, that young cells newly produced by division are active in protein and carbohydrate synthesis; then, as they age and become ripe to divide, the metabolism shifts more in favor of the production of reduced substances such as fats.

FREMONT-SMITH: Doctor Fogg, would it be worthwhile to mention that the nitrogen deprivation is also depriving the enzymes of nitrogen, and that, since the enzymes themselves are protein, when there is a protein depletion the enzyme systems are being affected directly?

FOGG: Yes, certainly. Perhaps in choosing nitrogen deficiency I haven't chosen the simplest sort of condition, but it's the one I know most about.

FREMONT-SMITH. It may be very pertinent, because it is the enzyme shift that is going to determine the pathways.

FOGG: Yes. In the case of the effect of nitrogen deficiency favoring the fat-synthesizing enzymes, I would suggest that probably it is merely that the other enzyme systems, such as that synthesizing carbohydrate, break down rather more easily so that there is no actual increase in enzymes but merely a selective inactivation.

If one particular condition may halt the cells in the young stage, and so result in a population in which carbohydrate and protein synthesis predominate, another condition may halt the cell cycle in a later stage and, perhaps, result in fat synthesis predominating. This is one possible mechanism for the response to a changed condition such as nitrogen nutrition. There must certainly be other mechanisms, of course.

At the third level, which I think we should consider, there may be actual production of enzymes which were not present before—an adaptation. This may occur by selection of a particular mutant or by production of adaptive enzymes in the way that bacteria do.

I used to think that algae were genetically rather stable and that if an alga were kept in culture, the chances were that it wouldn't change very much. I am now beginning to doubt this. One thing that has

shaken my faith is that shortly after I isolated *Anabaena cylindrica* in 1940, I deposited a subculture in the Cambridge Collection, but, when only two years ago, we had occasion to compare their strain with ours, it was very clear that they are now quite different. The Cambridge one still produces spores; ours no longer does. There are certain biochemical differences also.

One of my research students, Mr. H. Kumar, has recently been trying to produce strains of a blue-green alga, *Anacystis nidulans*, adapted to high concentrations of antibiotics. He has found this quite easy to do by subculturing in successively higher concentrations of things such as chloramphenicol, penicillin, and streptomycin. He has produced strains which can resist up to 1,000 times the concentration of streptomycin that was previously lethal. These strains, once produced, seem to be quite stable. A streptomycin-resistant strain has been passed through 40 subcultures in the absence of streptomycin but still retains its resistance. Incidentally, by means of these strains he has been able to obtain what seems to be the first clear evidence of sexuality in blue-green algae.²³⁸ So, I think that we must envisage the possibility that phytoplankton may change genetically quite considerably both in culture and in the sea. *Anacystis* hasn't much in the way of morphology, but Mr. Kumar has obtained strains which differ somewhat in mean cell length, and I'm sure that with more elaborate forms it should be just as easy to obtain strains which have quite definite morphological differences.

Doctor Braarud, would you like to speak on morphological responses?

BRAARUD: If one studies populations in the sea, certain groups cause considerable difficulties from the taxonomy point of view, because there are a great number of related forms and, usually, as we heard earlier, the descriptions are based on a few specimens which may be inadequate. How to delimit the species is always a problem. In many cases, one has a combination of genotypical and phenotypical variations, but very seldom is one able to distinguish between the influence of the environment and the genotypical variations which exist. For this reason, the situation, especially in certain genera of dinoflagellates, is very unsatisfactory because we don't know what the species is.

In our laboratory, we have made a study of the *Dinophysis* population from the Norwegian Coast, based on material from quantitative samples. It is possible to show that certain species which have been described from other waters actually fall within the phenotypic variation of one species. In material from a large area, it is, also possible to show that there is no real discontinuity in the characters which have been used. This would undoubtedly be found for many

other dinoflagellates also, and I think the influence of the environment upon morphology is one of the main reasons for the unsatisfactory condition of taxonomy in, for instance, certain *Peridinium* species, in *Gonyaulax* and in other genera.

This is one example²³⁹ of the extent of the morphological variation due to environmental factors. We don't know much about the main factors that influence the morphological characters. In the case of the *Dinophysis* species, it seemed to be salinity. Temperature may influence size, but it did not have a great effect on the morphological characters.

I would like also to comment on the question of the stability of the species. I have had an experience similar to Doctor Fogg's, namely, that *Peridinium trochoideum*, which for many years was cultured not in bacteria-free culture but in persistent culture in our laboratory, formed resting spores regularly when the cultures were crowded. After a certain number of years, it stopped forming resting spores. We have been unable to find the reason but some change had obviously taken place.

FOGG: You looked after it so well it found it wasn't necessary to form resting spores. (Laughter).

BRAARUD: One general problem in this connection is that of the morphological changes related to the life cycle, which doubtlessly is strongly influenced by the environment. Take the question of sexual reproduction in diatoms, where we have the auxospore formation as the result of sexual reproduction. We know very little about auxospore formation in nature, and the work that has been done suggests that it may be a very important feature for the general biology of the species.²⁴⁰ The studies on *Rhizosolenia*, the *styliformis-semispina* group, indicates that in this group of species the auxospore formation takes place at intervals of one or more years. We don't know much about the situation with other species, although in some we can very readily see auxospores, for instance, in *Thalassiosira* populations when they are in active growth. We know very little about *Skeletonema* auxospores, one feature we certainly would like to know more about in order to control our cultures. I am quite convinced that the occurrence of auxospores is closely related to environmental factors.

The life cycle of diatoms doubtlessly influences their flotation capacity, because the small and large forms have different floating capacities.

In other plankton groups, for instance, dinoflagellates, the mode of reproduction is very obscure. There is practically no example of sexual reproduction in the marine dinoflagellates, except *Noctiluca*, but I don't think that means it does not take place. Little is known about how the life cycle, including the formation of resting spores, is in-

fluenced by the environment. That it is determined by external conditions is indicated, for instance, by the observation that in *Gonyaulax polyedra*, a very common coastal species that has been observed by many planktonologists for several years, resting spores were not described until 1951.²⁴¹ Then suddenly the whole Oslofjord was full of the beautiful spiny resting spore of *Gonyaulax polyedra*. So, probably, certain rather special environmental conditions affect this part of the life cycle.

Coccolithophorids, again, provide a very striking example of the necessity of studying the life cycle and also of obtaining information on environmental influences. Mary Parke has shown²⁴² that two very different species of coccolithophorids, with extremely different microstructure of the coccoliths, are part of the same life cycle. This is probably true for many others. *Coccolithus huxleyi* is one of the commonest of all coccolithophorids and a very important producer in the sea, but we do not actually know how it reproduces, nor how the cells divide. This is an example of how little is known about the life cycle and environment.

Another enigma is *Halosphaera viridis*; but it is now being studied in Plymouth so we may soon learn more about it.

There is one more small point about morphological changes and that, again, concerns *Skeletonema*. FIGURE 30 showed a beautiful collection of chains of *Skeletonema* which, according to my experience, came from a very dense culture with not too good a supply of silica. This conclusion is based upon the fact that this species changes its form from beautiful chains with long appendages between the cells, to cells with shorter appendages. When the end of the reproductive season is reached, the cells don't look like *Skeletonema* at all. This is an extreme case of morphological change brought about by environment. There are other cases.

The thickness of the cell wall changes considerably according to the environment. Just what is decisive, whether it is the silica supply, temperature, or nutrient supply as a whole, I can't tell, but one finds very great changes in some species and much smaller variations in others. On the basis of such changes, a method has been adopted for estimating the number of cell divisions or the rate of reproduction by measuring the cell wall of diatoms over a certain period of time. I think the method can be applied perhaps only in a very restricted area, where there is a steady supply of silica or where the concentration is ample all the time.

STRICKLAND. Can anybody tell me what the cells are that appear in cultures that look like glass balls with a little brown material attached to the side? When cultures of *Skeletonema* or other centrics are subjected to periods of light and darkness, there is not much

effect, as the dark period increases with respect to the light. The division rate decreases a little, but the cells look rather better with 12 hours dark and 12 hours light than with 24 hours continuous light. *Skeletonema* grows quite well with only eight hours light, but immediately when we give it five hours light and the rest dark, the whole culture changes to the "glass ball" phase. When you put it back on 12 hours light and 12 hours dark cycle the wretched things won't hatch back out again, although sometimes we see them grow horns and reform *Skeletonema* chains. Are the glass balls resting spores?

BRAARUD: I would suggest they are auxospores.

STRICKLAND: Well, then, this is a way to produce auxospores quickly and conveniently.

STEEMANN NIELSEN: I had a Japanese guest, Doctor H. Takano,* who was working with *Skeletonema*. He found just auxospores and studied their formation.

STRICKLAND: The auxospore stops them from getting too small during asexual reproduction, does it?

STEEMANN NIELSEN: Yes, and from becoming large again.

DROOP: I have my doubts as to whether these "balloons" or "glass balls" are sexually-produced auxospores. *Skeletonema* has motile male gametes, and, very occasionally, we see them in cultures. But this sort of phenomenon, ballooning, frequently appears in cultures toward the end of active growth, particularly in defined media. It is a similar physiological response, as it were, to auxospore formation, but without the nuclear phenomena to go with it, I suspect. This enlargement is seen in quite a number of different diatoms. I recall Gross' work on *Ditylum*²⁴³ where he was studying what he called auxospore-formation. According to Doctor Von Stosch of the Botany Department, University of Marburg, Germany,† he was studying an asexual process. It has the same result: enlargement of cell diameter. But, I have never seen these "balloons" germinate. Be that as it may, one way of getting broad cells in cultures of *Skeletonema* is to use an inoculum with a very large percentage of "balloons"—a very old inoculum, just before it bleaches. The chances are that no culture will be obtained at all, but if one is, after a lag of one week or 10 days, it will be a fine culture of broad, straight chains. As I say, I have never seen the "balloons" germinate, but I suspect some of them do.

PROVASOLI: I am puzzled. I would like to have a definition of auxospore.

BRAARUD: The auxospore is the result of sexual reproduction. When the egg cell is fertilized, a zygote is produced which grows out to a

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†Personal communication

globular body within a thin wall. Within this, new cells are formed whose diameter may be three times as large as that of the mother cells. The auxospore is so called because it grows to give a larger cell. These auxospores may, of course, also be formed asexually, as is the case with several other diatoms.

LUND: Doctor Braarud has said what I have said, namely, that the definition of auxospore has nothing to do with sex.

GUILLARD: What is an example of auxospores formed asexually?

LUND: There are a number of examples in fresh water species.

GUILLARD: I believe the research of Doctor von Stosch²⁴⁴ and of other workers with more centric diatoms shows that they are oögonous and form auxospores sexually.

LUND: Pennate diatoms are also sexual, with every possible variance of sex, but, equally, there are some which are asexual. Fritsch,²⁴⁵ Geitler,²⁴⁶ and Patrick²⁴⁷ have reviewed this.

DROOP: Von Stosch^{248,249} has shown that there is sexual reproduction, and auxospore formation in that sense, in *Ditylum*, and also this nonsexual enlargement which Gross observed earlier.²⁴⁷

HUTCHINSON: Is there any connection between temperature and whether the organism will be sexual or asexual? In the equivalent situation with *Cladocera* and *Rotifera* where a resting egg is produced, the cases of a pseudosexual parthenogenetic nesting egg are confined to a few species living in cold areas that are ice-free for only a very short time, and they apparently go through a cycle in some cases without any subitaneous parthenogenetic eggs but produce nesting parthenogenetic eggs that look like the sexual eggs of allied species. This has been shown for rotifers and for *Daphnia meddendoriana*.

LUND: I can't think of any example among fresh water diatoms where there are two methods of producing auxospores, sexual and asexual. It is true that both sexually- and asexually-produced auxospores have been recorded for the same species,²⁴³ but Geitler's²⁵⁰ work on *Cocconeis* shows that the possibility must be borne in mind that the diatoms concerned may not be the same. So far as I know, two types of auxospore formation have not been observed in a clone. Geitler found that his varieties of *Cocconeis placentula* which differed sexually, also differed morphologically.

GUILLARD: I have noticed in a number of cultures, especially in *Melosira nummuloides*, that these "glass balls" do occur, and afterwards, chains of large sized cells appear. In this *Melosira* culture, I have observed the motile sperm and also egg cells and it has occurred to me that perhaps these glass balls that don't germinate are the unfertilized oögonia—they get larger and just die. I have also tried transferring large numbers of these "glass balls" into fresh medium and

find they usually die overnight. Occasionally, a culture of large-sized cells results.

STRICKLAND: Have you seen horns sprouting out of the balls?

GUILLARD: I think that is the way they germinate.

DROOP: I don't think so. The horns are the old chain it is formed from; the cells are long and narrow.

STRICKLAND: No, I have seen the horns grow.

GUILLARD: I also have a mental picture of a chain coming from one of these big things.

BRAARUD: How can you distinguish whether these narrow chains are the old chains or new ones?

STRICKLAND: Because they are very large.

DROOP: The large chains do exist. Old cells look something like those shown in FIGURE 50, much like what we think of as *Skeletonema*.

GUILLARD: I have light microscope photographs of this sort of thing in a species of *Thalassiosira*. In this genus, most species have cells growing in colonies that look like buttons strung on a thread, with cells separated by a distance equivalent to about cell diameter. But in culture, when they are ready to make auxospores, the cells often get small in diameter and grow in chains with the valves of the cells touching; that is, with no slime thread showing. I have seen essentially what you have described here; the two valves of the old small cell on the sides of a great "bubble." The next day there were cells of about the diameter of the "bubbles" in the culture.

DROOP: *Melosira* does that, too.

BRAARUD: Those of us who have worked on cultures have found that as they age they look very ugly and untidy because of these small glass balls, and so on. Von Stosch²⁴⁴ has shown that this cell state is a result of an attempted sexual reproduction, that in culture may not always be successful. For this reason, rather abnormal situations may arise with a preponderance of all these aberrant forms, that may only be due to the very poor environmental conditions which we offered this organism.

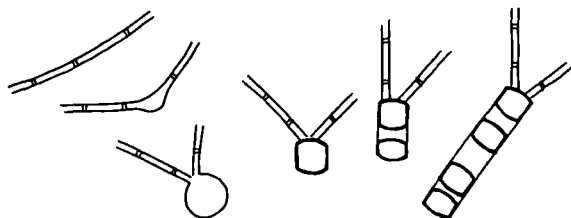


FIG 50. Formation and germination of auxospores in *Skeletonema* (Blackboard drawing).

STRICKLAND: The cultures of my *Skeletonema* are nutrient rich, though. The glass ball state was brought about by a light-dark cycle.

MCLAUGHLIN: With *Melosira*, the glass ball state appears when they are put in very low light and the temperature is lowered—one finds in these auxospores what morphologically appears to be glass balls with very little chlorophyll.

STRICKLAND: This happens with *Skeletonema* in low temperatures, too, with ordinary light.

BRAARUD. I have studied an old *Skeletonema* culture for about one hour. Some years ago, I certainly would have thought this culture was just dead, but, by examining it closely at high magnification, I was able to see all the stages of sexual reproduction. I hope someone will describe these stages and study which environmental factors influence sexual reproduction and auxospore formation. It is obvious that in *Skeletonema* these processes do not take place regularly at all seasons.

FOGG: Doctor McLaughlin, you told us earlier about responses in dinoflagellates. Would you like to add anything?

MCLAUGHLIN: As Doctor Braarud has indicated, phenotypic expressions from a single cell or from three or four cells from the environment may, in those particular cells, be identical, from which the taxonomist can draw a picture and compare it to previous four- or five-cell examples. But in a culture where there are many cells to see, even from a single cell isolate, the variation, for example, in plates can run from three to four plates in a hypocone. The position of the transverse sulcus can be a little up or a little down, so the phenotypic expression is a variable one can easily see as contrast to the natural situation. The variation in size in culture has been an eye-opener for us. A *Gyrodinium* sp. (we believe it is a new species), has a four to five-fold range in length. At its minimum size, it looks like a barrel. One sees small barrels swimming around, and a few very large barrels. The small barrels alone could be described as a separate species, giving length, width, etc. If the larger barrels are observed four to five days later, one might say they are very similar and set up a "var. magna" for such a population.

This size variation makes it very difficult to speciate wild forms merely by plate arrangement and dimensions. In the literature,⁹¹ for example, *Cochlodinium* is well defined and a very beautiful organism, and yet, as I indicated earlier, I believe we have a *Cochlodinium* where the cells are much larger, like the end segment of a tapeworm with a whole series of smaller cells following behind. But still when one shakes and breaks off individuals, they start swimming by themselves. This culture is then composed of size variations which would lead one to believe they had at least two or possibly more species.

Doctors Lee and Freudenthal¹⁰⁴ worked on *Glenodinium foliaceum*, supposedly a nonthecate form and, by using phase contrast, picked out a diaphanous theca, detected only because it does encyst. We thought we had a mixed culture because we didn't expect encystment. When we studied the vegetative form, there were two types: one obviously had a theca, the other ostensibly not. Yet by careful microscopy Lee and Freudenthal were able to distinguish the thecal structure here. So, to repeat what was said earlier, for good taxonomy study the organism in culture.

Of course, domestication does something to the organisms. I agree with you, Doctor Fogg, that after they have been domesticated for a while they may not be recognizable.

Organisms must go through cycles in the ocean, varying with temperature, salinity etc. In the laboratory, we keep them in conditions that we consider ideal; however, they may not think so. We keep them at 18C, with cool-white fluorescence light for five years, while their counterparts out in the ocean have had rugged lives., full of opportunities to evolve.

FOGG: We hamper their self-expression.

GOLDMAN: Does turbulence seem to make a difference in the morphology of the marine phytoplankton?

MCLAUGHLIN: Oh, yes. If some cultures are aerated all the organisms are killed.

GOLDMAN: I am thinking of something a little more subtle. I had cultures of *Scenedesmus quadricauda* Turp. growing in a north window at the University of Michigan some years ago. Through variations in the air-pressure supply for mixing, some of the cultures were agitated greatly and some were not, and I noticed that there was quite a difference in form of the *quadricauda* that were agitated, in that they developed longer spines on the ends.

MCLAUGHLIN: This is true of some chain-forming diatoms. If they are stirred or agitated, or subjected to different nutrients, they can be kept small. If silica is limited, it becomes even more obvious that these chains can actually be physically limited by deprivation of cell wall material. We know very little about what happens on domestication over a function of time.

HUTCHINSON: There is a very good case in animals, studied extensively by John L. Brooks at Yale University who finds that head length in *Daphnia* is to some extent determined by the turbulence of the water. New work by J. Jacobs, in Brooks' laboratory indicates that the phenomenon is quite complicated as turbulence seems ineffective in the dark. The animal when continually moved about would have to do extra work to maintain its orientation in a light field; this may effect its characteristics both physiological and morphological.

MCLAUGHLIN: We have an organism that forms a very heavy exudate, a very dense polysaccharide. If the culture is kept stationary the organism is trapped in the polysaccharide. If it is agitated, the culture pours out the polysaccharide but not as heavy a concentration.

The dinoflagellate *Katodinium dorsalisulcum* produces a mucoid layer along the culture bottom which entraps the organisms. They produce oxygen in this gelatinous mass, which eventually acts by buoyancy, to lift the organisms from the bottom.²⁵¹

JONES: At this point, I would like to add a remark to a point Doctor Fogg brought out in his summation, namely, that we have not said very much about light cycles. In our experience, and that of other investigators, organisms differ as to whether they divide during the dark period or the light period of a light cycle. Also, during a light cycle cells are going through a process of morphological and physiological development synchronized to the cycle imposed upon them. Similarly, the biochemistry of the cell will be changing. I think, therefore, that we should attempt to keep our laboratory culture conditions similar to the environmental conditions from which the particular organism was isolated. Growing cells in continuous light has certain advantages, but few from an ecological point of view.

DROOP: Is that particularly true of green algae?

JONES: No. I do not think it is limited to the green algae. From my own experience, *Euglena gracilis*, *Chlamydomonas moewusii*, *Porphyridium cruentum*, *Nitzschia closterium*, and *Carteria* are affected.

BRAARUD: Another example is the dinoflagellate *Ceratium*. In the Oslofjord, in the summertime there is a combination of very long days and short nights, and a very good supply of nutrients. Mrs. G. R. Hasle found that in this area the *Ceratium* species produced many abnormal forms with extra legs.

E. Nordli studied the same species in culture and found that, with continuous illumination, the same abnormal forms were found in the cultures as had been observed in the Oslofjord. In a joint paper²⁵² they concluded that the rhythmic division of the cells, which usually takes place during daylight, had been distorted or changed so that the cell did not complete its division before it was stimulated to start a new one. They had some information, also, on the nuclei but it was not very detailed. That is another example of how the light cycle influences the form.

MCLAUGHLIN: I believe it would be advantageous to study *in extenso* cycles and phases, in order to determine what phases represent physiologically. With the luminescent dinos, Hastings' work has shown that one can alternate their diurnal rhythm of luminescence.²⁵³ Do they have an inherent division rate which we change by putting them in constant light and not varying the intensity of light as it

varies in nature? More work should be done on this "phasing" to determine the nature of the inherent cycle of division and morphological change. Evidently, Doctor Braarud's organisms didn't know—they couldn't tell "time" because they were working on some internal division cycle. After a certain period of metabolism they automatically started to divide, regardless of whether the other half had completed its metabolic growth.

STEEMANN NIELSEN: Perhaps we should now turn to physiological variations. We have talked a great deal about *Skeletonema costatum*, and if I may, I will talk about some pitfalls. At the First Conference last year, I said I was of the opinion that we have two strains of *Skeletonema* in Danish waters. I thought at the time that we had one strain for low salinity and one for high salinity. But during the winter, our Japanese visitor, Doctor Takano, studied the response of *Skeletonema* to different salinities and found that it is able to adapt, but that it takes some time. It is able to adapt to and to grow at salinities between about 2°/oo S and 55°/oo S. Between 7°/oo S and 40°/oo S it reaches optimum growth.*

If a culture growing at 7°/oo, for instance, is put in ordinary seawater, the cell will die. It has to be transferred in steps over a period of a few days in order to get very nice cultures at different salinities growing at exactly the same rate.

KOCZY: Does it change shape or size?

STEEMANN NIELSEN: No.

FOGG: Is this a reversible adaptation, can it be brought back to the opposite salinity again?

STEEMANN NIELSEN: Oh, yes.

DROOP: Is it an adaptation or just a gentle easing onto—avoidance of osmotic shock, and so on?

STEEMANN NIELSEN: I think the shock comes when there is too great a difference between the salinities.

KOCZY: What is adaptation, by definition, then?

BRAARUD: In our salinity experiments, we have always tried to condition the species by transferring it in steps down to low salinities.²⁵⁴ It should not be moved from very high to very low salinities. *Skeletonema* should grow at very low salinities, according to the observations of Välikangas in the harbor of Helsingki.

STEEMANN NIELSEN: *Skeletonema* has been found growing at salinities as low as 2°/oo S, but not as well as it grows at 7°/oo S. I don't think we can generalize. In our species, there may, of course, be different salinity varieties, but we don't know about them.

*Unpublished observations.

ALLEN: A rather striking case of adaptation has been studied by my colleague, Doctor Alex Shrift.²⁵⁵ It may be of interest because I think it is one of the few cases where we have some evidence of what is going on during an adaptation. If the alga *Chlorella vulgaris* is placed in a solution of the amino acid analog selenomethionine, cell division is decreased, but not growth, and one gets enormous cells—20 to 30 times the size of a normal *Chlorella*. If the algae are left in this solution for a period of 20 to 30 days, they will begin to divide again. The cells resulting from such a culture (and Doctor Shrift has some very extensive experiments on this) have all become resistant to the growth inhibiting action of this amino acid analog. Moreover, the cells retain ability to divide. He has made more than 200 transfers in media free of selenomethionine and they still retain the ability to divide in the presence of this inhibitor.

In studies with radioactive sulphate and radioactive selenomethionine, Doctor Shrift has now found that there is a change in the permeability of the cells to methionine and its analogs. So, if the mechanism of uptake of amino acids is the same in algae as in bacteria, some sort of permease is being affected by this adaptation.

I would also like to say a word about genetic stability and instability in algae. One of the first well-documented cases of variation in micro-organisms was Beijerinck's work¹³ on *Chlorella variegata*, which is hardly a planktonic organism. It was isolated from the slime flux of an elm tree, but, during the several years it was studied, a continued instability in its pigment was displayed. There are now, in this country, two cultures of *Chlorella variegata* which are just about as different as one could expect, both of which seem to have become stabilized. The one in Doctor Richard C. Staff's culture collection at the University of Indiana is a typical green autotroph. The culture we have in our laboratory, which came from Beijerinck's growth by way of the Pacific Grove Culture Collection, is a pale yellowish-green creature with a very complex set of growth requirements that have not yet been fully worked out.

Both of these seem by now to be thoroughly stabilized. But from UV mutants we have isolated *Chlorella* strains *pyrenoidosa* in this case, which are identical to Beijerinck's original description of *Chlorella variegata*, and have, for a period of almost five years, maintained a continual phenotypic instability. If clonal culture is isolated and grown for a few months, then plated out again, the whole spectrum of color variation is observed in the population.

PROVASOLI: Something similar happens when *Euglena gracilis* is bleached with low concentrations of streptomycin: white, green, and yellow forms are produced. The white and green strains are stable,

giving, respectively, only white or green progeny. The yellow strain, when plated, keeps on giving white, green and yellow.

Mrs. Lewin has isolated several strains of diatoms which are identical morphologically under the electron microscope. She found that, within a species, one strain may have no vitamin requirements, another need B₁₂, and another thiamine or thiamine and B₁₂. There is also a strain of *Coccolithus* which does not produce coccoliths.

GUILLARD: The clone of *Coccolithus huxleyi* that Doctor Mary Parke, of the Plymouth Laboratory, England, has in culture does not usually produce coccoliths. However, I found that it will occasionally produce coccoliths in nitrogen-deficient medium. On the other hand, the clone I got from the Sargasso Sea regularly has coccoliths, but occasionally it will grow as a flagellate without them.

BRAARUD: *Gymnodinium veneficum* and *G. vitiligo*, which Ballantine²⁵⁶ has described, give an example of physiological differences in species which are very close morphologically. If one examined the cultures without knowing anything about the physiology, one would not think of referring them to different species. However, one produces toxin, the other does not.

DROOP: *Haematococcus pluvialis* is as an example of how morphology may differ in cultures from nature. In nature, *Haematococcus* is a motile chlamydomonad-like alga with the cell wall removed from the protoplast, but joined to it by fine protoplasmic strands. In nature, the space between protoplasts and the cell wall is very large, with high carotenoid content of the cells. Fairly small populations of stable populations without cell division are found in the small pools where it occurs. In cultures, when cell division occurs, there is a narrow space between the cell wall and the cell and little carotenoid formation. When division is stopped, carotenoid formation and encystment occurs. It is extremely difficult to get a numerically stable, motile carotenoid-forming population in cultures. The only way I have achieved it is by using something like a Rodhe medium #8,²⁵⁷ and diluting it by a factor of 100 with distilled water.^{258,35}

MCLAUGHLIN: Does anyone have any information on whether bacterized cultures maintain what we would consider a more normal morphology as compared to an axenic culture?

LUND: It is easier to obtain a bacterized culture. If the taxonomist or the morphologist wants to get what he calls natural-looking fresh water algae, he will start off with what I am sure you would all call an unscientific out-of-date method, namely, he will use a bacterized culture and, almost certainly, soil extract. This is the easy way to do taxonomy and morphology.

FOGG: We touched on aspects I am particularly curious about, that is, the importance of movement of the cell through the water in

bringing fresh fields for the cell to tap. I would be interested to know just how much validity there is in this supposed correlation of flagellate forms with nutrient-poor water. The converse is found, isn't it—Euglenas usually occur in nutrient-rich water?

GUILLARD: I mentioned that we had done some experiments and showed that algae can reduce the level of phosphate in seawater to a very low level, and I would like to bring up the question of the uptake of trace metals, particularly of iron, from a seawater medium. This is of great importance in culture and possibly in nature.

DROOP: I believe there were some observations which seemed to confirm that *Skeletonema* has rather feeble powers of catching iron from the medium as compared with the supralittoral organisms. The experiment, summarized in TABLE 9, does not tell anything about the mechanism of uptake. The idea was that the chelator N,N-dihydroxyethylglycine (DHEG) has a very high stability constant for ferric iron, but rather low constants for the other divalent metals, so it should be possible to add DHEG to a normally chelated medium and not interfere with the balance of any metal other than trivalent iron.

The chelator was added to an already chelated medium—with glycine, as a matter of fact—to find out whether *Skeletonema* could

TABLE 9
EFFECT OF FERRIC IRON AND N,N-DIHYDROXYETHYLGLYCINE
CONCENTRATIONS ON GROWTH OF *SKELETONEMA*
AND *PHAEODACTYLUM*

<i>Skeletonema costatum</i>				
DHEG:	10^{-4}	10^{-5}	10^{-6}	10^{-7}
10^{-4}	37 (± 8.4)			
10^{-5}	49 (± 5.4)	35 (± 11.8)		
10^{-6}	6 (± 5.4)	5 (± 4.8)	5 (± 2.0)	
10^{-7}		0	12 (± 2.6)	0
<i>Phaeodactylum tricornutum</i>				
DHEG:	10^{-4}	10^{-5}	10^{-6}	10^{-7}
10^{-4}	49			
10^{-5}	56	58		
10^{-6}	64	62	65	
10^{-7}		58	52	52

Note Growth as per cent absorption, standard errors in parenthesis; concentrations in log gram atoms or moles per liter. Basal medium S 76 less EDTA and iron, i.e. with otherwise normal complement of trace metals and glycine as sole chelator. (Reprinted from Droop, 1962.²⁵⁹)

be iron starved more easily than supralittoral, easily-cultivated organisms.

We have log gram atoms per liter, of ferric iron at the side, and of the chelator at the top. *Skeletonema* is in the upper table. In the presence of DHEG, at least 10^{-5} g-at iron/liter is needed to get good growth; i.e. 37,49,35 extinction. The figures are much lower when the iron is reduced below 10^{-5} g-at.²⁵⁹ The valves in the organisms are in per cent absorption, 100 minus per cent transmission.

The lower table is *Phaeodactylum*, and there is no significant difference between any of the entries. From the data, I inferred that the pelagic diatom *Skeletonema* under difficult conditions—very adverse conditions respecting iron, because of this chelator—was indeed less able to deal with the situation than the littoral organisms, and that its power of iron uptake must be feebler. Only *Phaeodactylum* is shown here, but I have also done the experiment with several other supralittoral species, and with them, also no difference can be shown; they also could not be starved of iron by this method—only *Skeletonema*. The cultures weren't depleted of iron very consciously before-hand. I inoculated from ordinary media.

GUILLARD: This more or less confirms what Doctor John Ryther and Miss Dana Kramer found.²⁶⁰ You may recall that Doctors Menzel and Ryther did some enrichment studies in the Sargasso Sea and found that C^{14} -uptake was often stimulated by the addition of chelated iron.²⁶¹ In more recent studies, it has been found that substances that cause precipitates in sea water—specifically, aluminum chloride—sometimes, but not always give the same stimulation as iron. In short, it would appear as if some but probably not all of the effect is due just to the precipitate.²⁶²

STRICKLAND: Would you say that again slowly, Doctor Guillard?

GUILLARD: In Sargasso Seawater, if one adds different nutrients, one frequently gets an increased C^{14} -uptake.

STRICKLAND: This is by adding noncomplex nutrients?

GUILLARD: Nitrogen, phosphorus, silicate, etc.; among these, if one adds complexed iron, one gets the increase of photosynthesis. It has been pointed out that sometimes the increase is brought about by just the chelator; i.e., EDTA, without the iron.

KOCZY: How is the C^{14} -uptake measured?

GUILLARD: In the usual way, trying to take into account that the light may cause absorption of C^{14} on the precipitate, etc. The point I am getting to, is that the stimulation of photosynthesis by a precipitate may in some cases be due to the absorption of phosphate on the precipitate.

STRICKLAND: Oh, you mean it is an artifact?

GUILLARD: It may be due to the precipitation, but it may not always be an artifact.

BRAARUD: Harvey²¹⁴ has reported iron uptake from particles which become attached to algae. I wonder whether that might be an explanation: Particles with bound iron adhere to the algae so that iron is more easily accessible than when it is finally dispersed or dissolved.

DROOP: Iron micells are brought within the influence of the cell membrane.

GUILLARD: I think so but whether it is iron or some other mineral, say phosphorus, that is brought in, I don't know.

GOLDMAN: I would like to bring up a cautionary consideration for culture work when iron is added. In some situations, say, working over 10 parts per billion (10 μg -liter), ferric hydroxide will be precipitated with carbon apparently occluded to it so tightly that it is not possible to remove the C^{14} , even with 0.01N acid rinses or exposure to fuming hydrochloric acid.²⁶³ This phenomenon isn't necessarily restricted to very high pH's, as we obtained this by prefiltering (HA Millipore) Lake Tahoe water of nearly neutral pH, which is even more surprising.

FIGURE 51 illustrates iron stimulation to a culture of Lake Tahoe phytoplankton at Fe levels below those where we found this precipita-

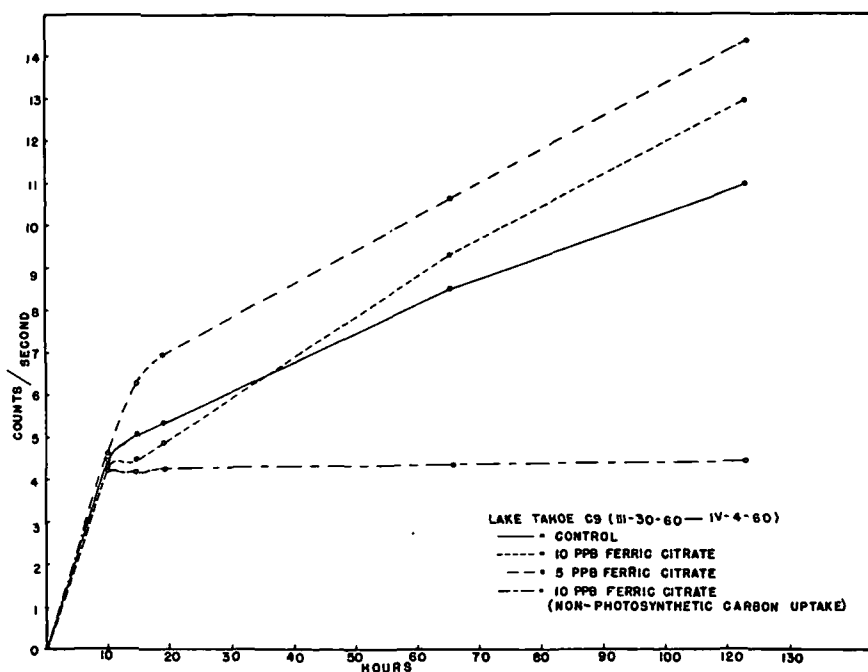


FIG 51. Photosynthetic carbon fixation in cultures of Lake Tahoe with iron additions of 5 and 10 ppb. A culture with 10 ppb iron was maintained in the dark as a measure of nonphotosynthetic carbon uptake (Ref. 107).

tion phenomenon.* The phytoplankton population is primarily diatoms. Dark controls in culture experiments may not be adequate, since there is some evidence that light is a factor influencing iron precipitation. In this experiment, the dark control had 10 parts per billion iron citrate. Also, there is an indication of some early inhibition at the 10 parts per billion level, as it was not as stimulating as five parts per billion.

We were unable to prevent Fe precipitation in prefiltered Lake Tahoe water at a 1 to 10, EDTA to iron level. It is necessary to go to about a 1:1 ratio to prevent precipitation phenomenon.

PROVASOLI: Have you any idea what makes the iron precipitate in the water?

GOLDMAN: Because of its restricted granitic watershed, Lake Tahoe is very low in dissolved organic and inorganic matter.

STRICKLAND: Is there silicate in it?

GOLDMAN: Yes. It is reasonably high. This precipitation of iron with C^{14} attached also occurred in Pacific Ocean coastal water and, more recently, in one of the very soft New Zealand lakes I studied.

HUTCHINSON: Is it a physically visible material?

GOLDMAN: In some cases, if there is enough iron the ferric oxide can easily be seen on the Millipore filters.

PROVASOLI: They are flocculus?

GOLDMAN: Yes.

PROVASOLI: Otherwise, a precipitate can't be seen even with the microscope.

GOLDMAN: It cannot be seen but C^{14} can be measured even though the culture medium was prefiltered when the C^{14} and iron were put in.

DROOP: Will you repeat for me the ratio of chelator to the iron put in?

GOLDMAN: We used ethylene diamine tetracetic acid at two ratios in this experiment. The ratios of 1:1 prevented the occlusion, apparently, of the carbon to the ferric hydroxide complex, but ratios of iron to EDTA of 10:1 do not prevent the precipitation.

DROOP: I would have thought that at least 50 times as much chelator as the iron would have been needed to hold it up²⁶⁴ at pH 8.0.

GOLDMAN: This was around pH 7.4.

PROVASOLI: Most marine media contain far less chelator and don't precipitate. The ratio of chelator to trace metals is almost 3:1.

DROOP: Not of iron.

PROVASOLI: If the molarities of all the trace metals are summed up, the ratio of chelator to trace metals is 3:1 in the PII metal mix, and

*Doctor Goldman wishes to acknowledge the support of the work reported on California lakes by NSF grant G8901.

about 1.5:1 in the TM-11. Iron is included in the sum of the trace metals.

DROOP: The ratio of chelator to iron in my metal mixture, TM-2, is 160:1, and in TM-12, 100:1. In a half-strength artificial seawater medium the overall ratio of chelator to metals is of the order of 1:200 if calcium and magnesium are included in the calculations.^{265,266}

PROVASOLI: If the pH doesn't rise above 8.2 there is no visible precipitate of iron in the medium, not even after a month. Later on, some visible precipitate may appear.

DROOP: I must correct myself in regard to the minimum iron/EDTA ratio. I forgot that Doctor Goldman was speaking of fresh water—mine was a seawater medium. Absence of calcium and magnesium reduces the ratio to nearly 1:1. That is right, if there is no calcium or magnesium in the medium. I was wrong.

GOLDBERG: Has anyone conducted an experiment where a radioactive chelating agent is added to seawater to ascertain if the chelating agent is incorporated into the protoplasm of the plankton?

KRAUSS: We have tried it and we found that some of the chelating agent is taken up by the cells.

GOLDBERG: Is it in the form of the chelating agent itself, or some degradation product? Is it absorbed or metabolized?

KRAUSS: It is a little hard to tell, but the evidence seems to indicate that it is in the form of the chelator. It is rather difficult to determine whether the cells are taking up the C¹⁴ from fragments or from the intact chelating agent. If it were taking it up from fragments, one would expect that there would be some indication of metabolism of the acetate, which is the moiety to be expected on degeneration.

In checking the gas evolved from cultures that are growing in labeled EDTA, there is no evidence of label in the expired gases. These were very dense cultures. Where there is a lot of respiration in the dark one would expect some labeled CO₂ to come off.

We have fractionated the cells to see where the EDTA occurs and it appears rather widely dispersed; some is in the lipid fractions, some comes out with the nucleic acids, some comes out with protein. I should say the EDTA molecule, which is what we think it is, is found pretty widely dispersed in the cell, but does not appear to be actually metabolized. FIGURE 52, taken from the work of one of my students, Eugene W. Test, illustrates this distribution.²⁶⁷

GOLDBERG: Is the iron still complexed with the chelating agent in the uptake process?

KRAUSS: I suspect that the iron has been removed from the chelate. There may be some other metals complexed. There are many other ions that can complex EDTA; the common ones are magnesium and calcium. Of course, the stability constants for iron are the highest of

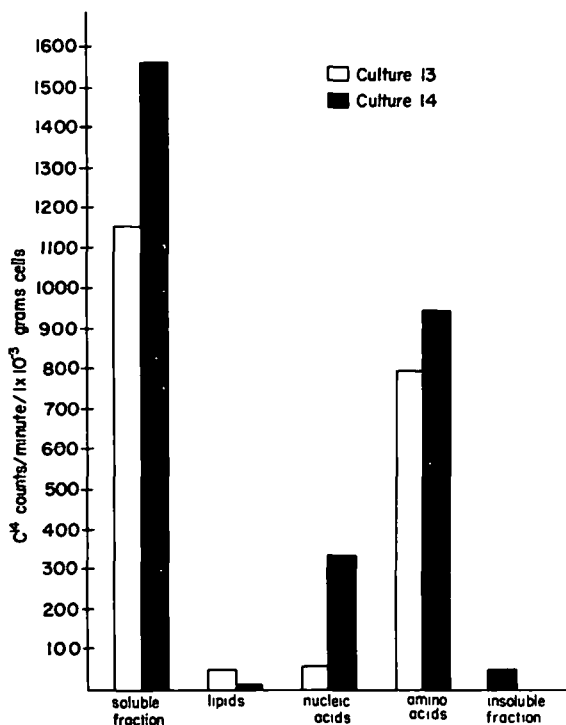


FIG 52. Distribution of radioactivity in the various fractions of *Chlorella pyrenoidosa*, van Niel's strain, grown in media containing EDTA labeled uniformly on the alpha carbon atom (from Test, 1962.²⁶⁷)

them all, but this does not mean that certain exchanges cannot be taking place.

RILEY: I think we moved away from the discussion of aluminum a little too quickly. We know that diatoms pick up and absorb particulate iron and, in the same way, they might pick up aluminum. In the meantime, the aluminum might have scavenged a lot of things that diatoms like, a process we know occurs naturally in the sea.

I would like to ask Doctor Goldberg to talk a little about the chemistry that is involved in this process, the physical chemistry, if you will.

GOLDBERG: I worked on this about six years ago and became aware of the complex problems one encounters in considering the uptake of iron by organisms in the marine environment.²²¹ I don't think we are going to get much further in such pursuits until the speciation of iron is established. Secondly, I think Doctor Riley has brought up a significant point regarding potential scavenging ability of any of the solid

phases in seawater. It is very difficult to *a priori* predict, for example, whether aluminum hydroxide will scavenge iron. One must ask the following questions: How did the solid phase form in seawater—was the aluminum hydroxide preformed? Are organic forms of iron scavenged? There are many parameters that affect the uptake of iron, say, on aluminum, and the subsequent transfer to the diatom.

It is very difficult today to talk sense about iron chemistry in seawater, knowing as little as we do about its true speciation. One sees statements in the literature, such as, "Iron phosphate formed," and, "iron exists in seawater as iron oxide or hydroxide." I see no evidence, really, for this. It is just a hope of the authors.

PROVASOLI: Who is going to solve this problem for us?

GOLDBERG: As I mentioned earlier, Sillen and Chow are working on it now in Sweden, attempting to define the forms of iron in seawater. I think the whole problem of chelating agents becomes even more complex when one considers that various equilibria can be set up. For example, if iron in the form of an EDTA chelate is added to seawater, ferric chelate decomposes into iron species and chelating agent. Further, a shift in this equilibrium can be brought about by the chelating agent plus calcium going to calcium chelate, and the subsequent release of iron to the seawater, possibly as a soluble species, possibly as insoluble species. Further equilibria with other charged cations and the chelating agent are possible.

PROVASOLI: Why should calcium chelates form? Because of mass action?

GOLDBERG: Yes.

PROVASOLI: The mass action will overcome the power of chelation.

GOLDBERG: Depending upon the stability constants of the chelates with the metal ions in question.

PROVASOLI: But the stability constant of trivalent iron is about 23, I think. For bivalent iron it is about 14, cobalt and nickel 18, and calcium down to 10. Would calcium, with a stability constant of 10, intervene by mass action and displace the iron chelate which has a much higher stability constant?

GOLDBERG: I didn't know the differences were that great.

KRAUSS: The problem here is that all stability constants have been worked out in essentially single metal solutions. I discussed this with both Melvin Calvin and Arthur Martell* a few years ago, hoping I would be able, somehow, to predict what sort of recombinations took place in complex media. Their reaction, was, that it is almost impos-

*Calvin, Melvin, University of California, Berkeley, California; and Arthur Martell, Clark University, Worcester, Mass.: Personal communications.

sible to predict—it is very, very difficult when a medium is composed of a number of salts, each, contributing ions which could be complexed. They were very pessimistic about an ultimate solution.

DROOP. There is a paper by Doctor Spencer on this very aspect.²⁶⁸

SPENCER. That paper is on the manipulation of stability constant equations. The big theoretical weakness is that the chelate stability constants are usually determined in 0.1M KCl or something similar. When one starts adding EDTA or any chelate for that matter, to a typical marine medium, one is working with a very difficult ionic background from pure systems. We just haven't got the activity data to make anything but a very rash sort of extrapolation.

At the moment, only an approximation can be made, but I think it is a realistic one, for the variation of the stability constants of the divalent cations with the ionic strength of the medium. This can be done on the basis of some work on the variation of the stability constant of the calcium—EDTA chelate with ionic strength, and the assumption that variations of the same magnitude will occur in the stability constants of the other divalent cations. By good fortune, in seawater containing EDTA it so happens that it is the divalent cations that are of greatest importance in the system of competing equilibria. Similarly, but working with a quasithermodynamic constant, one can compensate for changes in the activity coefficient of the very big polyvalent EDTA anion (the unchelated portion). These would be expected to be relatively enormous and cannot be assessed easily.

On the basis of such calculations, it can be demonstrated that when EDTA is added to seawater (or similar medium containing relatively much greater amounts of calcium or magnesium than of other divalent or trivalent cations), the greater part of the EDTA will be bound to the calcium or magnesium. Much higher concentrations of metals such as manganese or copper would have to be added than usual to compete effectively with the calcium or magnesium. The situation may be quite different in synthetic media with much lower concentration of calcium and magnesium to the trace metals, or with other chelators which bind calcium and magnesium less strongly than the trace metals.²⁶⁴

The question of the fate of iron in seawater to which EDTA has been added is difficult to assess. It is complicated in the first place by the photolability of the ferric-EDTA complex and, secondly, by uncertainties concerning the state of iron in the water. It seems fairly certain that, in spite of the very high stability constants for at least some of the iron-EDTA chelates, the addition of iron in the usual quantities will not affect the predominance of the calcium and magnesium chelates in the equilibrium mixture. I agree with Doctor Goldberg that, at the moment, one cannot say anything chemically about what happens to iron in seawater or media containing EDTA.

BRAARUD: I think it might be appropriate to say a word about the background of why we are so interested in iron, the need for iron by the organisms, and its occurrence in seawater. For 30 years, there has been discussion as to whether the very striking difference between the populations in coastal waters and in oceanic waters might have a nutritional background. In 1932, Graham¹⁰ published a paper in which he stressed that iron might be the nutrient which was decisive for this striking difference, and he conducted experiments with natural populations in flasks, adding various organic iron compounds and humates.

He obtained some very positive results, although some were not so good. Recent observations by Ryther and Kramer,²⁶⁰ have given indications that coastal species such as *Skeletonema* have a far higher demand for iron than such oceanic species as *Coccolithus huxleyi*. This observance may be very fruitful for explaining the differences in the distribution of the holoplanktonic and meroplanktonic species and, for this purpose, we need complete information about iron chemistry and the determination of iron in the sea.

KOCZY: In connection with the question of uptake and accumulation, I want to mention some of the experiments carried out by L. J. Greenfield²⁶⁹ and Robert Johnson at The Marine Laboratory at Miami. The problem is the uptake of calcium by organisms. The work is carried out in two directions. One is the study of calcium uptake by the conch, the other is the uptake of calcium by bacteria, and the main problem is the final precipitation or formation of calcium carbonate.

After it was found that almost all the metabolic energy was necessary to transport calcium in an ionic state through the cell wall, Greenfield looked for the organic compounds in the cell wall capable of chelating calcium. The culture medium was spiked with radioactive calcium and, after a few hours, killed. The cell wall was separated from the cell and hydrolized. Using chromatography, he found five different amino acids of which only one was radioactive. It seems that only one or two amino acids are the transporting calcium agent. They are also responsible for uptake.

FREMOND-SMITH: Which amino acids are they?

KOCZY: Alanine is one.

GOLDBERG: Upon the addition of a chelating agent to seawater, it may be possible to determine experimentally how much of the divalent ions it complexes in the following way: If it is assumed that the divalent ions have about the same stability constant—of course, they vary from chelating agent to chelating agent—one might use the electrodes that Professor R. M. Garrels of Harvard University has developed, that are sensitive to sodium, potassium, calcium, or magnesium. The electrodes for divalent ions are not very good but, nevertheless, it might be determined if the addition of a chelating agent does change

or lower the calcium or magnesium activity in seawater. This might be a very nice experiment to do.

SPENCER Yes, I think it would. I have looked at the competition between calcium and magnesium in seawater containing large quantities of EDTA (that is, up to about 5×10^{-3} M) by spectrophotometric techniques in the UV region. In this way, one can at least get a suggestion that the calculations are working out about right for these ions, and suggest that we are probably getting results of about the right order with the other divalent cations.

DROOP: How much calcium is there in seawater?

GOLDBERG: About 10 m mole/liter.

JOHNSTON: 10.2×10^{-3} M, that is, a higher calcium concentration than the EDTA that is added.

ECOLOGICAL IMPLICATIONS OF *IN VIVO* DATA**Discussion leader:**

G. E. HUTCHINSON

*Department of Zoology**Yale University**New Haven, Connecticut*

HUTCHINSON: In making a comparison between what happens in nature and what happens in the laboratory, it seems to me that the first thing that we have to consider is that laboratory vessels have walls round them, and that no one has yet invented a bottle that has only volume and no surface area in which to culture the organisms. This would be a nice insoluble topological problem for someone to think about. I suppose Doctor Strickland's bags are as near as we have managed to get, but, so far, they have to be kept in the ocean so they may be disqualified as laboratory vessels.

However, it does seem to me that one of the most important things to be considered is the spatial limitations that are imposed and all the various corollaries, the first one being that there is, inevitably, a surface and that all sorts of things may happen at surfaces. This should bring up (and perhaps will bring up) the question of how much surface per unit volume there actually is in the open ocean, because the sort of thing that Doctor Riley has been working on, marine snow, presumably provides a great deal more surface than one actually has at the edge of the surface of a culture flask. So, actually, there may be more surfaces involved in nature, than in culture flasks. In any event, we don't know what the story is here, and it is the first thing, I think, that has to be thought about.

Next, there is the whole question of stirring and turbulence and, here, again, we have obviously a great difference between the experimental and the natural situation, and apart from saying that there is a great difference, I suspect that we don't really know very much about it.

Then, there is the problem that, in a bottle, there are certain gradients but, inevitably, if one is going to imitate on one side or at the bottom of the bottle in ordinary laboratory glassware a situation that, say, corresponds to 100 meters depth, and at the top one that corresponds to zero meters, there will not be 100 meters between the two points.

The gradient problem has been quite ingeniously overcome in some animal experiments. I am thinking particularly of Harris' ingenious work on vertical migration in which the light gradients corresponding to about 50 centimeters, were made with Kaolin or with India ink suspension to correspond to the light gradient over 100 meters or 200 meters in nature. But there, again, the poor little *Daphnia* didn't have to swim as far as they would have to in the natural environment. Therefore, the scale effect of all kinds of gradients is going to be extremely important.

Then, we have the whole problem of the nature of nutrients. We have talked a great deal about this this morning and nearly everything that was said this morning is also relevant to this afternoon's discussion.

We have also talked a great deal about chelating agents. The main difference in the laboratory and in nature is that we know what these agents are when we put them in—we read the label on the bottle. We do not know what they are, very largely, in nature. Parenthetically, I might remark, in connection with the discussion this morning, that many years ago we attempted a fractionation of the various forms of iron in lake water and came to the conclusion that there was probably an organic compound of some sort that was a good deal more stable than any of the things we have so far discussed. It is not at all easily reducible and does not react with dipyrindine, nor is the iron liberated from it by dilute hydrochloric acid. It is quite clearly not the kind of thing that one gets as a ferric humate or humic acid complex. It is a great deal more stable.

I think, before we can get very much farther on the problem of iron and, possibly, of some of the other quite common materials, a lot more has to be done in nature to find out whether we are imitating the right sorts of situations.

Coming back to the question of the surface, there is always the possibility in anything but the most chemically refined vessels that one is adding an appreciable quantity of unrecognized materials such as vanadium, which leaches from some sorts of glass. We have not heard anything very much about trace elements other than iron here, and when I have stopped making these general remarks one of the things that I hope we shall hear about from Doctor Goldman, is the problem of other trace elements, because his work is a very interesting example of the interaction between our knowledge derived from the laboratory and our knowledge derived from nature.

I think, then, that we have a number of rather peculiar situations that have to be considered, even relative to the things, that, at least before this morning, we thought we knew all about, like phosphorus and nitrogen. I would very much hope that either Doctor Lund or

someone else perhaps, might throw a little bit of light on a situation that is rather puzzling. It seems reasonably common to find two species, one succeeding the other, in which the second one—and I am thinking here perhaps of *Tabellaria* in fresh water—is commonly supposed to be more parsimonious or thrifty of its nutrients than the one that has preceded it. This is partly the result of reasonable interpretation of field evidence, partly backed up by experiments, but neither the field evidence nor the experiments tell us why the more parsimonious one doesn't flourish at the higher nutrient levels. The whole question of a possible toxicity of a nutrient, as phosphorus in the case of *Dinobryon*, at a level that most people growing cultures would regard as rather inadequate, or in the past would have regarded as rather inadequate, is quite clearly a very significant thing. This has been mentioned on and off in connection with dilution, a rather vague phrase because it doesn't say what is being diluted. Perhaps it would be nice to hear a little bit more about it.

Then, we have the very obvious fact that, even in these "absolutely relatively" axenic cultures, indeed particularly in them, we are dealing with situations that are completely foreign to nature. We have to deal with them as a method of simplifying the situation to a point where we might hope to understand it, but there is the perennial problem as to whether that understanding will ever tell us anything about what happens in a natural community. Although this, perhaps, trespasses on some of the things that we will discuss tomorrow, I would like to stress the fact that hard as it may be to culture a single organism—and a great deal of ingenuity has been devoted to getting organisms in an axenic culture—when we want to make an association that is not one of a wanted alga and an unwanted bacteria but one of two wanted algae, then we are up against something that, so far as I know, is a matter solely for the future, except in some very special kinds of syllogistic cases. The whole problem of how to make a natural association is obviously one which will ultimately have to be faced if a real analysis is to be possible, and yet it is one on which virtually no progress so far as I know has been made.

My own feeling is, as I have probably expressed to some of you ad nauseam, that practically every association in nature, in the size ranges the alga-planktonologist deals with, are comparable to rather early stages in a batch culture; yet they are things that tend to grow rapidly, fairly rapidly, but hit a depleted environment fairly soon. They may undergo senescence at a level that would be very unrewarding to a laboratory worker, and then something else comes in and takes their place, but in no sense of the word can they be regarded as parts of what a bird or mammal ecologist, for instance, would regard as an equilibrium, or nearly equilibrium type of association. Particu-

larly in dealing with phytoplankton algae, we are dealing with situations that my friend Robert McArthur would call opportunistic associations rather than equilibrium ones, and the kinds of principles that the macrobiological ecologists have worked out probably do not apply very well in this sort of situation.

I think that it is a suitable introduction, and I would think that we might start by hearing a little bit about the interaction of experimental and field work on the trace elements that we have not discussed so far. Perhaps Doctor Goldman will tell us a little bit about molybdenum, perhaps something about boron, and he also intimated that he had a few remarks to make, that are quite interesting in this context, about surfaces and particulate inorganic matter.

GOLDMAN: I think I can best summarize briefly some of the trace element aspects I have investigated on a regional basis.¹⁰⁷ In Alaska, we have worked on some six lakes scattered along the Alaska Peninsula and north. Of these, four showed immediate molybdenum response, that is, an increase in carbon fixation as a result of the addition of about five parts per billion molybdenum. Four of the six showed a higher response to a mixture of trace elements. This trace element mixture included a chelating agent, sodium EDTA, manganese, zinc, cobalt, molybdenum, and boron. Almost inevitably, if there is any trace element response, a better response will be obtained if a spectrum is provided; this, of course, is evident also in laboratory work. The vitamins B₁₂, thiamin, and biotin were particularly stimulating in the Alaskan lakes.

Chronologically speaking, my work with trace elements started with the discovery of a molybdenum deficiency in Castle Lake, California.²⁷⁰ This is a high cirque lake which we could classify as mesotrophic. A summary of the general nutrient-limiting situation in this lake, based on about four years of study, appears in FIGURE 53. Certainly, we can't apply Justin von Liebig's law of the minimum in an absolute sense in this study, because, as I have said, we frequently get an interaction of limiting effects. In Castle Lake, carbon uptake is stimulated by the addition of molybdenum, potassium, or sulfate, singly, and to an even greater extent by the simultaneous addition of all three.

I should note, too, that five parts per billion molybdenum may be stimulating to a culture of Castle Lake water, particularly during the summertime when runoff is low. However, 10 or 15 parts per billion may be severely inhibiting during the period of spring runoff, when the natural rate of recharge of this trace element would be expected to be at its highest. In the same experiment, cobalt was added as sulfate. I think the cobalt sulfate response represents a small sulfate stimulation followed by a severe cobalt inhibition. Castle Lake happens to have

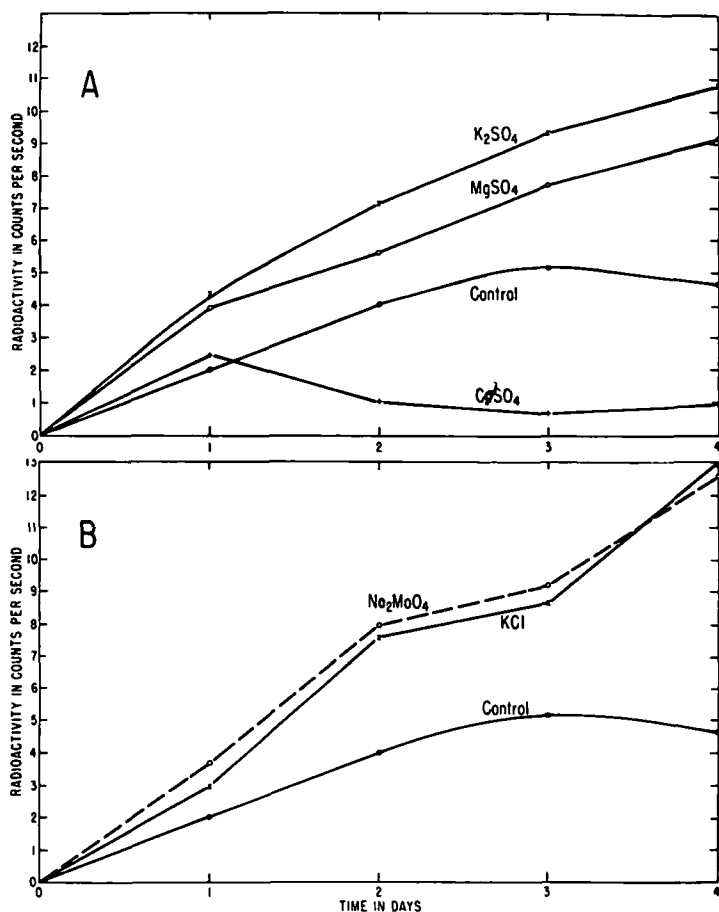


FIG 53. Response of cultures of Castle Lake phytoplankton to various nutrient additions. Relative rates of uptake were measured with carbon¹⁴. (Goldman, C. R. unpublished.)

about 15 parts per billion cobalt, the highest we have found so far in any lakes in California.

GOLDBERG: Molybdenum is here in the oxidized state. Did you ever try adding it in the plus three reduced state.

GOLDMAN: Yes, both sodium molybdate and molybdic acid were added and the latter initiates the same response.

LUND: What changes, if any, took place in the constitution of the population of algae from the beginning to the end of the experiment?

GOLDMAN: Nothing that I could demonstrate in the way of a population shift. These experiments were run in the lake at surface light

and temperature without any concentration of organisms—simply the natural population.

HUTCHINSON: Do you remember what it was?

GOLDMAN. A mixed population of diatoms and micro greens, and perhaps a few micro blue-greens, a very typical population in alpine environments; that is, predominantly very small organisms. Actually, if we ran this culture through a 5 to 10 μ filter we would retain about 20 per cent of the fixed carbon, so most of the organisms are very small.

MYERS: How should I understand the fact that potassium chloride and sodium molybdate gave the same response?

GOLDMAN: There will be stimulation with either potassium, sulfate, or molybdenum; when this particular set of experiments was run, the increases in carbon fixation were of about the same order of magnitude. When they are all added, it goes still higher.

HUTCHINSON. Do you interpret that to mean that some organisms are suffering from potassium deficiency and some from molybdenum?

GOLDMAN: I think that is certainly a possibility, if not a probability that, when one is working with a community of organisms, some of them need more sulfate, some more potassium, some more molybdenum, and perhaps some have a high requirement for all; this, of course, is where the interpretation of field experiments and mixed laboratory cultures is most limited.

An example of the sensitivity of Castle Lake phytoplankton in cultures under a meter of ice, to changes in their nutrient balance is presented in FIGURE 54. The immediate response, at essentially 0°C, initiated by the addition of 50 parts per billion molybdenum, and of potassium sulfate, is evident.

MYERS: Do I presume correctly that if one adds sodium chloride there is no response?

GOLDMAN: Yes.

MYERS: There are things we can add that will, in fact, give no response whatever?

GOLDMAN. Oh, absolutely. In fact, in these experiments each compound is checked with some related compound, providing the same amount of sodium, chlorine, or other companion element is present.

We know that molybdenum is involved in two enzyme systems; nitrogen fixation and reduction. The formation of the enzyme nitrate reductase presumably requires molybdenum as a hydrogen donor. I have tried adding molybdenum in combination with nitrogen, both as nitrate and as ammonium, with the nitrogen balanced, and have obtained the greatest carbon fixation rates with the nitrogen added as nitrate. This might suggest that the molybdenum is, in fact, in short supply in the reductase cycle.

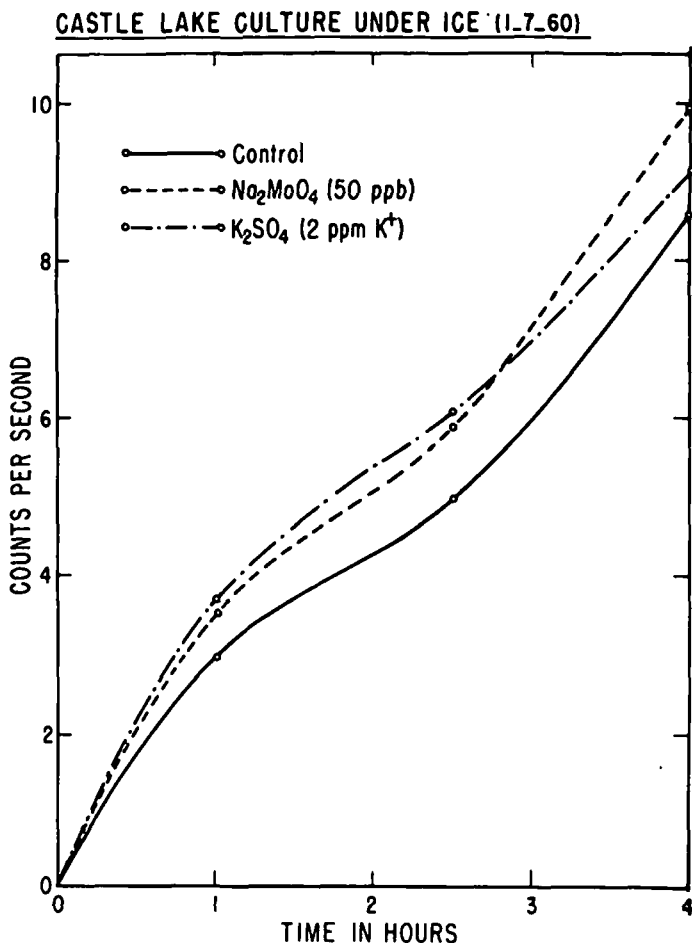


FIG 54. Response of Castle Lake winter phytoplankton to addition of the three most limiting elements (molybdenum, potassium, sulfate). Culturing was accomplished *in situ* under a cover of ice and snow. Relative assimilation was measured with Carbon¹⁴. (Goldman, C.R., unpublished).

I feel that a lake reflects the geology of the area in which it lies more, perhaps, than any other environment we might study. In effect, it is a concentrator for the ions from its entire watershed. In the Klamath Mountains of California, there is ultrabasic rock which would be expected to be low in molybdenum. This is characteristic of serpentine soils in general. Some other parts of the United States are high in molybdenum, and one might very well look for trace element toxicity in lakes of these regions.

Boron has produced positive response in only one of the lakes I have studied, Lake Nerka, Alaska. The New Zealand lakes show response not only to molybdenum, but also to almost every trace element added either singly or in combination. I have obtained response with zinc, manganese, cobalt, copper, iron, and the vitamins, as well as with iron chelated with EDTA. This is not really so surprising from our knowledge of New Zealand and Australian geology. The importance of cobalt additions in Australian agriculture is well known. This region of the southern hemisphere would be expected to be deficient in a variety of trace metals.

As to the problem of surface effects on algal populations, I have some observations which may be pertinent, particularly in relation to what Doctor Guillard mentioned earlier of the work at Woods Hole with iron and aluminum. These were C^{14} studies in Naknek Lake, Alaska, where the Savonoski and Ukak Rivers bring in a tremendous load of glacial flour and siliceous ash from the Valley of Ten Thousand Smokes.²⁷¹ The Katmai eruption of 1913 modified the climate of the earth for about 10 years with ash thrown up into the atmosphere. This ash is still coming into Naknek Lake, making it extremely turbid and reducing transparency tremendously, but, at the same time, apparently stimulating the photosynthesis of the organisms present. It is possible that this is a surface effect, since the ash has about the composition of pulverized granite. We know that some organisms are unable to grow unless they are in contact with a surface. I won't attempt to explain the physical chemistry of this, but it simply provides a better ionic gradient for the organisms, being in contact with the surface. I propose that perhaps this is the reason for the high productivity in Naknek Lake that I observed in 1957. It would appear that this may be a principle of general importance wherever we have precipitation or other solid surfaces for organisms to live with in close association.

VOLLENWEIDER: May I say that in this discussion perhaps we should distinguish between two kinds of limiting factors. It depends completely on the water with which we have to deal. Inland lakes often are very low in salt content, practically every element can be a limiting factor.

If a water has a very low conductivity, we ought not to expect any large production of plankton. In another type of lake with high nutrient complement, we may be in a quite different position. We can then add any kind of known growth factor and we get no response at all. Nevertheless, in this lake we must search for other certain limiting factors. It may be that in a short duration experiment we will find only the lack of a positive reaction but even a negative effect; but if the experiment goes on for several days it may be that the same factor

may have a positive effect. So, it seems to me we should face this problem of limiting factors with a much broader concept than only that a factor will react. How do we conduct experiments in this special situation?

GOLDMAN: I think your comment is quite pertinent. It is very frequently found, particularly in working at the low levels of trace-element addition, that the immediate response of the culture is a negative one that may last for only a matter of minutes or hours. At other times, if enzyme systems in the existing organisms are mobilized to utilize the limiting factor, growth is immediately stimulated; just as if enzyme templates are already set up, the trace metal plugs in, and its machinery at once begins working at an accelerated rate. Even nitrogen will react this way in a rather senescent culture. Probably the best experiment I have to illustrate this point on the kind of immediate response that can be obtained in a really nutrient-deficient culture was my first culture experiment with C^{14} (FIGURE 55). In this experiment, conducted at the University of Michigan in 1956, a nitrogen-deficient culture of *Scenedesmus quadricauda* Turp. was divided into two parts, after first adding C^{14} to both and nitrogen as calcium nitrate to one. Samples were filtered from both at five-minute intervals. FIGURE 55 illustrates the immediate increase in carbon assimilation initiated by the addition of a few parts per million nitrogen to this culture.²⁷² I believe it is the best straight line I have ever obtained.

VOLLENWEIDER: That is the kind of experiment I would call an oculte-limiting situation, but it may not be a true limiting factor for the lake. It might be a momentary situation, because a rich population existed directly previously that has used this one factor nitrogen. Here in this sense, the problem becomes a three-sided one.

GOLDMAN: Yes, and, as we know, from the transfer of phosphorus by organisms from cultures which have been grown in a phosphorus-rich medium, they apparently take the phosphorus with them. When we are dealing with enzyme systems, certainly the metals are not completely exhausted but are being recycled, as the system works at something less than its maximum rate. By adding the metal, we get an immediate stimulation, as the particular deficiency is relieved, but then the organisms' growth rate is controlled by the next limiting factor, which may be nitrogen, phosphorus, or any one of a host. So, we get stimulating effects, as well as essentially limiting effects.

HUTCHINSON: Since there are quite a lot of data from the experimental work indicating the essentiality of copper, zinc, and probably vanadium, perhaps Doctor Goldberg would like to say a little bit about the geochemistry of any elements that we might have to consider in the future, because I think people ought to be prepared for

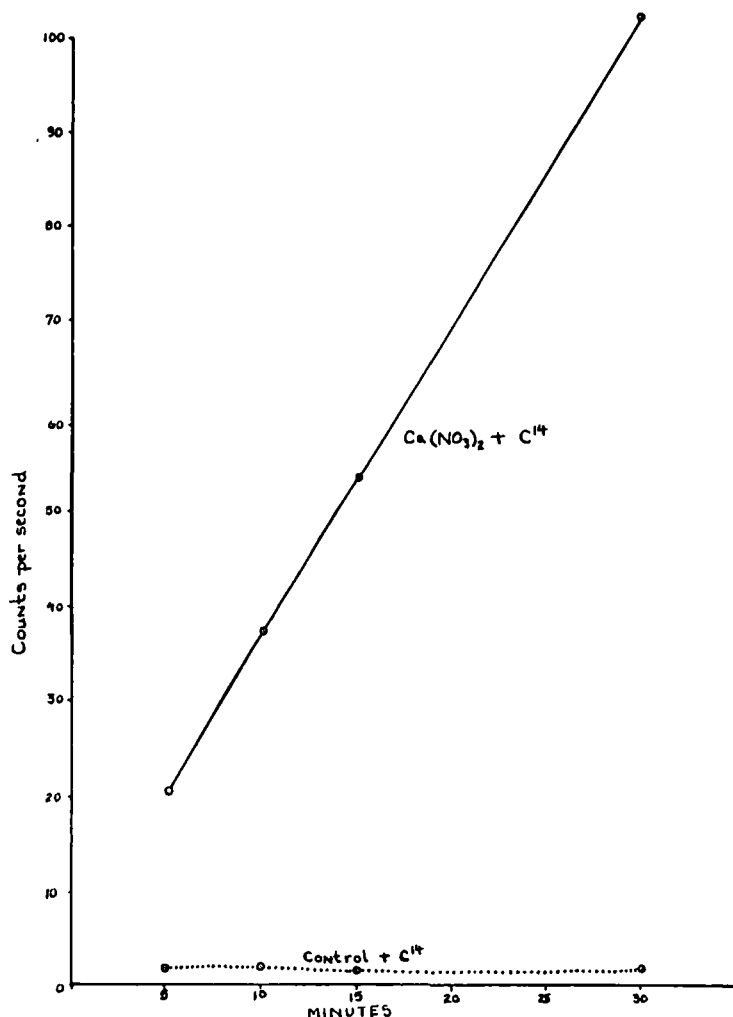


FIG 55. C^{14} uptake with the addition of $\text{Ca}(\text{NO}_3)_2$ to a nitrogen deficient culture of *Scenedesmus quadricauda* Turp., December 1956. (From C. R. Goldman, 1958. Primary productivity and limiting factors in three lakes of the Alaska Peninsula. Doctoral Dissertation, Univ. of Michigan.²⁷²)

this. It may be that none of them will be oceanographically limiting. They will not be ecologically important, in Doctor Droop's picturesque terminology, which I found so upsetting yesterday, but physiologically important. Still, I don't see why we shouldn't consider things from a slightly physiological point of view even if they are no good to the ecologist. Would you like to say a little about the type of work you are doing, Doctor Goldberg?

GOLDBERG: Let me make a few remarks about the distribution of heavy metals in seawater that have interested me lately. You are well aware of the observations made at the beginning and middle decades of this century that certain nutrients in the ocean showed increasing concentrations with depth—nitrate, phosphate, and silicate. Such distributions were interpreted as indicating the involvement of these species in biochemical cycles. Recently Doctor Chow and I looked at barium and found this same type of distribution—a depletion of barium at the surface and an increase in barium with depth, a change between the surface and 4,000 meter depth that was about a factor of five.²⁷³ More recently, I have looked at the rare earths and seen this same type of behavior: The rare earths are in smaller concentrations in surface waters than they are in deeper waters.

Studies in Japan²⁷⁴ indicate that mercury changes by a factor of five from the surface to deep water. I think such enrichments are due to a conveyance of these elements from the surface to the deeper waters by organisms or the remnants of organisms subsequent to their death. I would suggest that a chemical species suspected of being involved as a necessary reactant in productivity, such as manganese, nickel, cobalt or copper, would give a depth curve as mentioned. This may be something to question.

In looking at the data that is being reported for copper, zinc, and manganese in seawater, there apparently is a uniform or near uniform concentration with depth in the ocean, and no remarkable differences between coastal and deep sea waters. In addition, I think we are going to have to show more awareness to an effect, as yet not considered here: Man's alteration of the natural environment. I have discussed the fact that some trace metals usually show a decrease in concentration in surface waters while others are essentially constant with depth. On the other hand, recent analyses of lead in seawater have indicated that it shows higher concentrations at the surface than in deeper waters.²⁷⁵ The lead concentration is slightly less than 10 times higher in surface waters than in deep waters, in samples from the eastern Pacific Ocean. Such depth profiles are attributed to the burning of tetraethyl lead on land, the subsequent dissemination of this lead into the atmosphere, the transport by tropospheric winds, and the washout by precipitation back to the land and the ocean. Thus, lead, at a higher level in surface ocean waters today as compared to the past, may affect natural populations. Further, these surface concentrations are probably increasing.

The second contaminant that man has introduced into the marine environment is, of course, the syndets, the detergent-type soaps. Alexander Goetz, at the California Institute of Technology tells me that one can detect the syndets 500 miles off the coast. Is this

organic matter that man is introducing having any effect upon natural populations?

DROOP: How does that tie in with the Tween 80 effect on ciliates, which is usually seen at very high concentration?

OPPENHEIMER: Tween 80 is a nonionic detergent. The more biologically effective detergents are the cationic detergents which are currently effective at one to three parts per million for algal control. Ten years ago at Scripps, I experimented with Tide and other common detergents as a possible control mechanism for fish diseases, and found that they were extremely toxic to fish at one parts per million, with death occurring in a few hours. As the concentration of detergent was increased from one to 10 parts per million its lethality was increased. Some 25 different household detergents and quarternary ammonium compounds were tested and found lethal in one to 10 parts per million.

GOLDMAN: That is most surprising, considering the tremendous blooms of phytoplankton and large cladoceron populations found in oxidation ponds, where there are extremely high concentrations of detergents.

OPPENHEIMER: Detergents are rendered inactive very quickly by absorption.

ALLEN: In the oxidation ponds in California, where there is a fine growth of algae, the detergent concentrations in the effluent are so high so that great clouds of foam float all over the landscape.

HUTCHINSON: Are these special algae or ordinary things?

ALLEN: *Chlorella* and *Scenedesmus*, in the parts where the greatest oxidation is taking place, and a rather mixed flora of greens toward the effluent end.

JONES: Could these detergents be improving permeability of organic substances?

ALLEN: The algae, by the way, do not use the organic substrates directly in oxidation ponds.

JOHNSTON: I wonder if Doctor Goldberg is aware of very large local increases of lead in seawater. There are some small streams running into the sea from Cornwall, where 20 years ago they were working lead, and I believe for 10 or 20 miles off the coast the effect of these streams with high lead content can be detected.

GOLDBERG. I am not aware of that. In the United States alone, we burn somewhat over 10^{11} g/year of lead in gasoline. There are about two grams of lead/gal of gasoline, and apparently most of it is put into the atmosphere. That is roughly the same amount of lead that is being introduced to the world oceans by weathering each year. It is a fantastic amount.

JOHNSTON: Has this any effect on lung cancer?

literature a good deal to indicate that sometimes if you make a culture with natural lake water, the amount of phosphate that is needed is very much less than if you make your culture starting from distilled water and pure salts. Have you any views on this?

LUND: No, I haven't. I think both things are true. In Mackereth's experiments,²³⁰ there was no phosphorus uptake when he used distilled water. His test organism was *Asterionella*. If he used water from lakes in which *Asterionella* is often abundant or from those in which it is absent, there was uptake of phosphorus. It is noteworthy, too, that phosphate uptake was greater when water from a lake in which *Asterionella* flourishes (Windermere, FIGURE 56) was used than when water was taken from a very oligotrophic lake (Westwater, FIGURE 56) in which the organism does not exist. In his work on *Navicula pelliculosa* Lewin²⁷⁶ found that the uptake of silicon was suppressed if the cells were washed several times with distilled water, but restored if

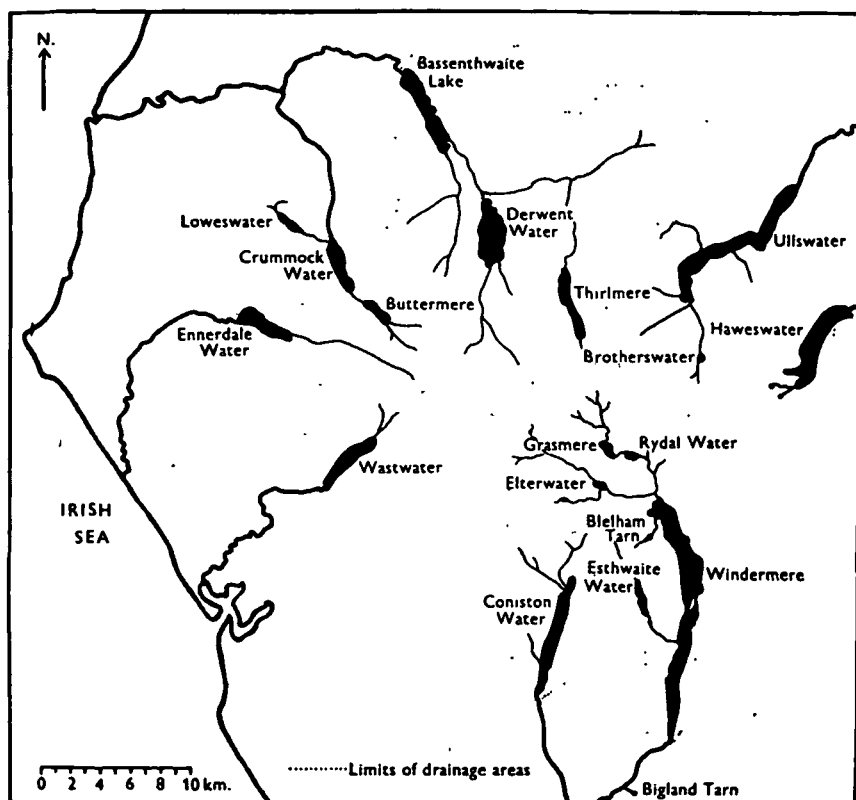


FIG. 56 Map of the English Lake District.

GOLDBERG: I don't know. I will give you a number for consideration though. In Los Angeles there is sometimes $1 \mu\text{g}$ of lead/ m^3 of air.

HUTCHINSON: Can you say what form it takes in the atmosphere?

GOLDBERG: W. C. McCrone²⁷⁵ suggests that the efficiency of conversion of lead tetraethyl in gasoline to atmospheric aerosols is about 100 per cent. Apparently it hydrolyzes rather rapidly, going to lead oxychlorides, etc.

HUTCHINSON: Twenty years ago Wollach and I noticed an accumulation of lead on the surface of *Lycopodium flabelliforme* growing in woodland about twelve miles from New Haven, Conn. Simply washing the plant carefully, reduced the rather high lead content by about 27 per cent, so that there must have been a thin layer of a rather lead rich material, possibly containing a lead soap, spread out over the surface of the plant. So far as I know, no one has commented on the effect of atmospheric lead on vegetation in natural stands, though areas in England which were the site of Roman lead works are said still to have a restricted flora.

JONES: May I ask a question of physiological interest in regard to this enhancement of C^{14} -fixation by trace metals? Is there any evidence that the addition of trace metals will result in enhancement of dark CO_2 fixation, or is it only applicable to CO_2 fixation in the light?

GOLDMAN: These experiments are controlled, with trace metals added in the dark. Sometimes there is a slight enhancement of dark fixation, but in these studies the dark fixation has been subtracted out, together with the background radiation, so we are just reporting the photosynthetic results.

KOCZY: We have made determinations of trace elements in sediments in order to find out with which component of the sediment the trace element is associated. Doctor P. S. Antal, of the Marine Laboratory at Miami, fractionated the sample by different methods. By extracting it with ethanol, part of the organic constituents could be determined. In this fraction were found 30 per cent of the total zinc and 35 per cent of the total uranium.

HUTCHINSON: There is one aspect of the difference between culture and nature that came up this morning, and I would rather like to ask Doctor Lund what his present views are about the phenomenon that Wilhelm Rodhe essentially discovered, although other people also were involved, about the so-called phosphorus-sparing factor in natural lake water. I got the impression this morning that perhaps you felt that this was not something that was a chemical entity but just a question of the conditions under which things were cultured, when you spoke of the great difference in phosphate required to start a culture growing under different conditions. As you remember, there is in the

certain reduced sulfur compounds were added. There is something in lake water, I believe, that makes this uptake of phosphorus possible, just as I think that this matter of what I call, probably wrongly, energy push, is very important as well.

HUTCHINSON: It would be very unlikely, I think, that this sort of situation would not occur in the sea if it is so universal in lakes. It is difficult to think of a reason why it shouldn't, even though it is difficult to think about it at all when one has nothing whatever to go on. I would be most interested to know if anyone else has any ideas about this particular problem.

FOGG: No, I suggested that stimulation by phosphate and perhaps trace elements might be due to glycolic acid, but that was a long shot and I haven't anything further to substantiate it.

HUTCHINSON: How did you feel glycolic acid would work in this context?

FOGG: In connection with the trace elements.

HUTCHINSON: Not in connection with phosphorus?

FOGG: Glycolate has been observed to stimulate phosphate uptake by *Ankistrodesmus* at concentrations of 1 mg/liter.²⁷⁷ We have tried to repeat this with *Anabaena cylindrica*, but have not had much success. It is just possible that some of the phosphate in natural waters may be in the form of phosphoglycolic acid, which is the precursor of glycolic acid in the photosynthetic carbon fixation cycle. I am not sure whether the glycolate which leaks out of cells may not be in this form.

GUILLARD: If I understand the experiments correctly, we may be looking at the problem the wrong way around. It may be that natural lake water does not make phosphate utilization more efficient, but that in artificial lake water the algae can't use phosphate completely for some reason. After all, if one puts a certain number of phosphate molecules into a culture flask, one would expect the algae to get them, whether in lake water or in an artificial medium. Yet apparently they don't.

HUTCHINSON: I am not at all sure that I understand your distinction, except as a verbal pattern. It seems to me that there are two possibilities, either that distilled water is never quite as good as it is supposed to be, containing something that inhibits phosphorus uptake, or that it lacks something that promotes phosphorus uptake. It does seem to me an important possibility that a rather widespread substance is involved. Some people have, however, supposed that the observed effects depended on the formation of insoluble calcium phosphate in the artificial media.

STEEMANN NIELSEN I should like to comment on a macronutrient. Carbon dioxide is a most important macronutrient. The easiest way to investigate the uptake of carbon dioxide, of course, is to have only free carbon dioxide in the water; that can easily be arranged if one works at pH 4. If one does so with *Chlorella*, the optimum rate of photosynthesis will already be at concentration of carbon dioxide of 2 $\mu\text{g-at/liter}$, which is rather low. It is very easy for carbon dioxide to be taken up by the cell; it can just diffuse into the cell.

There can be no direct comparison with the uptake of phosphate because the phosphate simply can't diffuse into the cell; but if it should be assimilated quantitatively in about the same proportion as CO_2 , and if we remember that about one-fiftieth of phosphorus compared with carbon is present in the cells, then a concentration of 0.04 $\mu\text{g-at/liter P}$ should be sufficient for the algae's growth. It seems to be at about this concentration in nature that phosphorus is more or less sufficient for a good growth of algae.

HUTCHINSON: Have you anything to say about bicarbonate as opposed to carbon dioxide?

STEEMANN NIELSEN: Yes. In some lakes, for instance where the water has a very high pH, it is necessary to have bicarbonate as a direct carbon source, because the concentration of free carbon dioxide in this case is much too low. Österling,²⁷⁸ in Sweden, has shown that *Scenedesmus* is able to assimilate bicarbonate, but that is the only plankton algae I know of that uses bicarbonate as a direct carbon source. Hood and Park²⁷⁹ have recently published a paper about marine plankton organisms utilizing bicarbonate, but I don't understand the paper.

[Since the conference, I have looked at the paper of Hood and Park again. I have prepared a critical review for *Physiologia Plantarum*, showing that none of the experiments made by Hood and Park give any evidence for bicarbonate uptake. I have also investigated the problems concerning a preferable uptake of carbon from carbamino complexes, and I have found no convincing evidence for it. The results of the experiment made by Smith, Tatsumoda and Hood²⁸⁰ may be explained as a result of NH_3 -poisoning.]

HUTCHINSON: There is an Hungarian paper in which it is claimed that some phytoplankton even use carbonate.

STEEMANN NIELSEN: I don't think that is possible. I have seen the paper.

HUTCHINSON: It may not be possible. However, it is ecologically rather attractive when one is dealing with extreme alkaline lakes with a pH of 11 or something of that sort.

STEEMANN NIELSEN: But there would be a lot of bicarbonate at

the same time.

HUTCHINSON: There would be quite a lot of bicarbonate there, yes.

STRICKLAND: The same couldn't apply to phosphorus, could it? I suppose people have thought of the possibility that only one of the three ions, presumably the monobasic one, is taken up readily, in which case a big pH effect would be expected with lake water.

I was just wondering if this whole phosphorus effect is a function of pH, in other words, that there is a reluctance to take up highly ionized forms of phosphate, and anything in the lake which will lessen the charge will make the phosphate more readily transported across the membrane.

STEEMANN NIELSEN: I can't tell about phosphorus.

HUTCHINSON: Do you think that this capacity to use bicarbonate is really very limited, that only a few angiosperms and a few algae do it?

STEEMANN NIELSEN: I think a lot of plants really do use it. When a long path for diffusion is present, it will be necessary to use bicarbonate. In a small plankton algae such as *Chlorella*, it isn't necessary. Of course, they could still have the ability, but the diffusion rate can easily afford enough carbon dioxide to be assimilated. Several workers have shown that *Chlorella* is unable to utilize bicarbonate directly as carbon source.

But with a plant like *Elodea*, for instance, the distance for the diffusion is so long that there isn't enough free carbon dioxide at pH 8, and at this pH there is about 100 times as much bicarbonate. If the bicarbonate is used at about the same rate as free carbon dioxide, then bicarbonate is of much more use for photosynthesis.

OPPENHEIMER: Do you think the bicarbonate is used, at pH 9?

STEEMANN NIELSEN: It is used at all pH's.

OPPENHEIMER: Then in the Bahama carbonate environment, the large plankton blooms occurring in the alkaline inland lakes (pH 8.5-9.5) must be utilizing bicarbonate.

STEEMANN NIELSEN: Oh, yes, I would say if the pH is near 10, they must use the bicarbonate.

OPPENHEIMER: The lakes are not quite as alkaline as pH 10—possibly about 9.5

STEEMANN NIELSEN: Up to about pH 9, a *Chlorella* can manage to grow optimally, with the same total concentration of carbon dioxide as in seawater. Above 9, and up to 10, it is necessary to have bicarbonate utilization. For instance, in some Danish lakes, during the summer the pH goes up to 10.4 or 10.5

HUTCHINSON: They are mainly calcium carbonate lakes, aren't they?

STEEMANN NIELSEN: There is a lot of calcium carbonate and there is precipitation of carbonate going on throughout the whole summer, due to photosynthesis.

HUTCHINSON: In the classical alkaline lakes, when photosynthesis raises that up to 10, all that starts happening is the precipitation of magnesium basic carbonate.

STEEMANN NIELSEN: In lake Søllerød Sø, when the rate of photosynthesis is extremely high on some late summer days, the bicarbonate uptake can effect an increase of pH to such an extent that the plankton algae will die as a result. I have taken water from this lake during the later afternoon and have found no photosynthesis at all. At the same time 200 or 300 per cent oxygen was found in the water, indicating that there had been a lot of photosynthesis going on during the day. When water is collected during the morning, some mixing has taken place. The very high increase in pH takes place only near the surface. Therefore, during the morning, again, living plankton algae will be at the very surface and photosynthesis can start again.

VOLLENWEIDER: In inland lakes I have found in the morning, only, pH levels of perhaps 8 or 9, because they are very alkaline; during the night there is a rather big shift. So I believe that there are more algae, mostly green algae, I would guess, which are able to use bicarbonate.

STEEMANN NIELSEN: I would suspect that, too, but *Scenedesmus* is the only one that has really been investigated.

HUTCHINSON: By and large, the sea would have just about the limit in pH where one might suspect either thing could happen. It would be indeterminate.

STEEMANN NIELSEN: Out in the sea there is enough free carbon dioxide. There, the concentration of free carbon dioxide is higher than necessary for producing optimum rates of photosynthesis in *Chlorella*. Of course, we don't know anything about marine plankton algae, but I would not expect such very big differences between the marine species and the freshwater *Chlorella*. It is a problem of diffusion, and there will never be long distances for diffusion in small plankton algae. Of course, they may, in some instances, be a little bit longer than in *Chlorella*.

HUTCHINSON: Doctor Provasoli, would you like to say something about the problem of the bottle versus the ocean? You must have had considerable experience that bears on this.

PROVASOLI: I don't think so. I seem to like the bottle more than the ocean. (Laughter).

HUTCHINSON: I have an idea that Doctor Strickland has what he calls "two penny worth" that he wishes to give us.

STRICKLAND: Doctor Braarud and Doctor Spencer are two of the few people who have actually counted, and done it the hard way, the

cell division rates of marine organisms in seawater. There has been remarkably little work done on cell division, the actual measuring of the number of cells at two consecutive times, and the work that has been done by Doctor Sorokin, Doctor Krauss,^{281,282} and others, pointing out the difference between cell division and growth is, I think, of some interest. The two processes are not necessarily simply related to each other, so we tried to devise an apparatus (and I will spare you all the troubles involved) in order to study cell division. As Doctor Jones has said, one should obviously do any work like this with varying temperature, light and nutrients. Not being able to make a four-dimensional apparatus, we have, to begin with, changed temperature and light simultaneously, and kept a nutrient environment which is probably not ideal but at least is fairly constant.

This has been accomplished by extending the type of experiment that Doctor Halldal did using an agar plate,²⁵ getting a temperature gradient over an aluminum block, but making holes in the block and putting in 80 small test tubes so that we could use liquid media. This then raises the problem of trying to get fairly parallel light, and if supraoptimal light is desired, one has to try to get parallel light of the intensity of sunlight filtered through a few meters of water. The technique is to start off with the block full of tubes with culture, decide on a light and dark cycle—we chose 16 hours on and eight hours off because it was convenient—and every day count the number of cells in the 80 tubes as they grow in the cross gradient of light and temperature.

Considerable criticism can be leveled at this technique; I can hear Doctor Steemann Nielsen already mentioning the fact that we probably didn't shake the tubes, and we undoubtedly created nutrient deficiencies. But, remarkable as it may seem, one still gets a fairly consistent, reproducible, and reasonable looking picture of growth. I would just like to mention a few observations which I think Doctor Jones is probably going to enlarge on later. One is that cell division may need more light than compensation photosynthesis.

If I may make a few sweeping generalizations, the picture is one of "shock" adaptation. In other words, algae do not like being moved from one condition to another, but if they are, and if they are given long enough to adapt, the general tendency is to come back to a fairly constant division rate. This is presumably a function of the species and, undoubtedly, a function of the medium.

Also, adverse growth conditions have an additive stress effect. If the cells are exposed to too high a temperature, they don't like too much light at the same time, but if the light intensity is lowered, the temperature can be tolerated, and so on. The other thing one sees is a low temperature adaptation. In other words, with the initial culture

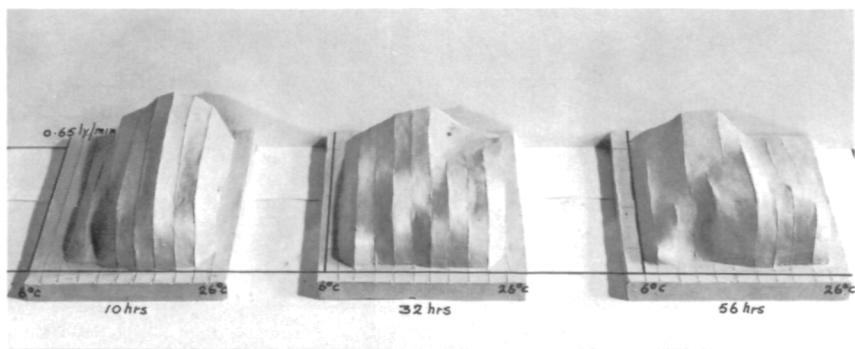


FIG. 57. Three dimensional models of *Monochrysis lutheri*. Cell division rate (vertical) as function of temperature and light at 10, 32 and 56 hours growth.

grown at 18°C and then put into the tubes, a low temperature adaptation of 4 or 5°C can be observed during two or three days of growth.

There does not seem to be much ability for high temperature adaptation. The low temperature effects seem to be fairly reversible, but eventually temperature will be reached below which cells will not divide. I still think it interesting that even after a week *Skeletonema* would not divide much below 6–7°C despite the fact that good growth is found in nature at 2–3°C. I think this is the same observation Doctor Braarud made with *Thalassiosira nordenskiöldii*,⁴ that the cells in culture behaved differently from the way one would expect from observations at sea. This may simply be a very long-term adaptation.

FIGURE 57 shows a three-dimensional model. The vertical scale is the division constant of *Monochrysis lutheri*. This organism normally has to take a pretty heavy beating of sunlight, and, I imagine, temperature also, doesn't it, Doctor Droop?

DROOP: Temperature, salinity, pH, sunlight.

STRICKLAND: These models are for each of the first three days of growth of *Monochrysis*. We have done experiments with *Amphidinium carteri*, which has a very narrow temperature range. This may be characteristic, perhaps of dinoflagellates.

We have studied *Dunaliella tertiolecta*, which has a very large temperature range. It grows at up to 37 or 38°C. From John Ryther's curves²⁸³ we expected to see a very low-intensity light inhibition, but we found no light inhibition at all! In other words, the rate curves found with C¹⁴ are not necessarily related to the long-term cell division picture. The temperatures in FIGURE 57 are from 6 to 26°C, plotted against zero to 0.65 cal (cm² min) of light, which has the spectral distribution we discussed earlier, that of sunlight through a few meters of

fairly clear coastal seawater. You will notice there is a marked adaptation to the lower temperatures, and a tendency for slowed growth at high light and low temperature. But where something like an optimum temperature is maintained, there is quite a wide light tolerance, and, at about the right light, there is really a very broad temperature tolerance, something like 12 to 14C, over which the rate doesn't vary by more than about 25 per cent from the mean. In other words, the Q_{10} is very small and there is a tendency for the cells to divide at about the same rate over a very wide range of temperature. Despite the defects of the technique, I think this type of experiment is of some importance and it enables one to choose interesting growth conditions which one can then study with other experiments with large culture volumes.

The other very interesting thing about this "stress" picture is that if the culture is poisoned very slightly, chemically—which we did by shaking the culture during incubation—the culture doesn't grow at all except right at the optimum of temperature and light. I imagine this is the kind of thing one would see with a whole combination of stresses.

HUTCHINSON: I am not absolutely certain how much detail one may legitimately interpret, but does it show the sort of effect that Wilhelm Rodhe called "the limiting optimum," namely, that at high temperatures or high light, or both, the culture went racing ahead at the beginning and then found that it couldn't keep it up?

STRICKLAND: Yes, and I am sure that in the succession of species, certainly of some of the spring diatom blooms, the effect of the correct sequence of temperature and light is worth considering. For example, the temperature may have to reach some critical value, which varies with each species, and if, for some reason, the temperature changes before much growth commences the algae are "shocked" and one species may suffer a set-back in preference to another species which has reached well into its growing range. There may be a reasonably simple explanation of the succession of some species without having to invoke a critical nutrient feature. The fastest division of *Monochrysis* that we got was about 17 hours. Does that sound ridiculous?

DROOP: It is a bit faster than I could get.

STRICKLAND: We have had *Skeletonema* growing for seven days and it shows very marked temperature adaptation. *Amphidinium* does a remarkable thing. It has a fairly restricted growth range and, then, after about four or five days, all around the growth area, where no division had occurred, a terrific out-burst occurred with a very rapid doubling time. In other words, it looks as if the cells had been saving up their division or were "hatching" or something.

HUTCHINSON: Would there actually be a depression where the hump had been, with a rim around it?

STRICKLAND: The hump in the three-dimensional model stayed the same, but just around the edges, after four days of no growth, the cells suddenly divided rapidly.

HUTCHINSON: In a model, would it be represented as a crater rim?

STRICKLAND: Yes, it would make a crater.

GUILLARD: To state it another way, if one variable (e.g. temperature) is held fixed, and the other varied, the growth curve will have a maximum, then a depression, then another maximum value.

DROOP: On the shoulder of the optimum.

GUILLARD: Yes. Schmitz²⁸⁴ studied the growth rate of *Thalassiosira fluviatilis* as a function of salinity in artificial river water, and found that if he changed the ratio of sulfate to chloride in the medium, the optimum salinity for growth changed; in fact, there were two optima, with a depression between them. The idea that there can be two optima for different values of the same variable is quite interesting.

DROOP: This is a question of time. In the early stage, cells grow fastest at the optimum and more slowly away from it, but those at the optimum often come to a halt and die earlier, while the suboptimal ones continue to grow and eventually reach the greatest density.

STRICKLAND: Yes, there is one very sudden division as if it were almost a cyst from bursting out. I think dinoflagellates can be terribly complicated, but I thought I would do one just for fun.

DROOP: This sort of interaction occurs—not growth rate measurements now. If the concentration of the major ions, sodium, calcium, magnesium, is varied simultaneously, the tolerance shown to any one of the ions is greatest when the others are at their optima, so a cruciform shape to the response surface is obtained.²⁸⁵

JONES. As I mentioned previously, by using continuous culture techniques with concentrations of organisms that are, shall we say, approaching those of the ocean, one can get the same type of interrelationships.

CONTINUOUS CULTURE VERSUS NATURAL POPULATIONS

Discussion leader:

L. PROVASOLI

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JONES: Doctor Braarud has brought out many times the difficulty of trying to relate batch culture data to what is going on in the ocean. There has always been this big gap, and I think it can be bridged by constructing similar types of environments to those found out in the sea. I think this can be done by the continuous culture method; but, unfortunately, in our experience, not every organism will grow in the continuous culture apparatus. There are one or two that, for their own physical reasons, just won't play ball.

Continuous culture techniques offer a number of advantages over batch culture methods, particularly because (a) concentration-dependent factors are controlled, (b) growth rates can be measured within short periods of time, (c) the measurement operation does not interrupt the culture regime, and (d) studies can be carried out at low cell concentrations. Of these, the last is of particular importance in the study of the ecology of phytoplankton organisms. I shall describe a continuous culture apparatus which has been employed in my laboratory for a study of some ecological parameters of *Nitzschia closterium* and *Carteria* sp.⁵⁴

The growth chamber (FIGURE 58) is a three-necked pyrex flask provided with an overflow tube in one neck which permits the escape of a compensating volume of culture when diluting medium is added, so that the working volume of culture remains constant. The one liter size used represents a compromise between, on the one hand, the desire to keep the surface to volume ratio small, and to provide a long light path for the turbidity-monitoring light. On the other hand, it avoids problems of keeping homogenous conditions within a larger volume and provides the very considerable quantities of sterile artificial medium which are required in proportion to the working volume used. A pyrex-enclosed impeller, driven by an external magnet, provides mixing in addition to the agitation produced by aeration through a fritted glass tube. The same fritted glass tube used for aeration also serves for addition of diluting medium, with the air

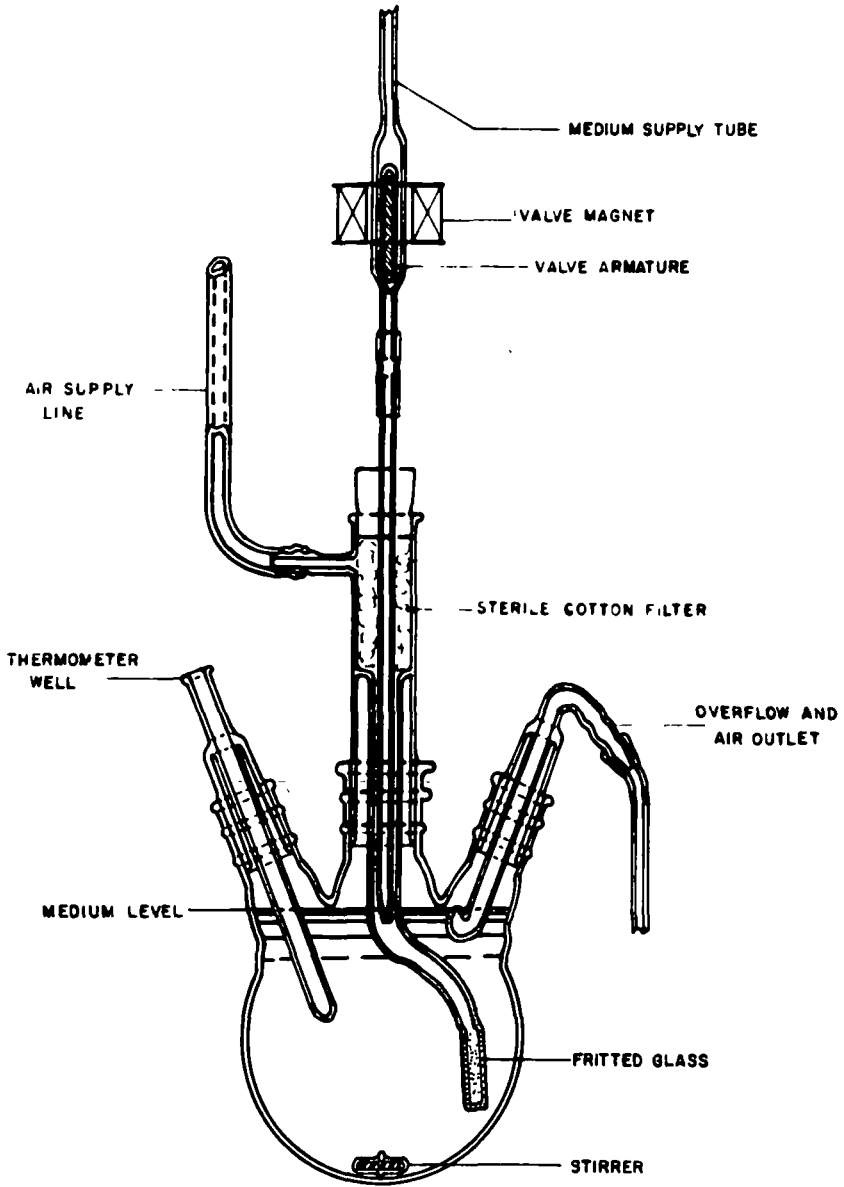


FIG. 58. Section through culture vessel and solenoid valve.

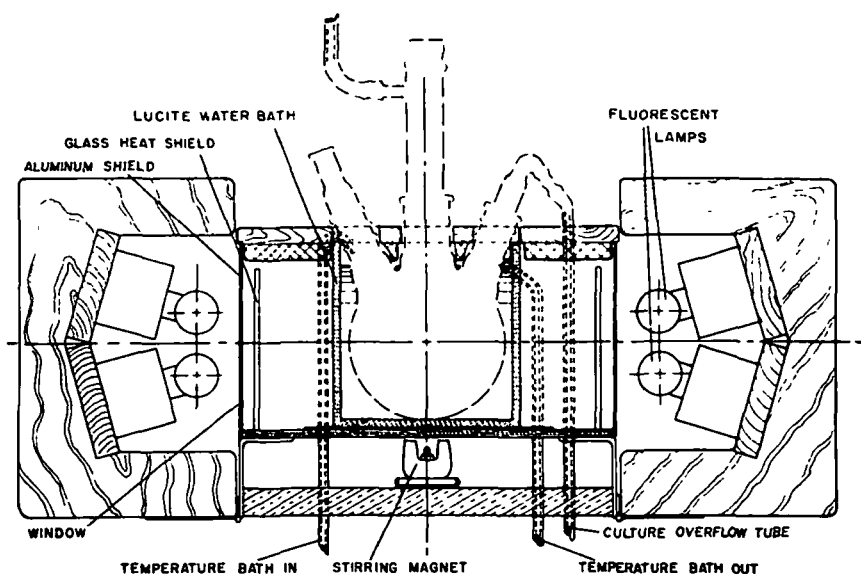


FIG 59. Cross section of culture apparatus showing arrangements of lights and water bath.

flow helping to assure uniform distribution of the freshly delivered medium through the culture, and also preventing any retention of stagnant medium in the delivery tube. A solenoid actuated pyrex valve controls the flow of medium into the flask. The third neck of the flask holds a thermometer well, and also may be used as an addition port for making inoculations.

The culture flask is immersed in a circulated temperature-controlled water bath constructed of lucite (FIGURE 59).

Light for photosynthesis is provided by four "cool-white" fluorescent tubes placed in pairs on either side of the lucite bath. The plane sides of the cubical path provide a good optimal match to the spherical growth flask both for the photosynthetic illumination and for the collimated monitoring light beam. Neutral attenuation of the light from the fluorescent tubes may be obtained by inserting various numbers of layers of metal-mesh window screening between the light tubes and the water bath.

FIGURE 60 illustrates the complete system. The medium supply is held in a constant head reservoir (an 8-liter pyrex bottle) above the culture flask, to which it is connected by a siphon to the solenoid valve. The only materials in contact with the culture or medium are pyrex glass and a minimum amount of exposed silicone rubber tubing used for connections.

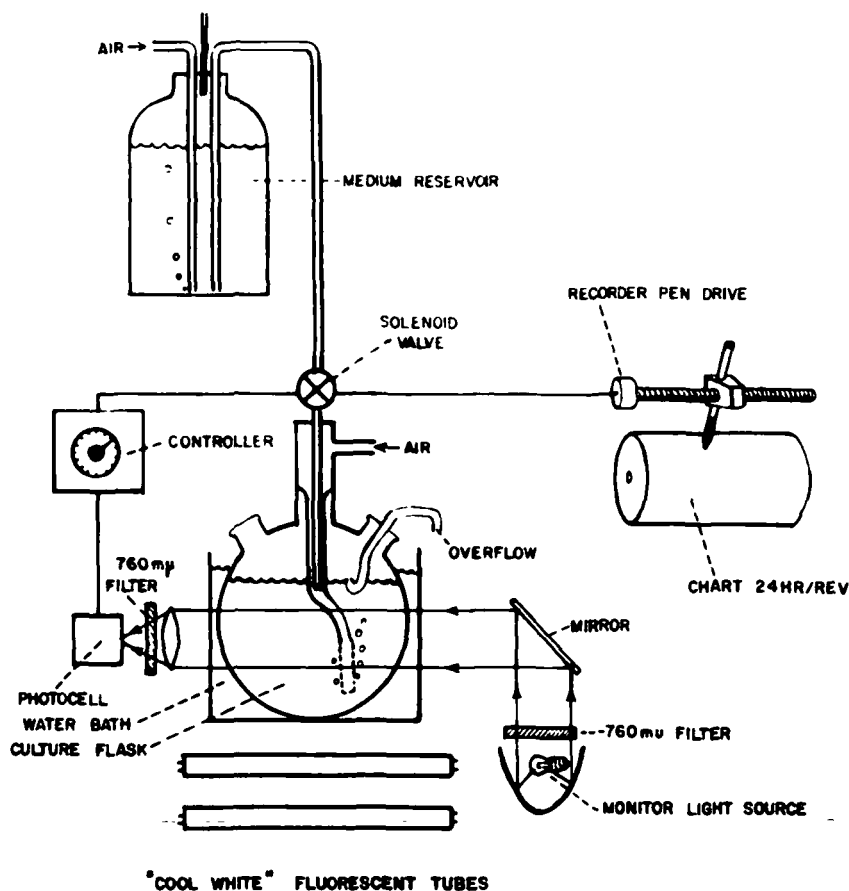


FIG 60. Scheme of continuous culture apparatus (Very diagrammatic)

The monitoring-light source is a 6.3 volt tungsten filament lamp, operated at 4.5 volts A.C. which greatly reduces aging of the lamp without seriously lessening emission in the red portion of the spectrum. The bulb is mounted at the focus of a parabolic flashlight reflector, and the resultant parallel beam passes through a near infra-red Kodak-Wratten # 88A filter before being reflected through the culture flask. A 10 cm focal length lens converges the light onto the active surface of a cadmium sulfide photoconductive cell after passage through the culture and water bath, with another infra-red filter interposed just after the lens. A second cadmium sulfide cell is placed facing the light source and first filter, to serve as a reference in order to reduce the effect of possible fluctuations in the intensity of the light

source. The use of the red filters which do not transmit light below $720\text{ m}\mu$, but quite well at $760\text{ m}\mu$ precludes any photosynthetic effect by the monitoring lights, eliminates errors due to pigmentation changes, and has the added feature of further reducing the small amount of light from the fluorescent tubes which might be scattered or reflected into the narrow acceptance angle of the monitoring optics system, since the "cool-white" tubes radiate very little energy above $700\text{ m}\mu$ which could be detected by the CdS cell. Because of this latter feature, the switching on and off of the fluorescent lamps does not affect the control system, even when very dense and highly scattering suspensions are in the culture flask. The overflow passes by sterilized tubing into a sterilized cotton-plugged measuring vessel. FIGURE 61

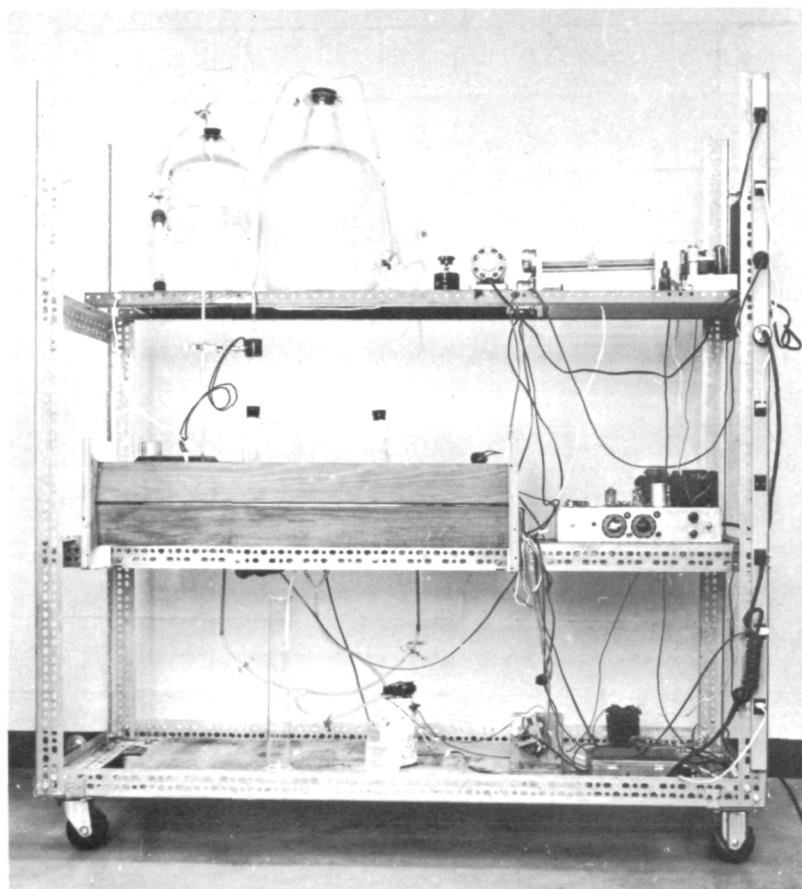


FIG. 61. Photograph of the equipment. The medium reservoir and solenoid valve are removed from the system on the right.

gives some idea of the actual instrument. As you can see, there are two culture vessels in the one unit.

Using this system, we have been able to grow populations of cells with very good accuracy down to 28 cells/mm³. If we try to go below that we get erratic recordings.

STRICKLAND: That is higher than marine levels, isn't it?

JONES: Slightly higher, yes.

MYERS: Doctor Jones, because of the general interest in this problem, may I raise a question concerning the sampling and the removal of cells: Are they taken from the surface of the culture?

JONES: Yes. The overflow comes from the surface of the culture.

MYERS: Our bitter experience with much denser cultures than yours is that when we do harvest from the surface we do not get a representative sample of the bulk culture. Do you have any data on this?

JONES: To overcome this, we tried to maintain a suitable maximum turbulence in the culture flask.

MYERS: It isn't maximum mixing; it is the fact that cells in the surface film are not at the same concentration as they are in the bulk of the liquid.

JONES: We have not found anything like that. We were worried about this at first and by experimentation we found no variation within the normal statistical variation.

FOGG: From our experience, it is a good idea to take the samples from the surface. If one continues to take the samples from the bulk, surface-active materials accumulate, the cultures froth badly, and one has to discontinue the experiment.

MYERS: I agree. There are two parts to the problem. One is that of removing a representative sample of solution, including surface-active materials. The other half is the problem of removing a representative sample of cells. I was concerned with this second part of the problem. If the cell concentration in the removed sample and in the bulk of the culture are not identical, then the data are invalid.

JONES: If we try to increase the population to something like 10⁶ cells/mm³ we find that frothing sometimes occurs and upsets the system. Originally, we used white light to monitor the cell concentration, but found that during the dark period of the light cycle, that is, when the fluorescent tubes were out, there was appreciable pigment built up in the cells which was picked up by the light beam. For this reason we then used light of 740 mμ to monitor the culture.

STRICKLAND: Do you use a photomultiplier that is sensitive to the red?

JONES: Yes.

KRAUSS: Is the whole apparatus sterilized?

JONES: Yes, every part of the culture apparatus can be sterilized and maintained under axenic conditions.

DROOP: Would you clarify the difference between this apparatus and the one that Doctor Fogg uses. They are essentially the same, are they not?

FOGG: Mine is essentially the same as Doctor Myers'.

JONES: Both Doctor Fogg's and Myers' apparatus are chemostat. We keep the population of cells constant at all times, in other words, we use a turbidostat.

SPENCER: May I try to clarify this? The turbidostat, as I see it, is essentially a means for controlling the population by an external device, which can be quite independent of the actual nutrient regime in the reaction vessel. The population is simply being controlled, I think, at a predesired level by turbidity monitoring. The chemostat principle works rather differently, in that a chosen nutrient is allowed to decrease to the point where it becomes limiting and, by holding it constant at this concentration, it is allowed to control the growth rate. One would expect the relationship between the growth rate of an organism and the concentration of any nutrient to be in the form of a rectangular hyperbola (or something similar) within the range of concentrations when it is growth-rate limiting. It is within this range of nutrient concentration and growth rates that a chemostatted culture operates. The growth rates used are always less than the maximum exponential rate appropriate to the physical conditions in the presence of excess nutrients.

DROOP: What I don't quite understand: Is equilibrium maintained in the turbidostat?

SPENCER: No, at least not in the sense that there is, of necessity, any relationship between the growth rate of the cells and the concentration of any nutrient in the medium in the reaction vessel.

STRICKLAND: Can the concentration of nutrient in a turbidostat be changed?

JONES: Oh, yes, at will.

STRICKLAND: However, it is a constant value for a long period?

JONES: Yes. The concentration of any one nutrient, or even combination of nutrients, in the reservoir can be changed and the change in growth rate of the cells in the culture flask can be measured as the new medium flows through. Thus, in the absence of one nutrient, one would expect that it would take longer for the one-liter culture to double.

KRAUSS: There is another order of complexity that can be introduced into a similar system. In both the chemostat and the other con-

tinuous culture devices in use, one is essentially washing fresh medium through the culture. Some of the culture is being taken out, as well as some fresh medium.

For certain purposes, it is interesting to see what happens when this isn't done—when the same water mass that one starts with is reused. So, we have built a device, called a "recyclostat," which has some features in common with the device shown by Doctor Jones in FIGURE 46. To it is attached an automatic cell-harvesting system that removes the cells and recycles the water that has come out of the device, now free of cells, back into the culture. If one knows what amounts of inorganic nutrients the organisms are taking out of the system, very small amounts of them in fairly concentrated solutions can be introduced at the same rate at which they are being removed. Consequently, the cultures can be maintained at a steady state in the same water in which they were grown.

We are using the apparatus for other purposes than to study marine ecology, but it might just conceivably be of some use to the marine scientist. It is another type of continuous culture apparatus.

GOLDMAN: Is there any evidence of metabolite production flowing from the system?

KRAUSS: We really have not explored this in as much detail as I would like, but I would say tentatively that it does not.

PROVASOLI: You were working with *Chlorella*?

KRAUSS: Yes.

PROVASOLI: Which is supposed to poison itself in some cases.

KRAUSS: There are certain strains of *Chlorella* that do. *Chlorella vulgaris*, especially the strain that Pratt studied,²⁸⁶ secretes a very strong auto-inhibitor, chlorellin. Correct me if I am wrong, Doctor Myers, but I believe this is the only species of *Chlorella* that has been shown to have a strong auto-inhibitor. The others do not.

GOLDMAN: Maybe harvesting would prevent that, since I think the chlorellin comes out as the culture gets old and senescent, doesn't it?

FOGG: What does the medium look like after *Chlorella* has grown in it for several weeks?

KRAUSS: It is fairly clear. If one does not clarify the medium, it tends to get a bit cloudy. The reason for this is that debris from cell walls and various other things are present. In trying to operate a completely cycled system sterile, one has the problem of sterilizing the water before returning it to the culture. The cells cannot be completely removed, at least with the centrifuging apparatus we have available, without introducing contamination. Therefore, after passing out of the centrifuge, the water is run through a Millipore filter which takes out any of the debris as well as chance contaminants.

So far as we have been able to ascertain, the water shows none of the brownish color that has been reported. The device is in its test runs at present, perhaps we can give you more information later.

SPENCER: Can inorganic nutrients be replenished automatically?

KRAUSS: Inorganic nutrients are replaced, but not, at present, automatically. It could very readily be made automatic.

RILEY: I simply wanted to draw certain ecological analogies that are involved in these two different kinds of instruments, each of which can serve an ecological purpose. In the ocean, during the autumn and early winter, we generally find that growth is limited by light penetration and by loss of plankton from the surface layer by vertical turbulence, whereas, in the spring and summer the chief limitation is nutrient deficiency. Thus, the turbidostat is attempting to simulate an autumn and winter ocean, although rather imperfectly, because the ocean is in what we would call a state of semi-washout due to deficient light at this time. The chemostat, on the other hand, simulates the spring and summer conditions and, for a very limited time at the beginning of the spring flowering, we have a batch culture.

JONES: The organisms we have tested in the turbidostat are the *Carteria* species that we got from Doctor Guillard: *Nitzschia closterium*, *Phormidium persicinum*, *Platymonas subcordiformis*, *Plectonema roseolum*, *Porphyridium cruentum* and *Prymnesium parvum*. Of these, the *Platymonas* was absolutely unsuited because, for some unknown reason, it tended to stick to the glass walls of the vessel, in spite of high turbulence and anything we tried to do. The *Phormidium* aggregated into small clumps and, therefore, proved to be unsatisfactory. All the other organisms grew very well, even the *Plectonema roseolum* was quite suitable since, although it broke up into small filaments, they remained uniformly distributed in the culture and could, therefore, be monitored.

STRICKLAND: Did you vary the temperature in the apparatus?

JONES: We could vary temperature, pH, any level of nutrient or salt, light intensity, and type of light cycle. In comparing continuous light to light and dark cycles, we noted for the *Nitzschia*, *Carteria*, and *Porphyridium*, that (a) these three organisms complete cell division during the first two or three hours of the light period, and (b) in the light and dark cycle, the rate of growth was never comparable to that obtained in continuous light.

HUTCHINSON: When the turbidostat is running completely smoothly, is there any tendency for an oscillation to develop?

JONES: We have noticed a slight tendency for an oscillation to develop, but we do not have enough information to relate this to a biological rhythm. I am not prepared to make any statement on this except to say that if *Porphyridium* is grown in continuous light and

then subject to a light cycle, its growth rate changes, becoming synchronized to the light cycle. However, on subjecting the organism to the original continuous light after a period of a few days, the periodicity of growth noticed in the light cycle is lost.

In continuous light, we have noticed a slight periodicity in the growth curves, but we do not know if they are at all significant.

PROVASOLI: Do you have other results on the interaction between ecological factors?

JONES: The ones that we have studied are primarily the ecologically significant ones, namely, light, temperature, and nutrient levels. We have some other data which I do not think at the moment are in a state for complete interpretation.

LUND: In the common freshwater species of *Ceratium*, the division of the nucleus takes place pretty largely in the dark, but actual cell cleavage takes place at dawn and afterwards, so it is rather important to know what you mean by cell division.

JONES: By division, I mean complete cell division. That is, when the original cell has completely divided to form two or more individual cells.

LUND: So, nuclear division may have started in the dark.

JONES: Yes. In fact, we have some evidence of this in *Porphyridium*. In the dark, what appears to happen is that the nucleus and the chloroplast divide first and then—

FREMONT-SMITH: Which comes first?

JONES: I cannot say. Observations are based upon light and electron microscope work. It is a case of looking at enough sections to make up one's mind. It appears, however, that the chloroplast and the nucleus divide in the dark period. At about the time of the light signal, the cell inside the polysaccharide wall divides into two, and then, during the first few hours of the light period, the polysaccharide wall of the organism cleaves and two individual cells are released. So, here we have a number of stages of division following one another.

These are the sort of things we are interested in now from a biochemical point of view. It is a big problem and things don't all happen at once. There is a definite time sequence. We do not have such information on the *Nitzschia* or the *Carteria*.

STEEMANN NIELSEN: You mentioned *Prymnesium parvum* as one of the organisms you cultivated. Did you investigate the production of poison by this organism?

JONES: We obtained this because of its ecological and economic significance in the ponds of Israel. We were interested in looking at the toxic materials, and in seeing what would happen when the organism was cultured with other cells.

STEEMANN NIELSEN: We had it in culture during the war, when there was a very severe growth of the algae in Denmark, but the culture we had didn't produce any poison. So, I am very curious to know if you were able to make it do so.

JONES: We have not done too much work with the *Prymnesium*, at present. So far, we have only tested its ability to grow in our culture apparatus.

PROVASOLI: Doctor Spencer, would you like to comment on your work with the chemostat?

SPENCER: I don't think so. I have been mainly concerned with using it to try to establish constant but very low concentrations of things like phosphate. Phosphate is the only ion I have used in this way to try to study the relationships between concentration and growth rate, and I reported those results earlier in the Conference.

MYERS: Could I ask Doctor Spencer if it is not possible, using his chemostat, to answer the question about the succession of organisms, of one growing better at low concentrations of phosphate? Would it not be possible to use both organisms, to set the thing at various levels of phosphate, and to examine the relation of cell concentration under these conditions?

SPENCER: Yes, I think in theory that would be possible. It is something I have had in the back of my mind. At the moment, the problem is one of culture technique. I know of no other marine diatom we can culture sufficiently well to allow really precise kinetic studies.

DROOP: Has anyone tried to grow two organisms together in a single chemostat? I believe it has been done with bacteria, but has it with algae?

JONES: We tried at one occasion to see if it would work. We used *Nitzschia* and *Carteria* species because of their different temperature tolerances. The experiment was started with a mixed population and we hoped that by varying the temperature we could eliminate one of the two species. Unfortunately, because of mechanical and electronic failures, we were unable to complete the investigation. However, in the little that was done, the *Nitzschia* outgrew the *Carteria*.

STRICKLAND: Could I have one point clarified? If one wants to calculate what the doubling time or the photosynthesis is at a small constant concentration of phosphorus, $0.3 \mu\text{g-at/liter}$, and what it is at $0.15 \mu\text{g-at/liter}$, should a turbidostat or a chemostat be used, or both?

RILEY: The turbidostat.

STRICKLAND: Will the chemostat only react with the desperately low values that probably can't be ascertained analytically?

RILEY: The concentration of nutrients in the chemostat is usually very low unless enough nutrient is added, so that something else

begins to limit growth, in which case it isn't a true chemostat. The results aren't very good.

If a series of levels of nutrients are to be tried, the only way to do it, I think, is by a turbidostat, and even then it will be necessary to try several different concentrations until an equilibrium condition at the desired nutrient concentration is reached.

STRICKLAND. The dual culture experiment that Doctor Hutchinson mentioned must really be done in a turbidostat?

RILEY: Yes, I think so.

STRICKLAND: What information does the chemostat give?

RILEY. The chemostat tells us approximately what we want to know about equilibrium conditions in the sea, where some one nutrient is low and extremely limiting. It will disclose, for example, what the chemical state of organisms will be under these conditions better than any other source.

STRICKLAND: So, it is a good harvesting machine.

DROOP. The difficulty is that it is working near what is called the critical washout point. At very fast flows, there are more residual nutrients, as the washout point is approached.

RILEY: That is true.

DROOP: It is very difficult to run near the critical point.

RILEY. May I answer your question in a different way, Doctor Strickland, because I am not sure I quite understand the conclusion we reached. I view these as mirror-image methods. If the chemostat principle of a constant dilution rate is used, the growth rate is set—it is preset—at any value less than the maximum growth rate of the organism. One then observes the concentration of cells which will be supported, and that concentration must be related to some concentration in the inflow of nutrients.

In the turbidostat method, the concentration of cells is set at a prescribed value, and then one sees what the growth rate comes out, so these are mirror images. I think either one will apply to the question, would it not?

STRICKLAND. In practice, isn't one more convenient than the other?

RILEY. I believe the turbidostat is the only reasonable way to work with a fairly high concentration of nutrients in the culture flask. On the other hand, in order to ascertain the nutrient uptake under conditions of relatively low nutrient concentration, the chemostat is a more useful tool.

BRAARUD: May I ask whether anyone has tried the ordinary plankton species in these apparati, for instance, to see how the coastal species of *Thalassiosira*, behave—if they can be used for experiments in these flowing systems?

JONES: The only species we have used is the *Carteria* sp. which Doctor Guillard isolated from the Sargasso Sea samples. Whether this is a true phytoplankton or not I am not prepared to say.

PROVASOLI: Doctor Riley has been working with *Skeletonema costatum*.

RILEY: Or, rather, Mrs. Wood has been working with *Skeletonema* and the chemostat, and there were some difficulties involved. She found considerable difficulty in keeping the organism in a constant state of growth over the long period that is required for this kind of work. There was some occasional tendency for the cells to settle around the outlet tube and, when that happens, there is nothing to be done except start over again.

I might say in this connection, in more general terms, that it is a very hard way to get experimental results. It is a long, slow job, using a chemostat, and I don't believe we will make any great progress until we have done a lot more instrumentation to develop fairly simple means of running a battery of these things at one time.

JONES: I think the problem may simply be that these two instruments were designed primarily to obtain populations of cells that were of the same physiological age or physiological state for certain biochemical investigations, and not for studying phytoplankton ecology. The time is now ripe, perhaps, for us to adapt this equipment to suit the needs of the phytoplankton ecologist.

BRAARUD: I am fully aware of the great importance of this physiological work, but I wonder how the coastal plankton species would behave, as they are difficult to grow in small flasks.

STEEMANN NIELSEN: May I call attention to a much simpler and much cheaper way of obtaining results similar to a chemostat. When using very dilute cultures, it is not necessary to have a constant flowing system. If the growth rate is, say, two divisions per day, then we are able to remove a part of the culture every day and make a new one. If there are only very few cells, then neither the concentration of ions nor the light conditions will change. For many experiments, this is quite enough and it is much, much less expensive than using a complicated method.

JONES: I agree with you. We have even tried experiments in small culture flasks with small populations of cells with the same light intensity, temperature, and nutrient levels. We find that we can duplicate, in short periods of time, the growth rate that we get with the machine; so it is only over an extended period of time that the machine may be more valuable.

STEEMANN NIELSEN: If, every morning, one just counts and then dilutes according to the counts, exactly the same results will be obtained. For culture work we use ordinary 100 ml. pipettes, in which

the outlet is bent to bring it into the same position as the inlet. It is a very cheap and easy way of doing culture work.²⁸⁷

PROVASOLI: I think Doctor Jones mentioned that there are difficulties in applying the present construction of either the chemostat or the turbidostat to organisms that have a low division rate. He said that more engineering is needed. What are the stumbling blocks?

JONES: I don't think there is any technical problem with regard to slow-dividing cells. I think the problem that arises is simply that certain organisms just will not grow in this type of system because of the way it is physically set up. They may do exactly the same sort of thing in a test tube.

PROVASOLI: Therefore, in this system the solenoid valve takes care of the small input of nutrient needed by slow-growing organisms, but the apparatus is not suitable for filamentous or clumping organisms.

JONES: I think it is more adaptable to unicellular organisms than to any others.

PROVASOLI: Doctor Riley, what are the main difficulties in your system?

RILEY: We used the standard Novick apparatus with some slight modification, the main one being to increase the size of the culture flask. I would say there were no great difficulties except that the solenoid valve and all of the works were rather badly engineered in the Novick machine and have had to be replaced or modified somewhat.

I think I used the term "engineering." I think you were quoting me, Doctor Provasoli, and all I meant, really, was that I think this apparatus should be modified for ecological purposes so that it will permit, for instance, the simultaneous culture of a number of flasks at once, because we simply can't get enough experiments done under the present circumstances to make it very worth while.

Mrs. Wood, who did the work on *Skeletonema*, the 12 requirements, worked for about two years to obtain what I would regard as very minimal results for the purpose. With a device that was better engineered, I think she could have done the same work in three or four months.

PROVASOLI: Is anyone planning to construct a cheaper and fool-proof apparatus? Do you plan to do it, Doctor Riley?

RILEY: We have thought about it, and have made some preliminary plans, but we have too many other things in mind and too few hands to do it immediately.

JONES: Since you mentioned cost I thought you may be interested to know that the instrument which I have described was built by a graduate student of mine by the name of W. S. Maddux, as part of his thesis work, for a cost of less than \$750. This of course does not take into consideration the price of actual "man hours."

DROOP: That is about what I paid for my chemostat, which is a 30-liter affair, for quite a different purpose, not experimental, It was homemade. It works out at just about £50 a unit, all told.

SPENCER: I agree with Doctor Riley, in general. The main technical problem I find is the maintenance of constant very low flow rates that are usually necessary for algal cultures. It is possible, of course, to increase the flow rate volume by increasing the volume of the reaction vessel, but this is often not desirable and involves problems of providing adequate illumination and the inconvenience of continually providing large amounts of medium. The solution, therefore, seems to be not very satisfactory unless there is a demand for the production of large amounts of cells. All my experiments have been made with culture volumes of 100 to 200 ml and with flow rates of about 12.5 ml/hour, and lower. I very soon found that I had to abandon any system that depended upon gravity flow and which was restricted and controlled by valves or capillary networks. Like Doctor Riley, I too have used the Novick hydraulic system and one instrument I built worked on this principle quite satisfactorily for some time. The system has one weakness in that it is not easy to change the amounts of medium dispensed at each delivery; something which is often desirable, as well as the facility for changing the frequency of delivery. My most recent apparatus depends upon a forced flow from a metering diaphragm pump with all plastic contacts, the delivery per impulse being infinitely variable from the maximum to zero.

STRICKLAND: I think most of you are aware of the Technicon Company in New York that has produced equipment capable of producing small monitorings for a very considerable period of time. They now seem prepared to sell the pumping unit separately, which they were reluctant to do previously, and I should imagine that this must be about the most highly tested device for delivering varying constant flow rates that is available on the market today.

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SUMMATION

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The great diversity of subject matter covered during this symposium precludes the possibility of a complete summary. The following account, although attempting to integrate the most important discussions of the group, undoubtedly reflects to some extent the special interests of the writer.

Populations of marine microorganisms pose many interesting questions, which are often extremely difficult to solve. Among these are two problems of measuring standing crop in the sea. The first is simply the technical problem of how to preserve algae, especially the delicate naked flagellates whose quantitative role in the productivity of the sea deserves special attention. The second is the more basic problem of meaningful quantitative measures. So much has already been written on the patch-manner in which plankton is distributed and the questionable reliability of most gear employed in sampling that I shall not attempt to review the problem here except to note the obvious advantages of the continual taking of plankton samples over the area of interest.

The problem of plankton preservation seems nearer solution, however, and lends itself more easily to controlled experimentation. One method which we have been trying in our laboratory is the exposure of fresh plankton to a Gamma (Cobalt⁶⁰) source for fixation and preservation. If this method should prove good enough, it might be practicable to have an adequately shielded cobalt source on shipboard, or in tow at an appropriate distance behind the vessel.

The value and long term economy of publishing rather extensive tables of plankton studies has been stressed by Doctor Braarud, who has noted that, although the text is soon out of date, the original data can be extremely valuable. A new trend for a more liberal editorial policy in this respect should certainly be encouraged. With much of biology entering a "computer phase" the exchange of data already punched out on IBM cards has practical merit. There has been a great deal of progress in machine counting in recent years and there is the possibility that some plankton might actually be identified on the

basis of mass, shape, or color through sufficiently sophisticated electronics. The autofluorescence of chlorophyll has been used to advantage in enumeration of algae, as has dye as an aid in distinguishing the living organisms from the dead. Where the dynamics of populations are under study, distinguishing the living material from the dead is of great importance.

The nutrient requirements of algae in nature and in the laboratory provide a field for investigation both fascinating and complex. The development of culture media since early days has often progressed more as art than science, with the more imaginative cooks developing the better broths. Addition of soil extract has frequently been necessary to supply some unknown growth factor, or perhaps suppress the toxic effect of some component of the culture medium. In recent years, with the availability of pure cultures and a better analytical understanding of the actual nutrient requirements of a variety of different algae, media are being defined with greater precision.

The extreme sensitivity of organisms to rather slight changes in nutrient levels points up the importance of avoiding physiological shock in experimentation. Both macro- and micronutrients may at times be quite inhibiting to growth even at rather low levels. Phosphorus, although essential, reportedly suppresses the development of the Chrysomonad *Dinobryon* when environmental levels exceed 5 mg/m^3 of phosphate phosphorus. Further, there is often a tendency to work on nutritional requirements in the laboratory at extremely high, and by this token, unnatural, concentrations of organisms which may compete for the available light and carbon dioxide within the culture container. Use of high cell concentrations was more necessary when growth measurements depended upon cell counts than it is now when growth rates can be measured in dilute suspensions by more sensitive methods such as Carbon¹⁴.

The role of precipitates in nutrient media has not been well understood. It has been observed that most of the phosphorus added to a culture can be recovered by Millipore[®] filtration but the 3 microgram atoms/liter that pass through the filter are sufficient for algal growth, and even levels of 0.1 microgram atoms/liter appear to be adequate in the North Sea. On the other hand, evidence for utilization of phosphorus from the precipitates at a greater rate than from the medium was found by Doctor Eppley, using P^{32} and *Porphyra* cells. It appears likely that a dynamic equilibrium may exist, with the precipitate providing a reserve supply for the culture, or perhaps even forming a substrate for growth. There is good indication that organisms may benefit from particulate matter suspended in culture media. Iron and aluminum provide examples of this in cultures of Sargasso Sea phytoplankton, and in Lake Naknek, Alaska, the phytoplankton

appear to derive some advantage from a suspension of glacial rock flour and volcanic ash.

Although any culture container must necessarily involve surface, there is a need for more knowledge on how much surface area is provided in nature by suspended material, and how important this is to the lives of associated microorganisms.

The difficulty, if not impossibility, of relating the kinetics of algal growth to the phosphorus concentration of the external environment has often been stressed, especially in relation to nitrogen. Doctor Spencer reports that *Phaeodactylum* grows at steady state in the chemostat with analytically undetectable phosphorus. This growth was at about half the rate that would be expected from the other experimental conditions. Beyond the well documented consumption of phosphorus far beyond metabolic requirements and the efficiency of phytoplankton in concentrating it from extremely dilute solutions, we have a demonstrated ability of the organisms for rather efficient inter- or intracellular recycling of the element. There is, however, a natural tendency among biologists to oversimplify rather complex natural situations, where the biota and environment are in constant interaction. The chemostat provides a valuable opportunity for investigating the interaction of a more limited set of variables than we encounter in nature.

The value of trace elements in the metabolism of natural waters has received great attention in recent years. There are two general approaches to this problem, one of which is to study natural populations *in situ* or in the laboratory, the other by working on the micronutrient and organic growth requirements of individual species isolated in pure culture. The latter has progressed to such an extent that the nutrient requirements of at least the more common phytoplankton forms are fairly well known. The work with natural populations, aided by the high sensitivity of isotope techniques, provides considerable promise for better understanding the role of trace elements and vitamins in nature. The extreme sensitivity of the organisms to the slightest change in certain growth factors points up both their value in bioassay, and the necessity for extra caution in experimentation. In C^{14} uptake experiments with added iron, abiotic precipitation of the iron, which is probably ferric oxide, resulted in the coprecipitation of the label. Without adequate prefiltered controls the high activity might be misinterpreted as photosynthetic stimulation.

The probable importance of iron to the development and distribution of marine phytoplankton has long been recognized. This relationship has been hard to study in the laboratory because of the difficulty of maintaining sufficient levels of iron in cultures. The use of freshly prepared iron hydroxide has been necessitated because of its trans-

formation with time into a very much less soluble, crystalline form. The main problem is essentially an unsolved chemical one involving the complexity of the speciation of iron. Ion exchange resins have been used successfully in cultures by Doctor Johnson, and chelating agents are, of course, in general use for making a variety of metals more available. It is important that EDTA, although widely dispersed in the cell, does not appear to be metabolized by the algae. The relative strength of the solubilizing effect on the different metals is complicated by such factors as the pH, and the variability in composition of natural waters and culture media.

Concentration of silicon poses an exceedingly interesting problem in natural waters, with some diatoms able to adjust their cell wall thickness in accord with the amount available. Where weak beta emitters like C^{14} are used in tracer experiments involving diatoms, special care should be given to calibration of counting efficiency. Not only is the barium carbonate extrapolation to zero thickness subject to question, but also the tacit assumption that the self absorption and/or the geometry of algae can be neglected. Some labeled diatoms I have counted give an appreciably lower efficiency than flagellates, and there appears to be some change associated with the age and size of the cells. Counting efficiency should therefore be based on absolute activity determined by combusting some of the samples to carbon dioxide, subsequent counting in gas phase or by liquid scintillation, and comparison with the counts from the conventional solid sample counters. This procedure allows for realistic correction for self absorption of the sample and provides the best measure of the counting efficiency with the population under study.

Probably the best documented studies on diatom population fluctuations are those of Doctor Lund, which have been continuous for about 18 years in Lake Windemere. The limiting silica level for *Asterionella* growth in this lake on an annual basis is evident at about 0.5 mg/liter. This limiting effect of low silicon would appear to be modified by the availability of phosphorus and light, as the same diatom is able to continue growth in the laboratory, although at a slower rate, to the point of removing all analytically detectable silicon from the medium when supplied with a small additional amount of phosphorus. With high light intensity the "energy push" suggested by Doctor Lund is apparently more than the organisms can adjust to in the face of lowered nutrient levels, and a population decline results. Doctor Strickland has also noted that cultures are better able to take abnormally elevated temperatures if they are at the same time subject to lower light concentration, while a combination of high light and temperature is likely to be harmful. The interaction between the demand of the organism for nutrients under the influence of

prevailing conditions of light and temperature with the rate of supply from the environment is certainly of great importance in the rise and fall of plankton populations. It appears likely that the low light which prevails at high latitudes and altitudes during winter has a sparing effect on the plankton populations present.

The ability of algal cells to alter their composition during periods of nutrient depletion is evidenced by the large bag experiments of Doctor Strickland and his associates. Their studies in Pacific coastal waters indicate a decrease in the protein to carbohydrate ratio and a general decline in cell carbon, with the lipid fraction remaining about the same. Despite the decline in nutrients and reduction in parameters of actual growth, cell division continues. It has been pointed out in this conference that the general protein shift that accompanies nitrogen deficiency is almost certain to affect the enzymes themselves by virtue of their proteinaceous nature. A selective breakdown of these enzymes might well be the cause of the shift in cell constituents during nitrogen deficiency. Any real loss in protein constitutes a loss in what Doctor Myers has termed "the cells' working machinery" which is responsible for the primary productivity of the environment. The change in the quality of the phytoplankton crop, which may be expressed in terms of calories per unit volume, or the protein to carbohydrate ratio, is of importance to higher levels of the food chain, and may well impose limitations to growth of the herbivores.

It is important to express growth in terms of units of some particular mass, as cell division can occur without growth. There is evidence for continued cell division after transfer of *Chlorella* or *Dinobryon* to culture media without combined nitrogen. In *Dinobryon*, Doctor Fogg feels that further cell division is determined by the cells' capacity to synthesize DNA, and division can be continued at the expense of certain other nitrogenous cell components for about two divisions. This ability to divide in the face of environmental adversity has been suggested as having great importance in the survival of the species. If the cells can continue to divide and live they have the chance, even despite a greater tendency to sink, of being transported to more favorable conditions. Studies of both RNA and DNA concentration in natural populations might be illuminating if combined with studies of their dynamics.

Uncertainties of relationships between plant and animal in both marine and freshwater environments have attracted the attention of numerous investigators. The role of bacteria in the energy cycle as decomposers of both plants and animals and as a food supply for zooplankton and other filter feeders is just beginning to receive the attention it deserves.

Carbon¹⁴ studies evidence a significant secretion of dissolved organic matter into the environment by phytoplankton. Much of the extracellular products of photosynthesis appears to be in the form of glycolic acid. The use of this fixed carbon as a substratum for heterotrophic growth of both phytoplankton and bacteria lends itself to a most useful line of experimentation and could prove to be a very valuable contribution by aquatic ecologists.

The thought that underlies many biological studies is that although organisms undergo physical change with age, they are basically the same organisms and are genetically stable. The unreliability of even this assumption is evident from isolations of blue-green algae which in time may completely lose the ability to develop spores or to develop permanent resistance to antibiotics. Similarly *Chlorella vulgaris* has been shown to have considerable instability in pigment pattern. The combination of genotypical and phenotypical variation makes species in some genera of the dinoflagellates extremely difficult to identify. Phenotypic variation is often visible in a single laboratory culture. In the sea, phenotypic differences within a single species may be great over what is probably a spectrum of environmental variability.

Adaptation to a wide range of salinity has been discussed in relation to *Skeletonema*. Actual adaptation is difficult to differentiate from selection as it involves preventing osmotic shock in this case. In *Chlorella vulgaris* which develops and retains resistance to the inhibiting effect of the amino acid analog methionine on cell division, we have a better example of what is probably selective adaptation. This appears to have resulted from a change in cell permeability to methionine and its analogs.

The relationship between environment and reproduction of many kinds of phytoplankton such as the marine diatom *Skeletonema* is certainly of great interest and worthy of further study. Factors influencing the production of auxospores by marine and freshwater phytoplankton certainly deserve special attention. In general variability in morphology and size contributes greatly to errors in plankton taxonomy. These problems are often accentuated in the laboratory where it is obviously difficult, if not impossible, to duplicate natural conditions of light intensity, temperature, turbulence, and nutrition.

The turbidostat and the chemostat originally designed to obtain cells at the same physiological state have both been discussed at length. In general the former performs best at high nutrient levels and the latter at lower levels. Doctor Jones has stressed the desirability of experimenting with batch cultures which more nearly duplicate conditions in the sea. So far concentrations of 28 cells/mm³ appear as the lower limit for his continuous cultures. Growing two or more or-

ganisms together in a chemostat is likely to produce results of particular interest to the ecologist as this culturing device more nearly approaches natural nutrient levels.

The impact of man's activities on the environment is of ever increasing importance to the aquatic ecologist. There is a report of detergents in detectable levels some 500 miles at sea. The general increase with depth of metal content in the sea that has developed through decomposition of organisms in the tropholithic zone appears to be reversed in the case of lead. More lead appears in the surface waters and this is attributed to the burning of tetraethyl lead on land and its subsequent transport to the sea by precipitation. Doctor Hutchinson's observation of an enrichment of lead on plant surfaces lends support to this contention of appreciable lead in the atmosphere. The extreme sensitivity of some organisms to heavy metals might already have made this a factor in plankton succession where concentrations of lead are highest.

Although not discussed during the conference, atomic fallout and pesticide residues are generally recognized as other factors to which the seas are not immune. The biologist has a growing responsibility to keep abreast of these changes man is making in order to conserve the important resources of the sea.

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